

## **Appendix 1**

### **Essential Nutrients**

Essential nutrients are those that are required to properly drive biochemical reactions within the body. These nutrients may be required as a specific energy source, as structural components or as factors and cofactors in specific biochemical reactions or processes.

### **Energy**

The total amount of energy, or the gross energy contained within the feed, is broken into several fragments as it is metabolized in the body. During the process of digestion, potential energy sources are lost through the faeces, urine and urates. What remains is the metabolizable energy (ME), or that which is available for the body's metabolic processes. A portion of the ME is lost as heat (the heat increment). The remaining energy (net energy value of the food) is available for maintenance of the bird. Any energy that remains after satisfying the basic maintenance requirements is available for production activities such as growth of body mass and feathers, production of eggs and for exercise. That which is surplus to the needs of all these activities is deposited as fat.

The bird derives energy from proteins, fats and carbohydrates in the diet. Of these, protein is the least efficient source of energy. Carbohydrates are the most important energy source for the body because they are the only energy form that the brain can use. Of the carbohydrate family, energy is derived from starches (digestible polysaccharides), disaccharides (sucrose, maltose) and the simple sugars or monosaccharides (glucose, fructose, mannose, galactose). Carbohydrates also form the fibre fraction of the diet, broadly classified as indigestible carbohydrate. This fraction consists mainly of cellulose, which is essentially undigested because of the bird's lack of the enzyme *cellulase*. The required dietary fibre intake of varying species of companion breeds is undetermined.

Dietary fat is not only an important source of energy but it is the primary storage form of energy in the body. The ME in fat is concentrated with a value of 9 kcal/g, 2.25 times greater than that of either carbohydrates or protein. Fat is also easily absorbed into the body via the gastrointestinal tract, with its digestibility being dependent on the fatty acid composition.

### **Essential Fatty Acids**

Animals and birds have no requirement for fat per se, but they do have a requirement for the individual fatty acids that make up fat.

The primary essential fatty acid for animals and birds is linoleic acid. This compound cannot be synthesized in the body so it must be provided through the diet.

The essential fatty acids are used as structural components in the cell with particular importance in the cell membranes. Based on the general requirements for most other species, it can be safely predicted that the linoleic acid requirement for companion and aviary birds is 1.0 to 1.5% of the diet. In seed-based diets, this would rarely fall short.

### **Amino Acids and Protein**

Amino acids are the building blocks of the protein chain. The protein chain can contain up to 22 different amino acids. Of these, ten cannot be manufactured by the body, so they must be routinely provided by the diet (essential amino acids).

The quality of a protein is determined by two primary factors. The first is the balance of amino acids within that protein. To be optimally utilized, the protein should have an amino acid profile similar to that of the animal's body. The second criteria that affects protein quality is the availability of the amino acids within the foodstuff.

## **Vitamins**

The vitamins are chemically unique but share similar metabolic roles and modes of action and are therefore grouped together.

Generally, vitamins are defined as natural food components that are present in minute quantities, are organic in nature and are essential for normal metabolism and health. They will cause specific, characteristic deficiency symptoms when they are severely limited in the diet. Metabolism will generally be affected to a degree proportional to the level of the deficiency; therefore, in the case of mild deficiency, the symptoms are usually vague and non-specific, such as poor performance or compromised health. Vitamins are now subcategorized into two general groups based on their solubility characteristics.

The fat-soluble vitamins are comprised of vitamins A, D, E and K.

The water-soluble vitamins include thiamine (vitamin B1), riboflavin (vitamin B2), niacin, pyridoxine (vitamin B6), pantothenic acid, biotin (vitamin H), folic acid (vitamin M), vitamin B12 (cyano-cobalamin), choline and ascorbic acid (vitamin C).

## **Vitamin A**

Vitamin A occurs in several forms: retinol (alcohol), retinal (aldehyde) and retinoic acid, all having different metabolic activity.

Plants do not contain active vitamin A, but instead contain vitamin precursors. These exist in the form of carotenoid plant pigments, with the *carotenes* being the most important of the pro-vitamin A compounds. In the avian species studied, beta-carotene is the most active of the carotenoid compounds.. The sum of the vitamin A content (expressed in retinol equivalents or IU) and the contribution from carotene represents the total vitamin A activity of the food.

The most well understood function of vitamin A is its role in vision, but the most influential action of vitamin A in avian medicine is its effect on the growth and differentiation of epithelial tissues, with deficiencies resulting in keratinisation ( a thickening of the cells with loss of function), of the tissue. It is in this role that

vitamin A is obligatory for normal disease resistance because it is required for the maintenance of adequate mucous membranes and for the normal functioning of secretory tissues (eg, the adrenal glands for the production of corticosteroids).

Vitamin A also functions in the proper growth of bones and in the maintenance of normal reproduction. It is generally accepted that vitamin A improves the immune function of the body; however, its mode of action has not been totally elucidated.

The liver will typically contain over 90% of the total body stores of vitamin A and is mobilised for use as required.

### **Vitamin D**

There are two predominant forms of vitamin D: ergo-calciferol (vitamin D<sub>2</sub>), a plant derivative, and chole-calciferol (vitamin D<sub>3</sub>), produced exclusively in the bird's body. In all of the birds studied, vitamin D<sub>3</sub> is considered to be 30 to 40 times more potent than vitamin D<sub>2</sub> as a source of vitamin D activity. Therefore, plant sources of vitamin D are essentially disregarded when providing vitamin D to birds. Unlike most other vitamins, the active form of vitamin D<sub>3</sub> can be synthesized in the body by the conversion of 7-dehydrocholesterol in the skin and sebaceous secretions by irradiating with ultraviolet rays. Early studies in poultry showed that sufficient Vitamin D<sub>3</sub> could be formed to prevent rickets in growing chickens and maximize growth with 11 to 45 minutes of sunshine (not filtered by glass) each day.

The cholecalciferol formed in the skin is then transported by the blood to the liver, where it is converted. This new compound is then transported to the kidney, where it is again converted to the metabolically active form. Unlike other vitamins, the active metabolite actually acts as a hormone in the body, being transported to the intestines, bones and other target organs where it exerts its role in the metabolism of calcium and phosphorus.

## **Vitamin E**

Vitamin E is abundant in plant materials (particularly those high in oil) and in plant leaves. In cereal grains, vitamin E is concentrated in the germ. Alfalfa leaves are a particularly high source of vitamin E. However, as many of the oils in these plants are polyunsaturated, and attempt to oxidise as soon as they come in contact with air, (the process of becoming rancid), much of the vitamin E is used up combating this process.

Vitamin E is a compound with eight active forms. The compound of the greatest biologic importance in the avian species is alphanatocopherol.

Vitamin E is essentially a biologic antioxidant that functions at the intercellular and intracellular level by preventing the oxidation of saturated lipid compounds in the cell, thereby maintaining membrane integrity. Working in conjunction with vitamin E are several metallo-enzymes. These enzymes incorporate manganese, zinc, copper, iron and selenium as active components.

Vitamin E is absorbed through passive diffusion and is dependent upon normal lipid digestion requiring presence of bile salts and pancreatic juices. Any malabsorption syndrome will decrease uptake. Vitamin E enters the portal circulation in association with chylomicra, (microscopic fat particles) but is readily transferred to plasma lipoproteins for transportation to the liver. Initial storage occurs in the liver, being released primarily in lipoproteins. Liver and plasma stores of vitamin E are the most readily accessible to the body in times of need. Vitamin E stores of the body tend to be relatively stable, that is not easily mobilised, and may not be effective in preventing a vitamin E deficiency from occurring. It appears that the breakdown (lipolysis) of fatty stores may be required for vitamin E to be released.

## **Vitamin K**

Vitamin K actually represents a large number of related compounds that possess widely varying degrees of anti-hemorrhagic characteristics, all being forms of the compound naphthoquinone.

Vitamin K comes from three sources: 1) green plants (phyloquinones - K1 series), 2) bacteria (menaquinones - K2 series) and 3) synthetic forms (menadione - K3)

Natural vitamin K compounds require the presence of dietary fats and bile salts for proper absorption from the gastrointestinal tract; therefore, altered micelle formation (eg, decreased pancreatic and biliary function) will impair the normal absorption of vitamin K. Menadione salts are fairly water-soluble so they are less reliant on micelle incorporation. Absorption of the K2 and K3 forms occurs by passive diffusion throughout the intestines and also in the colon, while K1 is absorbed via an active transport process in the proximal small intestine. Vitamin K then enters the portal circulation and, in association with chylomicra, is transported to the liver. Generally, vitamin K is stored only briefly in the liver before it is released into the body and transported to all tissues via lipoproteins.

A number of plasma clotting factors (eg, prothrombin) are dependent on vitamin K for their synthesis.

## **Thiamine (Vitamin B1)**

Thiamine is fairly common in food sources, but generally at only low concentrations but is readily available when normal amounts of gastric hydrochloric acid are present. Thiamine is absorbed both by an active transport system and at high concentrations, by passive diffusion. After absorption, thiamine is transported via the portal vein to the liver, predominantly bound to serum albumin. Thiamine is not stored for any length of time in the body. It is excreted primarily through the urine and in lesser amounts through the faeces. About 80% of thiamine in the body is present as thiamine pyrophosphate

## **Riboflavin (Vitamin B2)**

In foods, riboflavin is generally bound to proteins in the form of flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD). Riboflavin contained in plant materials is generally less available than from animal sources because of decreased digestibility of the flavin complexes in plants.

These forms are broken down in the gastrointestinal tract, absorbed and then transported to the liver and other tissues, where riboflavin enters the cell in the free form.

Very little riboflavin is stored in the body; the highest concentrations are found in the liver, kidney and heart. Unlike other tissues, the egg contains predominantly free riboflavin. Laying chickens have been found to have specific riboflavin-binding proteins in the plasma. These are produced in the liver under the influence of oestrogen and are believed to be involved in the transovarian passage of free riboflavin.

Riboflavin as part of the coenzymes FMN or FAD (flavoproteins) act in a large number of enzyme complexes that are responsible for essential reactions in the utilization of carbohydrates, fats and proteins. The flavoprotein enzyme complexes often contain a metal ion (eg, iron, molybdenum, copper) and function to help regulate cellular metabolism, the metabolism of carbohydrates, the breakdown of amino acids, the formation of uric acid, the formation of ascorbic acid, fatty acid biosynthesis and degradation, oxidation of various substrates in drug metabolism and other functions.

## **Niacin**

Niacin exists in two major forms, nicotinic acid and nicotinamide.

Niacin is widely distributed in foods, but that found in plants has low bioavailability. The greatest concentrations of niacin compounds are in the liver, but no true storage occurs.

The coenzymes NAD and NADP are important components in carbohydrate, fat and protein metabolism, being especially important in the energy-yielding reactions of the body. These functions are critical to the generation of energy for the body as well as for normal tissue integrity, especially of the skin, alimentary tract and the nervous system.

### **Pyridoxine (Vitamin B6)**

Vitamin B6 refers to the group of three compounds: pyridoxal, pyridoxamine and pyridoxal phosphate. Pyridoxal is the form predominantly found in plants, the other two are found mainly in animal tissues. Large amounts of vitamin B6 in foods are bound to proteins or complexes, some of which have very low bioavailability. After digestion to free the vitamin from these protein complexes, vitamin B6 is absorbed by passive diffusion throughout the entire small intestine and is transported to the liver. Minimal amounts of the vitamin are stored in the body.

The metabolically active form of vitamin B6, pyridoxal phosphate, is involved in a number of enzyme systems as a coenzyme. It is required in essentially all major areas of amino acid utilization and in the formation of antibodies. A deficiency of pyridoxine creates a deficiency of many other important metabolites and hormones such as serotonin and histamine

### **Pantothenic Acid**

Pantothenic acid is present in feeds in both the bound form (predominantly CoA) and free forms.. CoA is one of the most critical coenzymes in tissue metabolism, forming the compound acetyl CoA.. Acetyl CoA acts as the entry point into the citric acid cycle for carbohydrate metabolism, a point of entry for amino acid degradation and as an essential component in fatty acid biosynthesis and degradation, the synthesis of triglycerides and phospholipids, as well as in the formation of compounds such as acetylcholine, mucopolysaccharides, cholesterol, steroid hormones and many more.

## **Biotin**

Biotin is widely distributed in foods but generally at low concentrations. A relatively large portion of naturally occurring biotin is present in a protein-bound form with varying degrees of biological availability. There is evidence that suggests that the synthesis of biotin by intestinal microflora is important. Microbial-derived biotin would be manufactured and absorbed in the large intestine.

## **Folic Acid (Folacin)**

Folates are generally widely distributed in foods and are present as the polyglutamic derivatives of folic acid. These are converted by hydrolysis to free folic acid and absorbed by both an active transport system and passive diffusion in the duodenum and jejunum. The absorption process is only moderately efficient (<50%).

Folic acid's primary metabolic role is in the transfer of single-carbon moieties in a wide variety of reactions. This function is particularly important in amino acid metabolism. Because of folic acid's requirement in the synthesis of three of the four nucleic acids, a deficiency results in impaired cellular division and an alteration of protein synthesis. This is particularly noticeable in the young growing animal.

Additionally, due to impaired cell mitosis in a deficient bird, females do not physiologically prepare for breeding, as noted by a lack of oviduct hypertrophy in the presence of oestrogen. Further, there is an effect on normal red blood cell maturation, resulting in the characteristic macrocytic anemia. Similarly, deficiencies result in immune system impairment due to the effects on cell replication and protein synthesis. Folic acid is involved in the formation of uric acid, so there is an increased requirement when high-protein diets are provided. Folic acid is required for the production of white blood cells and a severe deficiency can reduce immunologic response through decreased WBCs or reticuloendothelial cells. In some species, a deficiency of zinc has been found to impair the utilization of dietary sources of folic acid. A zinc deficiency decreases the absorption of folic acid because of impaired activity of the mucosal enzyme that creates an absorbable form of folic acid.

Vitamin C and iron may improve the bioavailability of folates in food.

## **Vitamin B12**

Vitamin B12 or cyanocobalamin is a product of bacterial biosynthesis and therefore must be obtained by consuming a bacterial source or animal tissues that accumulate the vitamin. The only exceptions are a few plants, such as peas, beans, spirulina and kelp, that may be able to synthesize minute amounts of this vitamin, although this accumulation is likely due to their close symbiotic association with bacteria.

Naturally occurring vitamin B12 occurs in the coenzyme form bound to protein. This complex is broken, primarily through the normal action of pepsin and trypsin. Free vitamin B12 is absorbed by the intestinal tract via an efficient active transport system involving a vitamin B12 specific-binding protein. At very high levels, simple diffusion occurs throughout the small intestine.

Most of the vitamin B12 in the body is found in the liver with secondary stores in the muscle. Lesser amounts (but high concentrations) are contained in the pituitary gland, kidney, heart, spleen and brain. Vitamin B12 is stored efficiently, with a long biological half-life (approximately one year in humans).

Vitamin B12 is a critical component of a large number of metabolic pathways. It interacts with several other nutrients such as folic acid, pantothenic acid, choline and methionine. Vitamin B12 deficiencies result in an impairment of protein synthesis causing failure or delay of normal cell division. This affects growth rate and feed intake, may result in nervous disorders and poor feathering, perosis (bone thinning), anaemia and fat accumulation in the heart, liver and kidneys. Deficiency of vitamin B12 can also create a folic acid deficiency.

## **Choline**

Natural sources of choline are widely distributed and occur primarily in the form of phosphatidylcholine (lecithin). Phosphatidylcholine is readily broken down in the intestinal lumen and is absorbed by the mucosa via both active transport and passive diffusion, depending on luminal concentrations. Of the free choline that is ingested,

up to two-thirds may be metabolized by intestinal microorganisms. The remainder is absorbed intact.

Choline is found in all tissues as a part of the membrane phospholipids, with the greatest concentrations in organs such as the brain, liver and kidney. Choline can be synthesized in the body but in the avian species tested to date, it cannot be synthesized at high enough levels to meet the needs of the young bird. It appears that with age, the synthetic abilities improve, thereby meeting most of the body's needs.

Choline has four general metabolic functions: 1) As a component of phospholipids, choline is an essential part of the cell membrane and is required for maintaining cell integrity; 2) Choline is required for maturation of the cartilage matrix of bone; 3) Choline is involved in fat metabolism of the liver by promoting fatty acid transport and utilization, and is therefore necessary to prevent hepatic lipidosis in the normal bird; 4) Choline is acetylated to form the neurotransmitter acetylcholine.

Because of their interrelated functions, the requirement for choline is dependent upon the levels of folic acid and vitamin B12 available to the animal. Excess protein increases the choline requirement, as do diets high in fat.

### **Vitamin C (Ascorbic Acid)**

Vitamin C has not been demonstrated to be a required nutrient for any of the avian species, except for a few highly evolved, largely frugivorous species (Willow Ptarmigan and Red-vented Bulbul). Vitamin C is easily manufactured in birds with the enzyme L-gulonolactone oxidase.. Biosynthesis of ascorbic acid can be inhibited by deficiencies of vitamin A, E and biotin.

The metabolic functions of vitamin C are related to its ability to act in oxidation and reduction reactions. Its best understood role is in the synthesis of collagen. Collagen, the major component of skin and connective tissue and also the single most abundant protein in the body, is critical for proper cell structure and integrity. In species

requiring vitamin C in their diet, the breakdown of this function produces the classic deficiency symptoms (scurvy, capillary fragility, gum and bone alterations and poor healing). Vitamin C is also an excellent antioxidant, acting to neutralize free radicals that are produced in the body. Ascorbic acid can also regenerate vitamin E (the active lipid antioxidant).

## **Minerals**

Minerals are essentially classified in one of two groups: macro minerals and trace or micro minerals.

The macro minerals can be classified based on their use in the body. Calcium and phosphorus act primarily in the body's skeletal structure, while sodium, potassium and chlorine (along with phosphates and bicarbonates) function to maintain homeostasis in the body (acid/base balance and proper osmotic pressures). The required trace minerals are magnesium, manganese, zinc, iron, copper, iodine, selenium and, in certain situations, cobalt and molybdenum. These trace elements have their primary function as parts of enzymes, hormones or as enzyme activators.

As the normal digestion process breaks food into its components, the minerals are liberated, and the cationic elements are converted to chloride salts in the presence of gastric hydrochloric acid. Once in the intestinal tract, they are able to easily dissociate and be absorbed. There is also considerable complexing with other minerals or chelating agents. An example of this is the calcium and phosphorus precipitate that is formed by excess levels of these minerals while in the alkaline conditions of the small intestine. This complex can then adsorb manganese or zinc, causing excretion of the trace mineral, and subsequently, an increased requirement.

Mineral (particularly trace mineral) concentrations of foodstuffs are largely dependent on the original mineral source.

Concentrations in plant products are dictated by the soil mineral content, while those of animal products are dependent on the diet consumed.

## **Calcium**

Calcium is the predominant mineral in the body (approximately 1.5% of body weight) with primarily skeletal system containment. Calcium is also contained in the body fluids, where it plays an essential role in blood coagulation and membrane permeability, and maintains normal excitability of the heart, muscles and nerves. Several enzyme systems are also activated by calcium. Ionic calcium( $\text{Ca}^{++}$ ) is the physiologically active form. Low  $\text{Ca}^{++}$  concentrations result in a decrease in electrical resistance and an increase in membrane permeability (to sodium and potassium) of nerve tissue, which causes hyper-excitability of neural and muscle tissue and can result in spontaneous fibre discharge.

Calcium absorption occurs predominantly in the upper small intestine by an active transport system involving a calcium-binding protein. This is regulated by the active metabolite of vitamin  $\text{D}_3$  in response to low plasma calcium levels. A lesser amount of absorption also occurs in the lower small intestines through passive diffusion.

High-protein diets and acidification of the intestines aid calcium absorption.

Compounds such as phytate (in cereal grains), oxalates (in spinach, rhubarb and related vegetation) and phosphates will decrease absorption of calcium due to the formation of complexes. Similarly, high intestinal concentrations of free fatty acids (from very high-fat diets or because of impairment in fat digestion) will result in the formation of insoluble calcium soaps.

Once absorbed, calcium is carried by the plasma as ionized calcium, protein-bound calcium and a small amount of chelated calcium (chelated with citrate and phosphate). Regulation of calcium metabolism involves parathyroid hormone, calcitonin and vitamin  $\text{D}_3$ .

The calcium content of dried, fat-free bone is approximately one-third of the total weight, predominantly present in the form of calcium phosphate, with lesser amounts

of calcium carbonate. In egg shells, calcium carbonate is the structural compound. For maintenance of proper bone tissue, the calcium to available phosphorus ratio should be approximately 2 to 1. A range of 0.5:1 to 2.5:1 can be tolerated. The further this ratio deviates from the ideal level, the more critical proper vitamin D<sub>3</sub> levels become. Vitamin D<sub>3</sub> is essential to regulate absorption and metabolism of calcium and phosphorus, especially when dietary levels are unbalanced. During growth of most species, ratios of approximately 1:1 are required to support adequate growth, 1.5:1 to maintain normal serum calcium and phosphate and alkaline phosphatase values, and 2:1 to achieve maximum bone density. High egg-producing hens (poultry) may be provided with dietary ratios in excess of 10:1 in order to support daily shell production. This must not be confused with the significantly lower needs of a hen (most companion birds) that produces a periodic clutch of eggs. This ratio is based on the amount of phosphorus available to the bird, not the total phosphorus content of the diet. As much as 70% of the phosphorus in certain ingredients can be present in a form that is unavailable to the bird. Therefore, an estimation of the diet's available phosphorus is essential in order to balance these two minerals.

Levels of over 1.0% calcium in the diet have been observed to decrease the utilization of proteins, fats, vitamins, phosphorous, magnesium, iron, iodine, zinc and manganese. Where there are marginal intakes of one or more of these nutrients, increased calcium intake can induce a deficiency state.

## **Phosphorus**

In addition to being an important bone constituent, phosphorus is also a component of proteins, carbohydrates and lipid complexes that perform vital functions in the body. Phosphorus has a wider range of biological functions than probably any other element.

Phosphorus is widely distributed in nature, occurring as phosphates, orthophosphoric acid salts and organophosphates. Absorption of phosphorus in the orthophosphate

form takes place primarily in the duodenum, with efficiency of adsorption being dependent on the metabolic requirement and affected by a number of factors such as its source, calcium:phosphorus ratio, intestinal pH and dietary levels of vitamin D, potassium, magnesium, manganese, iron and fat. Once absorbed, it is readily incorporated into bone and other tissues, with bone acting as the reservoir. Like calcium, circulating levels are regulated by parathyroid hormone and the hormone calcitonin, with plasma levels being inversely related to plasma calcium levels. Excretion of excess amounts of phosphorus takes place primarily through the kidneys.

In plant sources, phosphorus is often complexed with phytin, making it unavailable to all monogastric animals because of their lack of the enzyme phytase.. As a general rule, phosphorus from animal products or inorganic supplements is almost completely available, while that from plant sources is generally considered to be approximately 30% available. These typical values can be used to generate an estimation of the available phosphorus in the diet.

When kept within the range of acceptable calcium:phosphorus ratios, moderately higher phosphorus does not create a significant problem. Amounts of phosphorus outside these acceptable ratios, however, will cause decreased performance and will interfere with the absorption of calcium from the gastrointestinal tract. Additionally, high serum phosphorus levels can induce nutritional secondary hyperparathyroidism by suppressing serum calcium, resulting in stimulation of the parathyroid. In some species, increased excretion results in the development of urolithiasis (urinary stones). It is estimated that the level of available phosphorus, when balanced with calcium and vitamin D, can be supplied at approximately two times the requirement without adverse effects. Amounts greater than this level have resulted in increased mortality in a number of species.

## **Magnesium**

Most of the body's magnesium is present in the bone, complexed with calcium and phosphorus. In the body fluids, the majority of magnesium is found in the blood cells, whereas calcium is predominantly associated with the plasma. Magnesium (like potassium) is found at the highest concentrations in soft tissue cells (intercellularly) such as liver, striated muscle, kidney and brain. In these tissues, magnesium serves as an activator for many of the enzymes involved in metabolism.

Magnesium is absorbed in a manner similar to calcium and phosphorus, with the efficiency of absorption dependent on the concentration in the gastrointestinal tract. With low levels, absorption tends to be very efficient, with decreasing efficiency as levels become higher. Most of this mineral appears to be absorbed in the small intestine. Levels of calcium and phosphorus in a diet affect the magnesium requirement, with high levels of either of the former tending to increase the requirement of the latter.

## **Potassium**

Potassium is widely distributed in most foods, making deficiencies unlikely in adult animals. Unlike sodium, potassium is located primarily intracellularly, and is found at the highest levels in muscle, erythrocytes, brain and liver. Potassium is the primary intracellular cation, affecting acid-base balance and osmotic pressure. It is also involved in protein biosynthesis, cellular uptake of amino acids and as a cofactor in a number of enzyme systems. In the extracellular fluids, potassium reduces muscle contractility and induces relaxation, therefore having the opposite effect of calcium. Potassium is absorbed predominately in the upper small intestine by passive diffusion, although absorption occurs to a lesser extent throughout the entire intestinal tract. Excess potassium is excreted through the kidneys under the influence of sodium and the hormone aldosterone.

The minimum requirement of potassium is influenced by the dietary levels of sodium, total chlorides, the energy content of the food and possibly the protein content.

## **Sodium**

Sodium is the primary cation of the extracellular fluid, and is predominantly responsible for the regulation of the body's acid-base equilibrium by associating with either chloride or bicarbonate. Sodium is critical in the maintenance of the proper osmotic pressure in the body, protecting against excessive fluid losses. It is also involved in the transmission of nerve impulses, the permeability of cells and acts to inhibit mitochondrial enzyme systems that are otherwise activated by the intercellular ions, potassium ( $K^+$ ) or magnesium ( $Mg^{++}$ ).

Sodium salts are readily and efficiently absorbed by the body (primarily in the ileum), and can be efficiently conserved when the dietary supply is limited. Excess sodium, on the other hand, can be efficiently excreted through the kidneys by an increase in water consumption. Sodium retention is regulated by the adrenal hormone, aldosterone, which maintains proper plasma sodium levels and regulates sodium excretion.

Depending on the species, bone will contain between 25 and 50% of the total body sodium, which is bound to the inorganic matrix of the bone. The rest of the sodium is predominantly found in the extracellular fluid of the body, with highest concentrations in plasma, nervous tissue and muscle tissue.

High levels of sodium intake result in poor feathering, polydipsia, polyuria, nervousness, oedema, dehydration and mortality.

## **Chlorine**

Chlorine, metabolically active as the chloride ion, is closely associated to sodium in foods, in the body and in metabolic processes, and both will be excreted under the same conditions. Chloride is also essential in maintaining the body's acid-base balance, osmotic pressure and water balance. It is a component of the hydrochloric acid that is produced by the body as a primary gastric secretion. In the body, chloride is concentrated in spinal fluid and blood.

It is critical to evaluate the overall dietary sodium, chlorine and potassium levels together. In the diet there must be a balance of the total sodium and potassium content with the total chloride and sulphate content in order to maintain the proper acid-base balance in the blood. This becomes particularly important with the addition of relatively high levels of dietary supplements that are complexed with one of these ions (such as high levels of choline chloride or lysine hydrochloride), especially when the chloride or sulphate form increases the acidity of the diet.

## **Essential Trace Minerals**

### **Iron**

The functions of iron in the body are almost entirely related to the cellular respiration processes. In the body, iron exists as haem iron (which is chelated with a porphyrin group) and non-haem iron (which is found bound to proteins). Iron is present in the body at approximately 50 to 100 parts per million and is unique in that body reserves are conserved and recycled very efficiently with negligible excretion. The primary method of iron depletion is through bleeding. Any iron found in the faeces is generally a result of unabsorbed iron from the diet. Because the body has no normal pathway for the excretion of excess iron, intestinal absorption is carefully controlled to prevent accumulation. Under normal situations, the absorption of iron from the gastrointestinal tract is poor, however, if the body becomes marginally deficient, the absorption is improved until the situation is corrected. (c.f. Iron-storage disease in birds)

Normally, haem iron (from animal sources) is considered to be approximately 20-25% available to the animal, while non-haem, vegetative sources are usually less than 5% available. Additionally, the non-haem iron present in most foods is in the ferric form ( $\text{Fe}^{+++}$ ), which is poorly absorbed. This can be present either as the free ferric ion or loosely associated with an organic compound. In order for proper absorption to take place, ferric iron must be reduced to the ferrous state ( $\text{Fe}^{++}$ ). In the ferrous form, iron becomes more soluble and therefore absorption is improved. This can be accomplished by any reducing compound in the food, with ascorbic acid (vitamin C)

being one of the more efficient agents. Proteins also enhance absorption, probably by forming soluble amino acids chelated with the iron. Additionally, absorption may be improved by dietary organic acids (eg, citrate, lactate), fructose and vitamin E, as well as by diets low in phosphorus. Normal gastric secretion is necessary to solubilise iron and increase its availability. Total iron absorption from a variety of mixed diets has been observed to range from 2 to 20% across a number of species.

In the normal, healthy animal there should be no toxicity symptoms from moderate excesses of dietary iron because of the efficient controls the body has over iron absorption and metabolism. Excess iron can reduce performance, however, by creating interactions with a number of nutrients. Chronically high iron intake can result in elevated blood levels, increased tissue concentrations (especially of the liver and spleen) and the eventual development of hemosiderosis and possibly hemochromatosis (skin pigment changes). Liver damage and sometimes pancreatic fibrosis occur in this condition, which in other species is most often due to a genetic anomaly (extremely efficient absorption). Iron storage diseases have been predominantly seen in mynahs and toucans, possibly being caused by a combination of genetic and dietary factors.

## **Copper**

The copper content in the bodies of most species is approximately two parts per million. The largest concentrations are in the liver. Copper is a component of several proteins, enzymes and certain natural pigments. It is required for hemoglobin synthesis, proper collagen (bone), elastin and keratin formation and maintenance of the nervous system.

Copper is well distributed in normal feedstuffs, so the likelihood of a copper deficiency on a mixed, practical diet is not great. Availability can be affected by the chemical form as well as the copper status of the animal, with more efficient

absorption occurring when the animal is deficient or when the dietary concentration is low.

### **Zinc**

Zinc is critical to the animal for growth, reproduction and normal longevity because of its involvement in tissue repair and wound healing. It functions in a number of reactions in protein and carbohydrate metabolism, cell division and mucopolysaccharide formation. It also functions in the mobilization of vitamin A from the liver. Zinc is required in a large variety of enzymes, either as an enzyme activator or as a component of certain metalloenzymes.

Zinc is widely distributed in foodstuffs, but generally is not present in adequate supply to fill the needs of the young or producing animal. In plant sources, phytate can actively bind with inherent zinc, producing varying degrees of zinc availability. Some high-phytate ingredients, such as wheat bran or buck-wheat, may also bind zinc from other dietary sources. Additionally, zinc requirements are increased with added calcium in the diet.

### **Manganese**

Manganese is present in most plant sources at moderate to poor levels. Compounding the problem of marginal levels is its relatively poor availability. The formation of chelates appears necessary for the proper absorption of manganese, which occurs throughout the intestinal tract. Bile salts are important in the absorption, excretion and reabsorption of this mineral. Recycling appears to occur several times before the mineral is finally excreted in the faeces. In addition to the constantly recycling pool in the intestines, the primary storage sites for manganese are bone, kidney and liver. With high dietary intakes, the skin and feathers will accumulate large quantities of this element.

Manganese has several functions in the body. It is essential for normal bone structure, being required for the formation of the organic bone matrix through involvement in the synthesis of chondroitin sulphate.

### **Iodine**

Iodine's sole metabolic function is for the biosynthesis of the thyroid hormones. Thyroid hormone functions to control the rate of energy metabolism in cells. In this way it influences growth and tissue differentiation or maturation, with resultant effects on other endocrine glands, neuromuscular function, skin and tissue development and nutrient metabolism.

Iodine is easily absorbed from the gastrointestinal tract and is transported by loose attachments to plasma proteins. A large portion of the ingested iodide is excreted by the kidney, while the remaining amounts are taken up primarily by the thyroid gland. Small amounts can also be found in the salivary glands, stomach and other locations. The iodide uptake by the thyroid is stimulated by thyroid stimulating hormone (TSH) produced by the pituitary.

### **Selenium**

To a greater degree than other trace minerals, selenium content in foods is largely dependent upon the soil selenium content in which they were grown.

The absorption of selenium is dependent upon its chemical form. The bioavailability of selenium in most plant products ranges from 60 to 90%, while in animal products it is less than 25%. Of the different chemical forms of selenium, selenite has the highest availability followed by selenomethionine, selenide and lastly, elemental selenium. The efficiency of absorption is also dependent upon the levels in the diet, with absorption higher during a deficiency situation. Once absorbed, selenium is carried in association with plasma proteins and transported to all tissues. Although selenium is distributed throughout the body, it is found in the highest concentration in the

kidneys, pancreas, pituitary and liver. Other than the enzymatic form, there are no stores of selenium.

Selenium's metabolically active form is as a component of glutathione peroxidase. This enzyme is located in cells and acts as a biological antioxidant. It protects membrane lipids and other cellular constituents by preventing oxidative damage by neutralizing any hydrogen peroxide and fatty acid hydroperoxides that are formed in the body.

Vitamin E and selenium are interdependent, each having the ability to spare the other. The protection of lipid membranes from exposure to free radicals is not only important for the cell membrane, but also for the membranes of the mitochondria and microsomes. Because these act to both fuel and protect the cell, it is necessary for adequate vitamin E and selenium to be present for the cell to maintain its defence mechanisms.