8

Minerals

Information concerning mineral nutrition of fish and crustaceans is limited compared to most other nutrient groups. In addition, there is much less information on mineral requirements of aquatic species compared to terrestrial animals, in part because of complications in that fish can absorb some minerals from the aquatic medium in which they live, in addition to from their diet. Nevertheless, the basic metabolic functions of the various mineral elements are the same for aquatic and terrestrial animals with the exception of osmoregulation (Table 8-1). The large discrepancy in knowledge of mineral nutrition between terrestrial and aquatic species is due to the relative infancy of aquatic animal nutrition and the related difficulty of conducting research on mineral nutrition of aquatic species. Problems associated with the quantification of mineral requirements include identification of the potential contribution of minerals from the water, leaching of minerals from the diet prior to consumption, availability of suitable test diets that have a low concentration of the targeted mineral, and limited data on mineral bioavailability. Despite these problems, mineral requirement data for a variety of species originating from diverse environments are becoming established (Tables 8-2, 8-3, and 8-4).

The metabolism of various minerals by aquatic organisms is influenced not only by dietary concentrations but also by the concentration and relative composition of dissolved ions in the aquatic medium, because they may influence the organism's osmoregulation, ion regulation, and acid:base balance (Moyle and Cech, 2000). Numerous minerals can be absorbed by the gills and contribute to meeting metabolic requirements. The uptake of minerals from the diet or aquatic medium and excretion of minerals in the urine and feces are influenced by osmoregulatory processes in response to the salinity of the aquatic medium. Organisms in freshwater are characterized as being hyperosmotic to the environment. They continually lose small ions from the gills, and water is passively taken up such that they do not drink water but excrete large quantities of dilute urine. In contrast, organisms in seawater are hyposmotic to the environment and thus lose

TABLE 8-1 Minerals and Some of Their Prominent Functions and Deficiency Signs Observed in Fish and Shrimp

Mineral	Functions	Deficiency Signs
Macromineral		
Calcium	Skeletal tissues, membrane permeability	Impaired growth and hard tissue mineralization
Chloride	Osmotic balance	Impaired growth
Magnesium	Enzyme activator	Tetany, muscle flaccidity
Phosphorus	Skeletal tissue, phospholipids	Impaired growth, reduced hard tissue mineralization, skeletal deformities, fat accumulation
Potassium	Osmotic balance, acid-base equilibrium	Convulsions, tetany
Sodium	Osmotic balance, acid-base equilibrium	Impaired growth
Micromineral		
Copper	Metalloenzymes	Impaired growth and reduced activity of copper-containing enzymes
Cobalt	Vitamin B_{12}	Anemia
Chromium	Carbohydrate metabolism	Impaired glucose utilization
Iodine	Thyroid hormones	Thyroid hyperplasia
Iron	Hemoglobin	Impaired growth, anemia
Manganese	Organic matrix of bone	Impaired growth, skeletal abnormalities, cataracts
Molybdenum	Xanthine oxidase	Reduced enzyme activity
Selenium	Glutathione peroxidase	Impaired growth, anemia, exudative diathesis, reduced activity of glutathione peroxidase
Zinc	Metalloenzymes	Impaired growth, cataracts, skeletal abnormalities, reduced activity of various zinc metalloenzymes

TABLE 8-2 Continued

Mineral	Species	Recommended Supplement (g/100 g diet)	Rearing Condition[a]	Reference
Ca:P ratio	*I. punctatus*	1.5:0.8	FW Practical diet	Andrews et al. (1973)
		No relationship	FW (14 mg Ca/L)	Lovell (1978)
	Chrysophrys major	0.34:0.68	SW	Sakamoto and Yone (1973)
		No relationship	SW	Sakamoto and Yone (1976a)
	Cyprinus carpio	No relationship	FW (20 mg Ca/L)	Ogino and Takeda (1976)
	Oncorhynchus mykiss	No relationship	FW (20–23 mg Ca/L)	Ogino and Takeda (1978)
	Oncorhynchus keta	No relationship	FW (20 mg Ca/L)	Watanabe et al. (1980)
	Chinook salmon (*Oncorhynchus tshawytscha*)	0.6–1.2	FW	Shearer (1988)
Potassium	*I. punctatus*	0.26	FW (4 mg K/L)	Wilson and El Naggar (1992)
	Chrysophrys major	Dispensable	SW	Sakamoto and Yone (1978b)
	I. punctatus	Dispensable	FW Practical diet	Murray and Andrews (1979)
	Oreochromis niloticus × *Oreochromis aureas*	0.2–0.3	FW	Shiau and Hsieh (2001a)
Sodium/ chloride	*Oncorhynchus mykiss*	Dispensable	FW; Levels up to 11.6% produced no adverse effects	Salman and Eddy (1988)
	Red drum (*Sciaenops ocellatus*)	2	FW and BW (6‰)	
		Dispensable	35‰	Gatlin et al. (1992)
Sodium	*Chrysophrys major*	Dispensable	SW	Sakamoto and Yone (1978b)
	Oreochromis niloticus × *Oreochromis aureas*	0.15	FW	Shiau and Lu (2004)
	Oreochromis niloticus × *Oreochromis aureas*	Dispensable	SW	Shiau and Lu (2004)
Magnesium	*I. punctatus*	0.04	FW (1.6 mg Mg/L)	Gatlin et al. (1982)
	Oncorhynchus mykiss	0.06–0.07	FW (3.1 ppm Mg)	Ogino et al. (1978)
		0.05	FW (1.2 mg Mg/L)	Knox et al. (1981)
		0.06	FW (1.3 mg Mg/L)	Shearer (1989)
	Nile tilapia (*Oreochromis niloticus*)	0.059–0.077	FW (1.0 mg Mg/L)	DaBrowska et al. (1989)
	Mozambique tilapia (*Oreochromis mossambicus*)	Dispensable	FW	van der Velden et al. (1991)
	Oreochromis aureus	0.023	FW	Reigh et al. (1991)
	Cyprinus carpio	0.06	FW (9.4 mg Mg/L)	Dabrowska et al. (1991)
	Chrysophrys major	Dispensable	SW (0.012% basal diet)	Sakamoto and Yone (1979a)

[a]Information about diet and/or water used in the experiment. FW = freshwater, BW = brackish water, SW = seawater.

water (but gain monovalent ions from the environment) such that they must drink water. The excess salts ingested are primarily excreted by specialized chloride cells in the gills and opercular skin epithelia via active transport, while the kidney excretes primarily divalent ions in small volumes of urine and other salts are concentrated in feces. In addition to maintaining stable internal osmotic concentrations relative to the aquatic environment, organisms also exert energy to maintain appropriate ionic and acid:base balance via active and passive processes in various organs including the gills, kidney, and gastrointestinal tract (Moyle and Cech, 2000). As such, these various processes may directly affect the metabolism of certain minerals.

The functions of macrominerals, those required in the diet and body at relatively high concentrations, include the formation of skeletal structures and other hard tissues (e.g., fin rays, scales, teeth, and exoskeleton), electron transfer, regulation of acid:base equilibrium, the production of membrane potentials, and osmoregulation. Six minerals, including calcium, chlorine, magnesium, phosphorus, potassium, and sodium, are the most commonly recognized macrominerals. Specific information about each of the macrominerals will be detailed in individual sections later in this chapter. Trace minerals or microminerals, which are typically required in the diet and body at much lower concentrations than the macrominerals, are important components of hormones and enzymes, serve as cofactors and/or activators of a variety of enzymes, as well as participate in a wide variety of biochemical processes. The most commonly recognized trace minerals include chromium, copper, iodine, iron, manganese, selenium, and zinc. Detailed information about each of these microminerals also will be provided in individual sections later in this chapter. Among the most important dietary minerals, eight are cations: calcium (Ca^{++}), copper (Cu^{2+}), iron (Fe^{2+}), magnesium (Mg^{2+}), manganese (Mn^{2+}), potassium (K^{+}), sodium (Na^{+}), and zinc (Zn^{2+}). Five are anions or are

TABLE 8-2 Macromineral Requirements of Fish

Mineral	Species	Recommended Supplement (g/100 g diet)	Rearing Condition[a]	Reference
Calcium	Channel catfish	1.5	FW Practical diet	Andrews et al. (1973)
	(Ictalurus punctatus)	Dispensable	FW (14 mg Ca/L)	Lovell (1978)
		0.45	FW (Ca-free)	Robinson et al. (1986)
	Blue tilapia	0.17–0.65	FW (Ca-free)	Robinson et al. (1984)
	(Oreochromis aureus)	0.7	FW (Ca-free)	Robinson et al. (1987)
	Red sea bream	Dispensable	SW	Sakamoto and Yone (1976a)
	(Chrysophrys major)			
	Common carp	Dispensable	FW (20 mg Ca/L)	Ogino and Takeda (1976)
	(Cyprinus carpio)			
	Rainbow trout	Dispensable	FW (20–23 mg Ca/L)	Ogino and Takeda (1978)
	(Oncorhynchus mykiss)			
	Chum salmon	Dispensable	FW (20 mg Ca/L)	Watanabe et al. (1980)
	(Oncorhynchus keta)			
	Hybrid tilapia	0.35–0.43	FW (27–33 mg Ca/L)	Shiau and Tseng (2007)
	(Oreochromis niloticus × Oreochromis aureas)			
Phosphorus	*I. punctatus*	0.8	FW Practical diet	Andrews et al. (1973)
		0.45	FW (0.03 mg P/L)	Lovell (1978)
		0.33 (available P)	FW (0.04 mg P/L)	Wilson et al. (1982)
	Cyprinus carpio	0.6–0.7	FW (0.002 mg P/L)	Ogino and Takeda (1976)
	Oreochromis aureus	0.5	FW (Ca-free)	Robinson et al. (1987)
	Oncorhynchus mykiss	0.7–0.8	FW (0.002 mg P/L)	Ogino and Takeda (1978)
		0.54–0.61	FW	Ketola and Richmond (1994)
	Oncorhynchus keta	0.5–0.6	FW (0.002 mg P/L)	Watanabe et al. (1980)
	Atlantic salmon	0.6 (available P)	FW (< 0.5 mg P/L)	Ketola (1975)
	(Salmo salar)		0.7% dietary P from plant sources	
		0.83–0.93	FW	Vielma and Lall (1998)
		1.0 (0.9 available P)	FW	Asgard and Shearer (1997); Lall and Bishop (1977)
	Hybrid striped bass	0.5	FW (150 mg/L hardness as $CaCO_3$)	Brown et al. (1993)
	(Morone chrysops × Morone saxatili)			
	Chrysophrys major	0.68	SW	Sakamoto and Yone (1978a)
	Milkfish	0.85	SW	Borlongan and Satoh (2001)
	(Chanos chanos)			
	Red drum	0.86	BW (5–6‰)	Davis and Robinson (1987)
	(Sciaenops ocellatus)			
	Yellow croaker	0.89–0.91 (available P)	SW	Ma et al. (2006)
	(Pseudosciaena crocea)			
	Haddock	0.96 (0.72 available P)	SW	Roy and Lall (2003)
	(Melanogrammus aeglefinus)			
	Japanese flounder	0.6–1.5 (total P)	SW	Choi et al. (2005); Wang et al. (2005); Uyan et al. (2007)
	(Paralichthys olivaceus)			
	Black sea bream	0.55 (available P)	SW	Shao et al. (2008)
	(Acanthopagrus schlegeli)			
	Japanese sea bass	0.86–0.90	SW	Zhang et al. (2006)
	(Lateolabrax japonicus)			
	Orange-spotted grouper	1.09	SW	Ye et al. (2006)
	(Epinephelus coioides)			
	Gilthead sea bream	0.75	SW	Pimentel-Rodrigues and Olivia-Teles (2001)
	(Sparus auratus)			
	European sea bass	0.65	SW	Olivia-Teles and Pimentel-Rodrigues (2004)
	(Dicentrarchus labrax)			

TABLE 8-3 Micromineral Requirements of Fish

Mineral	Species	Recommended Supplement (mg/kg diet)	Rearing Condition[a]	Reference
Copper	Channel catfish	1.5	FW	Murai et al. (1981)
	(Ictalurus punctatus)	5	FW	Gatlin and Wilson (1986a)
	Common carp	3	FW	Ogino and Yang (1980)
	(Cyprinus carpio)			
	Rainbow trout	3	FW	Ogino and Yang (1980)
	(Oncorhynchus mykiss)	3.5	FW	Julshamn et al. (1988)
	Atlantic salmon	5–10 (as supplement)	FW; 3.5 mg Cu/kg	Lorentzen et al. (1998)
	(Salmo salar)	≥ 500 toxic	FW (0.6 µg/L)	Berntssen et al. (1999)
	Hybrid tilapia	4	FW	Shiau and Ning (2003)
	(Oreochromis niloticus × Oreochromis aureas)			
	Malabar grouper	4–6	SW	Lin et al. (2008a)
	(Epinephelus malabaricus)			
Iron	*I. punctatus*	30	FW (0.43 mg Fe/L)	Gatlin and Wilson (1986b)
	Cyprinus carpio	199	FW (dietary levels 1 and 199 mg Fe/kg)	Sakamoto and Yone (1978b)
	Red sea bream	199	SW (dietary levels 1 and 199 mg Fe/kg)	Sakamoto and Yone (1976b)
	(Chrysophrys major)	150	SW	Sakamoto and Yone (1978c)
	Oreochromis niloticus × Oreochromis aureas	85	FW	Shiau and Su (2003)
Zinc	*I. punctatus*	20	FW (25 µg Zn/L)	Gatlin and Wilson (1983)
		150	FW with 1.1% phytate	Gatlin and Wilson (1984c)
	Cyprinus carpio	15–30	FW (10 µg Zn/L)	Ogino and Yang (1979)
	Oncorhynchus mykiss	15–30	FW (11 µg Zn/L)	Ogino and Yang (1978)
		20–40	FW	Satoh et al. (1987)
		40	4% Tricalcium phosphate	
		80	7% Tricalcium phosphate	
	Blue tilapia	20	FW (4 µg Zn/L)	McClain and Gatlin (1988)
	(Oreochromis aureus)			
	Red drum	20	BW (6‰)	Gatlin et al. (1991)
	(Sciaenops ocellatus)			
	Nile tilapia	30	FW	Eid and Ghonim (1994)
	(Oreochromis niloticus)			
	Oreochromis niloticus × Oreochromis aureas	26–29	FW	Lin et al. (2008c)
Manganese	*I. punctatus*	2.4	FW (2 µg Mn/L)	Gatlin and Wilson (1984a)
	Cyprinus carpio	12–13	FW	Ogino and Yang (1980)
	Oncorhynchus mykiss	12–13	FW	Ogino and Yang (1980)
	Mossambique tilapia	1.7	FW	Ishac and Dollar (1968)
	(Oreochromis mossambica)			
	Oreochromis niloticus × Oreochromis aureas	7	FW	Lin et al. (2008b)
Selenium	*I. punctatus*	0.25	FW; adequate vitamin E	Gatlin and Wilson (1984b)
	Oncorhynchus mykiss	0.15–0.38	FW (0.4 µg Se/L); adequate vitamin E	Hilton et al. (1980)
		0.07	Prevented overt deficiency	Poston et al. (1976)
		3	May produce toxicity	
		13	Toxic	
	Malabar grouper	0.7	SW	Lin and Shiau (2005)
	(Epinephelus malabaricus)			
Iodine	Chinook salmon	0.6–1.1	FW (0.2 µg I/L)	Woodall and LaRoche (1964)
	(Oncorhynchus tshawytscha)			

[a]Information about diet and/or water used in the experiment. FW = freshwater, BW = brackish water SW = seawater.

TABLE 8-4 Mineral Requirements of Crustaceans

Macromineral	Species	Dietary Protein	Requirement (g/100 g)	Reference
Calcium	Kuruma prawn (*Marsupenaeus japonicus*)	Casein—egg	Dispensable	Deshimaru and Yone (1978)
		Squid meal	1.2	Kitabayashi et al. (1971)
		Casein	1.0–2.0	Kanazawa et al. (1984)
	Pacific white shrimp (*Litopenaeus vannamei*)	Casein—gelatin	Dispensable	Davis et al. (1993a)
	Tiger shrimp (*Penaeus monodon*)	Casein—gelatin	Dispensable	Penaflorida (1999)
Phosphorus	*M. japonicus*	Casein—egg	2.0	Deshimaru and Yone (1978)
		Casein	1.0–2.0	Kanazawa et al. (1984)
		Squid meal	1.0	Kitabayashi et al. (1971)
	L. vannamei	Casein—gelatin	0.03% dietary Ca, ≤ 0.34% P	Davis et al. (1993a)
			1% Ca, 0.5–1.0% P	
			2% Ca, 1.0–2.0% P	
		Casein—gelatin	0.5% Ca, 0.93% total P (EAP[a] = 0.77% P)	Cheng et al. (2006)
			1.5% Ca, 2% total P (EAP = 1.22% P)	
		Fish meal, soybean meal	> 1.33%, practical diet	Pan et al. (2005)
	P. monodon	Casein—gelatin	0.5% (0.74 total) at low Ca	Penaflorida (1999)
Ca:P ratio	American lobster (*Homarus americanus*)	Casein, yeast	0.56:1.10	Gallagher et al. (1978)
	H. americanus (juvenile)	Casein—fish meal	1:1	Gallagher et al. (1982)
	H. americanus (adult)	Casein	1:1	Kanazawa et al. (1984)
	M. japonicus	Squid meal	1.24:1.04	Kitabayashi et al. (1971)
	Yellow leg shrimp (*Penaeus californiensis*)	Soybean meal, shrimp head meal, and fish meal	2.06:1, < 2.42:1	Huner and Colvin (1977)
Potassium	*L. vannamei*	Casein—gelatin	Unclear	Davis et al. (1993a)
	M. japonicus	Casein—egg	1	Deshimaru and Yone (1978)
		Casein	0.9	Kanazawa et al. (1984)
	P. monodon	Casein	1.20	Shiau and Hsieh (2001b)
		Casein	1.09	Zhu et al. (2006)
Magnesium	*M. japonicus*	Casein—egg	Dispensable	Deshimaru and Yone (1978)
		Casein	0.3	Kanazawa et al. (1984)
	L. vannamei	Casein—gelatin	0.26–0.35	Cheng et al. (2005)

Micromineral	Species	Dietary Protein	Requirement (mg/kg diet)	Reference
Copper	*M. japonicus*	Casein	Dispensable	Kanazawa et al. (1984)
	Fleshy prawn (*Penaeus orientalis*)	Fish meal, peanut meal	53	Liu et al. (1990)
	L. vannamei	Casein—gelatin	16–32	Davis et al. (1993b)
	P. monodon	Casein	10–30	Lee and Shiau (2002)
	Fleshy prawn (*Fenneropenaeus chinensis*)		25.3	Wang et al. (1997)
	M. japonicus	Casein—egg	Dispensable	Deshimaru and Yone (1978)
Iron	*M. japonicus*	Casein	Dispensable	Kanazawa et al. (1984)
	L. vannamei	Casein—gelatin	Dispensable	Davis et al. (1992b)
	M. japonicus	Casein	Dispensable	Kanazawa et al. (1984)
Manganese	*M. japonicus*	Casein	Dispensable	Kanazawa et al. (1984)
	L. vannamei	Casein—gelatin	Required	Davis et al. (1992a)
Selenium	*L. vannamei*	Casein—gelatin	0.2–0.4	Davis (1990)
Zinc	*P. monodon*	Casein	32–34 (growth)	Shiau and Jiang (2006)
			35–48 (immunity)	
	L. vannamei	Casein—gelatin	15 (32 total)	Davis et al. (1993c)
			200 (218 total) in the presence of phytate	

[a]Estimated available phosphorus.

usually found in anionic groupings: chloride (Cl^-), iodide (I^-), molybdate (MoO_4^{2-}), phosphate (PO_4^{3-}), and selenite (SeO_3^{2-}) (Scott et al., 1982). Other trace minerals such as aluminum, arsenic, cobalt, fluorine, molybdenum, nickel, silicon, tin, and vanadium are typically required in such small amounts that dietary supplementation is not required.

The remainder of this chapter will summarize pertinent information about mineral nutrition of fish and crustaceans, including general functions of the macro- and microminerals, dietary essentiality and/or quantitative requirements, bioavailability and dietary interactions, and general recommendations for dietary supplementation.

CALCIUM AND PHOSPHORUS

Calcium and phosphorus are two of the major constituents of the inorganic portion of diets. Quantitatively, calcium and phosphorus function primarily as structural components of hard tissues (e.g., bone, exoskeleton, scales, and teeth). In addition to its structural functions, calcium is essential for blood clotting (vertebrates), muscle function, proper nerve impulse transmission, osmoregulation, and as a cofactor for enzymatic processes (Lall, 2002). Phosphorus is a component of a variety of organic phosphates, such as nucleotides, phospholipids, coenzymes, deoxyribonucleic acid (DNA), and ribonucleic acid. Inorganic phosphates also serve as important buffers to maintain normal pH of intra- and extracellular fluids (Zubay, 1983).

Dietary deficiencies of most macrominerals such as calcium have been generally difficult to produce with fish species because of the presence of these ions in the water. However, supplementation of phosphorus in fish diets is usually most critical because its presence in the water and utilization by fish is limited. Dietary deficiency of phosphorus impairs intermediary metabolism, resulting in reduced growth and feed conversion. Various skeletal malformations associated with reduced mineralization of hard tissues also occur at suboptimal phosphorus intake (Sugiura et al., 2004).

The influence of excreted phosphorus on eutrophication of receiving waters has resulted in a considerable amount of research being focused on phosphorus nutrition in recent years with the aim of minimizing phosphorus excretion, especially for salmonid species cultured in flowing-water or net pen systems where effluents have direct effects on the surrounding waters. The experimental measurement of urinary phosphorus concentration has been a sensitive indicator of dietary phosphorus metabolism in fish (Sigiura et al., 2000). Various dietary manipulations, such as reducing total dietary phosphorus (e.g., Green et al., 2002a) or increasing the availability of phosphorus in the diet by adding the enzyme phytase (see Chapter 10; reviewed in Gatlin and Li, 2008) or other additives such as citric acid (Sugiura et al., 1998; also see Chapter 10), have been effective in reducing urinary and fecal phosphorus excretions. Such nutritional manipulations have been shown to reduce solid and dissolved phosphorus wastes produced by rainbow trout by more than 50% (e.g., Green et al., 2002a,b). Phase-feeding strategies also have been evaluated in conjunction with different dietary phosphorus levels in an effort to minimize dietary phosphorus inputs but maintain adequate production characteristics of the cultured organism (Lellis et al., 2004). The occurrence of spinal deformities in commercially produced Atlantic salmon, such as compression of the vertebral column, has been recognized for several years as a problem due to the reduction in processed fish quality (Sullivan et al., 2007a) and likely was caused by inadequate mineral nutrition. Extensive investigations have led to the conclusion that this condition does not have a genetic basis (Sullivan et al., 2007b), but is primarily caused by phosphorus deficiency in first-feeding fry (Sullivan et al., 2007c). In addition, elevated calcium and phosphorus in the diet of early seawater smolts decreased the incidence of vertebral deformities (Fjellhal et al., 2009). Another recent study on rainbow trout fry revealed that a phosphorus deficiency not only induced phosphorus depletion in whole-body tissues, but also lowered or delayed ossification of both the endochondrial and dermal skeleton (Fontagne et al., 2009). This study also reported that calcium deficiency delayed the ontogeny of skeletal development without affecting final bone mineralization, but did lead to modifications in the size and shape of vertebrae.

Models have been developed using multiple regression approaches to accurately predict the availability of phosphorus in diets formulated with a wide variety of feedstuffs and phosphorus supplements (Hua and Bureau, 2006). Factorial models also have been developed to predict phosphorus waste output in salmonid culture systems (Hua et al., 2008).

In contrast to calcium, concentrations of phosphorus in natural waters are generally very low (Boyd, 1981). Consequently, absorption of significant amounts of phosphorus from freshwater and saltwater is unlikely (Lall, 1991), making a dietary source of phosphorus potentially more critical for both fish and shrimp.

The dietary phosphorus requirements of fish species have been reported to range from 0.3 to 1.5% of diet (Lall, 2002). Some of the variability in these requirement values may be due to differences in phosphorus availability of diets used in quantifying requirements. Available phosphorus requirement values as low as 0.3 and 0.34% of diet have been reported for subadult (Eya and Lovell, 1997a) and fingerling (Wilson et al., 1982) channel catfish (*Ictalurus punctatus*). A higher value of 0.56% of diet was reported for rainbow trout (*Oncorhynchus mykiss*) (Rodehutscord et al., 1995). Values of 0.45% and 0.58% were reported for hybrid striped bass (*Morone chrysops* × *M. saxatilis*) (Brown et al., 1993) and striped bass juveniles (Dougall et al., 1996). The European whitefish (*Coregonus lavaretus*) was reported to have available phosphorus requirements of 0.62 to 0.65% of diet based on maximum growth and vertebral ash content, respectively (Vielma et al., 2002). The minimum available phosphorus requirement value for Japanese sea bass (*Lateolabrax*

japonicas) was reported to be 0.68% of diet based on weight gain; higher values of 0.86 and 0.90% were estimated based on whole-body and vertebrae phosphorus (Zhang et al., 2006). An available phosphorus requirement of 0.7% of diet for maximum growth and minimum phosphorus excretion was reported for juvenile common carp (*Cyprinus carpio*) (Kim et al., 1998) as well as for silver perch (*Bidyanus bidyanus*) (Yang et al., 2006). The same requirement level was estimated for the marine yellow croaker (*Pseudosciaena crocea*) based on weight gain with a high value of 0.9% based on phosphorus deposition in vertebrae or whole body (Ma et al., 2006). A similar requirement value of 0.72% of diet was determined for haddock (*Melanogrammus aeglefinus*) (Roy and Lall, 2003) while a value of 0.86% of diet was reported for red drum (*Sciaenops ocellatus*) (Davis and Robinson, 1987). The phosphorus requirement of black sea bream (*Acanthopagrus schlegelii*) was reported to be 0.55% of diet based on weight gain but 0.81, 0.87, and 0.88% of diet based on phosphorus deposition in whole fish, vertebrae, and scales, respectively (Shao et al., 2008). A much lower requirement of 0.44% of diet was estimated for yellowtail (*Seriola quinqueradiata*) based on nonfecal phosphorus excretion (Sarker et al., 2009).

The effects of dietary phosphorus on immunity and disease resistance of fish have been investigated to only a limited extent to date. Eya and Lovell (1998) reported that phosphorus deficiency in channel catfish reduced their antibody production and resistance to *Edwardsiella ictaluri* infection. The minimum phosphorus requirement for maximum protection from *E. ictaluri* was 0.4% of diet, which is similar to that required for maximum weight gain, while phosphorus at 0.5% of diet maximized antibody production (Eya and Lovell, 1998).

Phosphorus requirement estimates for crustacean species in early studies were much higher than the values previously reported for various fish species. For example, phosphorus requirements of 1% (Kitabayashi et al., 1971), 1 to 2% (Kanazawa et al., 1984), and 2% (Deshimaru and Yone, 1978) of the diet were recommended for kuruma prawn (*Marsupenaeus japonicas*). Pan et al. (2005) supplemented graded amounts of calcium phosphate monobasic to practical diets for *Litopenaeus vannamei* and found that total phosphorus at 1.33% was required for optimal weight gain and feed efficiency. However, Davis et al. (1993a) and Cheng et al. (2006) demonstrated that the dietary phosphorus requirement of *L. vannamei* was dependent upon the calcium content of the diet. In the absence of calcium supplementation, Davis et al. (1993a) observed the basal diet (0.03% Ca, 0.34% P) contained adequate phosphorus for normal growth. Cheng et al. (2006) reported that *L. vannamei* weight gain was optimal with available phosphorus at 0.77% of diet in the absence of supplemental calcium, but an increase to 1.22% available phosphorus was needed with 1% supplemental calcium. Similarly, Penaflorida (1999) reported that based on weight gain, *Penaeus monodon* required 0.5% supplemental phosphorus (0.74% total phosphorus) when the diet was not supplemented with calcium, and higher phosphorus supplementation was required with calcium supplementation. Ambasankar and Ali (2002) fed semipurified diets containing graded levels of phosphorus with 1.25% calcium to *Penaeus indicus* and reported that weight gain and body composition was optimal for shrimp fed the diet containing 1% total phosphorus, and shrimp maintained their body phosphorus to calcium ratio at 1:2 regardless of dietary phosphorus level.

Gallagher et al. (1978) examined the effects of varying dietary calcium-to-phosphorus (Ca:P) ratio on juvenile lobsters (*Homarus americanus*). Based on growth and histological evaluation of the endocuticle, a Ca:P of 0.51 (0.56:1.10) was found to be best for lobster juveniles, with Ca:P of 1.55 and greater resulting in abnormalities of the endocuticle. In *Marsupenaeus japonicus*, a dietary Ca:P of 1:1 was recommended (Kitabayashi et al., 1971; Kanazawa et al., 1984). Davis et al. (1993a) reported that supplementation of calcium to the basal diet appeared to inhibit phosphorus bioavailability, and the Ca:P did not totally explain the inhibitory effects of calcium. Based on these studies, dietary calcium may affect phosphorus availability such that calcium levels in excess of 2.5% should be avoided. Although there does not appear to be a fixed Ca:P ratio that will produce optimal results, it appears that a ratio < 2:1 (Ca:P) provides good results in commercial formulations for shrimp as well as for fish such as Atlantic salmon (Vielma and Lall, 1998) and grouper (*Epinephelus coioides*) (Ye et al., 2006).

In contrast to phosphorus, calcium deficiency has been difficult to establish in fish species such as common carp (Ogino and Takeda, 1976) and channel catfish (Andrews et al., 1973; Lovell, 1978) cultured in freshwater with normal calcium hardness because of their ability to absorb waterborne calcium via the gills; however, overt deficiency signs such as reduced weight gain, feed efficiency, and bone mineralization were produced in low-calcium water (Robinson et al., 1984, 1986, 1987). Uptake of waterborne calcium occurs principally from the gills, although other tissues such as fins and oral epithelium also have been associated with this function. Adequate calcium hardness is an important characteristic of freshwater for fish culture, and calcium ions are abundant in seawater; therefore, metabolic requirements for calcium are commonly met by uptake from the aquatic medium. Studies conducted in low-calcium freshwater have established dietary calcium requirements of channel catfish and blue tilapia (*Oreochromis aureus*) of 0.45 and 0.7%, respectively (Robinson et al., 1986, 1987). Shiau and Tseng (2007) reported a dietary calcium requirement ranging from 0.35 to 0.43% for hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) reared in water with 27–33 mg Ca/L based on weight gain, bone calcium, and scale calcium. Calcium concentrations as low as 0.34% of diet were reported as required for common carp and Japanese eel (*Anguilla japonicas*) (Ogino and Takeda, 1976). Atlantic salmon (*Salmo salar*) in seawater utilized waterborne calcium such that dietary

supplementation was unnecessary (Lall and Bishop, 1977). In contrast, red sea bream (*Chrysophrys major*) were unable to obtain enough calcium from seawater and required calcium at 0.34% of diet (Sakamoto and Yone, 1973, 1976a). More recently, a series of experiments was conducted on different marine species by Hossain and Furuichi (1998, 1999, 2000) in which a dietary supply of calcium from calcium lactate was needed to support normal growth of tiger puffer (*Takifugu rubripes*) and scorpion fish (*Sebastiscus marmoratus*), but not required for black sea bream (*A. schlegelii*).

Calcium requirements of various crustacean species have been reported (Kitabayashi et al., 1971; Gallagher et al., 1978; Kanazawa et al., 1984); however, *L. vannamei* raised in seawater did not require supplemental calcium (Davis et al., 1993a). Even in low-salinity (2‰ [parts per thousand]) water, a dietary calcium requirement of *L. vannamei* was not apparent, but the amount of calcium in the diet affected the dietary phosphorus requirement (Cheng et al., 2006) as previously observed with *P. monodon* (Penaflorida, 1999). Although the diet plays an important role, it appears that crustaceans also can absorb some minerals from the water via drinking and by direct absorption via the gills, exoskeleton, or both (Deshimaru et al., 1978; NRC, 1993).

Many factors influence the absorption, distribution, and excretion of calcium and phosphorus. Dietary calcium is primarily absorbed from the intestine by active transport. In vertebrates, the vitamin 1,25-dihydroxycholecalciferol functions in the maintenance of serum calcium and phosphorus levels by altering the rate of intestinal absorption (via Ca^{2+}-binding protein), renal resorption, and bone mobilization (Zubay, 1983). There is some evidence that vitamin D and its metabolites may affect calcium homeostasis in teleosts. However, O'Connell and Gatlin (1994) observed that dietary supplementation of cholecalciferol (vitamin D_3) was not required for blue tilapia to utilize dietary calcium for growth and tissue mineralization in low-calcium water. In rainbow trout the major regulator of phosphorus metabolism was dietary phosphorus as compared to vitamin D_3 and its metabolites or the calcium phosphate cotransporter (Coloso et al., 2003). The role of vitamin D_3 in intestinal calcium uptake of fish, therefore, is not well established as it is in terrestrial vertebrates. The control of extracellular calcium concentrations in marine invertebrates appears to be rather limited (Cameron, 1990).

MAGNESIUM

In vertebrates, approximately 60% of total body magnesium is located in bone, of which about one-third is combined with phosphate and the remainder is adsorbed loosely on the surface of the mineral structure (Pike and Brown, 1975). In soft tissues, magnesium occurs both intra- and extracellularly. Magnesium is essential for maintenance of intra- and extracellular homeostasis in fish (Moyle and Cech, 2000) and crustaceans (Mantel and Farmer, 1983). In addition, magnesium is essential for cellular respiration and phosphate transfer reactions involving adenosine tri-, di-, and monophosphate. It is an activator for all thiamine pyrophosphate reactions and is involved in the metabolism of fats, carbohydrates, and proteins.

Dietary magnesium deficiencies have been documented for a variety of freshwater fish and include poor growth, anorexia, lethargy, muscle flaccidity, convulsions, vertebral curvature, high mortality, and depressed magnesium levels in the whole-body, blood serum, and bone (Lall, 2002). Fish in freshwater, which contains 1 to 3 mg Mg/L, have been shown to require 0.025 to 0.07% magnesium in the diet (Lall, 2002). Rainbow trout were able to uptake waterborne magnesium (1.3 mg Mg/L) to meet a portion of their metabolic requirement (Shearer, 1989). Seawater typically contains high levels of magnesium (1,350 mg/L), and magnesium is excreted by marine crustaceans and fish, resulting in blood levels lower than that of the external medium. Thus, marine species may not require a dietary source of magnesium (Dall and Moriarty, 1983). Atlantic salmon reared in brackish water (54 mg Mg/L) needed at least 0.01% magnesium in the diet to maintain normal magnesium concentrations in whole-body and serum as well as for proper bone mineralization (El-Mowafi and Maage, 1998). In contrast, red sea bream reared in seawater showed no signs of deficiency when fed diets containing as little as 0.012% magnesium (Sakamoto and Yone, 1979a).

The effects of dietary magnesium on immune responses of fish have received limited consideration to date. Atlantic salmon vaccinated against *Vibrio anguillarum* and fed graded levels of magnesium had similar antibody titers, lysozyme, and serum complement hemolytic activity regardless of dietary magnesium level (El-Mowafi et al., 1997). However, both lysozyme and serum hemolytic activity were elevated in vaccinated fish compared with unvaccinated fish.

In crustaceans, early research with *M. japonicus* indicated that supplementation of 0.3% magnesium did not improve the nutritive value of a semipurified diet (Deshimaru and Yone, 1978). Kanazawa et al. (1984) reevaluated the magnesium requirement of *M. japonicus* and reported that dietary supplementation of 0.1 to 0.5% magnesium improved growth responses. However, in this series of studies, weight gain was very low (< 100%) and a dietary essentiality was not established. Davis et al. (1992a) reported a depression of hepatopancreas magnesium levels in *L. vannamei* in response to the deletion of magnesium from a semipurified diet; however, weight gain and magnesium levels of the carapace were unaffected. Based on the high levels of magnesium in seawater and the magnesium requirements of freshwater fish, a dietary magnesium requirement for marine species would not be expected. More recently, the magnesium requirement of *L. vannamei* cultured in low-salinity (2‰) water was estimated to be 0.26 to 0.35% of diet based on weight gain (Cheng et al., 2005). Another study in artificial low-salinity (4‰) water did not observe an improve-

ment in growth of *L. vannamei* when magnesium chloride was supplemented at 150 and 300 mg/kg (Roy et al., 2007).

In terms of practical diet formulations, most feed ingredients, especially those of plant origin, are high in magnesium, and supplementation of magnesium to practical diets is generally not necessary. However, because of the low bioavailability of magnesium from white fish meals (Watanabe et al., 1988), some diets containing this ingredient may require magnesium supplementation.

SODIUM, POTASSIUM, AND CHLORIDE

Sodium, potassium, and chloride are recognized as being essential for a number of physiological processes including acid:base balance and osmoregulation (Lall, 2002). Dietary deficiencies of sodium and chloride have been difficult to demonstrate in fish (NRC, 1993). These minerals are typically abundant in water and feedstuffs, and thus metabolic deficiencies have rarely been observed.

The supplementation of high levels (4.5 to 11.6% of the diet) of sodium chloride (NaCl) to the diet has been reported to inhibit feed efficiency of rainbow trout raised in freshwater, presumably due to nutrient dilution (Salman and Eddy, 1988). Additionally, there were no positive or negative effects of sodium chloride supplementation on channel catfish raised in freshwater (Murray and Andrews, 1979) or on Atlantic salmon cultured in freshwater or seawater (Shaw et al., 1975). However, hybrid tilapia reared in freshwater required approximately 0.15% Na in purified diets for optimal weight gain, gill Na^+-K^+ ATPase activity, and whole-body sodium retention, whereas no dietary requirement was apparent in seawater (Shiau and Lu, 2004).

Research with the euryhaline red drum demonstrated that at low salinities, the supplementation of sodium chloride at 2 to 10% of diet resulted in increased growth (Gatlin et al., 1992). Similar improvements in growth and feed efficiency were reported for European sea bass (*Dicentrarchus labrax*) (Eroldogan et al., 2005) and Asian sea bass (*Lates calcarifer*) (Harpaz et al., 2005) in freshwater with the addition of sodium chloride to the diet at 3 and 4%, respectively. One possible explanation for this positive response is an increase in amino acid absorption. Boge et al. (1983) demonstrated that, in addition to being energized by a Na^+-gradient, amino acid transport by brush-border membrane vesicles prepared from enterocytes of the European sea bass is dependent upon the presence of Cl^- ions. The addition of dietary sodium chloride at low salinities may increase the absorption of amino acids and/or satisfy other metabolic requirements, thus resulting in a physiological advantage for some species. Harpaz et al. (2005) reported that addition of dietary salt in freshwater increased the activity of several brush-border enzymes including alkaline phosphatase, lactase, and leucine amino peptidase, with the effect most pronounced in the pyloric caeca.

The supplementation of sodium chloride to practical diet formulations at 7 to 10% also has been found to reduce osmoregulatory stress and increase survival of fish being transferred from freshwater to saltwater (Zaugg et al., 1983; Al-Amoudi, 1987; Duston, 1993). This was presumably through the stimulation of osmoregulatory function and gill sodium and potassium ATPase activity (Al-Amoudi, 1987).

Due to the recent interest in culturing shrimp such as *L. vannamei* in low-salinity ground waters, the effects of dietary supplementation of sodium chloride and other minerals have been evaluated with this species in natural and artificial low-salinity water. Sodium chloride at 1 and 2% of diet did not improve weight gain of *L. vannamei* in artificial water of 4‰ salinity (Roy et al., 2007).

Dietary potassium requirements have been identified for channel catfish (Wilson and El Naggar, 1992) and Chinook salmon (*O. tshawytscha*) in freshwater (Shearer, 1988), but not for red sea bream in seawater (Sakamoto and Yone, 1978b), possibly indicating that marine fish can obtain adequate levels of potassium from the water. Channel catfish and Chinook salmon both absorbed waterborne potassium but could not meet their metabolic requirements without a dietary supply. Dietary potassium requirement estimates for channel catfish and Chinook salmon were 0.26 and 0.8%, respectively. Hybrid tilapia reared in freshwater required potassium at 0.2 to 0.3% of diet for optimal weight gain, gill Na^+-K^+ ATPase activity, and whole-body potassium retention (Shiau and Hsieh, 2001a). More recently, the Asian sea bass or barramundi was reported to require more dietary potassium at hypoosmotic salinity (5‰) compared to iso- or hyper-osmotic environments (Partridge and Lymbery, 2008). One study with African catfish (*Clarius gariepinus*) indicated that a dietary sodium to potassium ratio of 1.5:2.5 produced the best growth and nutrient utilization, while excess dietary potassium in relation to sodium reduced growth.

Potassium nutrition of crustaceans also has been studied to an appreciable extent with marine shrimp. Kanazawa et al. (1984) reported that diets containing 0.9% potassium improved growth of *M. japonicus* as compared to diets containing 1.8% potassium. Deshimaru and Yone (1978) recommended dietary supplementation of 1% potassium based on the comparative growth of shrimp fed a diet without magnesium and potassium supplementation; however, potential interactions between potassium and magnesium were not evaluated. Davis et al. (1992a) reported that the individual deletion of potassium from a semipurified diet did not result in a significant depression in tissue potassium or growth of *L. vannamei*; however, tissue levels of magnesium were affected, indicating a potential interaction. In another study, *P. monodon* reared in water of 19 to 21‰ salinity (360 mg K/L) showed a minimum potassium requirement of 1.2% of diet based on weight gain and protein efficiency ratio (Shiau and Hsieh, 2001b). *L. vannamei* responses to graded levels of dietary potassium in conjunction with different concentrations of potassium in seawater at 30‰ were monitored in a recent study (Zhu, 2006). Dietary potassium had limited

influences on shrimp responses compared to potassium levels in the water. No interaction between dietary and waterborne potassium was observed, possibly due to limited assimilation of dietary potassium at 30‰ salinity. As mentioned earlier, culture of marine shrimp in low-salinity environments has increased in recent years and prompted more research investigating dietary and environmental mineral requirements of these organisms. Dietary supplementation of chelated potassium at 1% improved weight gain of *L. vannamei* in low-salinity (4‰) water in at least three experiments, but addition of potassium to the water to correct imbalances with sodium and chloride appeared to be more effective in improving growth and survival (Roy et al., 2007; Saoud et al., 2007). Thus, with the exception of culturing marine shrimp in low-salinity water, most freshwater and all seawater probably contains sufficient amounts of sodium, potassium, and chloride ions to satisfy the physiological needs of various cultured species (Lovell, 1989). Those ions are also found in substantial amounts in most feedstuffs, making the necessity of dietary supplementation unlikely. However, commercial diets may be supplemented with sodium chloride at 1 to 2% because of its relatively low cost.

CHROMIUM

Chromium, which is found predominantly in trivalent form, has been recognized since the late 1950s as an essential nutrient for humans and animals. One of its major metabolic roles is in association with the glucose tolerance factor, an organometallic molecule that potentiates the action of insulin in carbohydrate metabolism (NRC, 1997). In addition, chromium has been shown to be integral in activating various enzymes, influencing lipid metabolism, as well as maintaining the stability of proteins and nucleic acids (NRC, 1997).

To date, research on the influences of dietary chromium on fish has been rather limited with even less effort directed toward crustaceans. An early study with rainbow trout in which chromium chloride was supplemented to chemically defined diets at 0, 1, 3, or 6 mg/kg did not produce differences in weight gain or changes in tissue chromium distribution (Tacon and Beveridge, 1982). In another study with rainbow trout, supplementation of a practical diet with chelated chromium at 0.5 mg/kg increased blood glucose clearance but did not affect weight gain or protein and energy retention (Bureau et al., 1995). Gatta et al. (2001a) fed semipurifed diets supplemented with up to 4.1 mg Cr/kg from chromium yeast to rainbow trout and reported increased serum lysozyme as well as increased phagocytosis and respiratory burst of head-kidney macrophages after 6 weeks. However, organic chromium supplementation did not affect growth performance, carcass and fillet composition, or hepatic xenobiotic-metabolizing enzymes of gilthead sea bream (*Sparus aurata*) (Gatta et al., 2001b).

Several other studies with hybrid tilapia (*O. niloticus* × *O. aureas*) have shown that supplementation of chromium, especially in diets containing glucose compared to corn starch, caused significant increases in weight gain, energy deposition, and liver glycogen, as well as altered postprandial plasma glucose concentrations (Shiau and Chen, 1993; Shiau and Lin, 1993; Shiau and Liang, 1995). Only one subsequent study reported no significant effect of chromium picolinate supplementation at 2 mg/kg on growth or carbohydrate utilization of hybrid tilapia (Pan et al., 2003). In the other studies, chromium supplementation was effective at doses as low as 2 mg/kg from chromium chloride (Shiau and Lin, 1993) and as high as 0.5% of chromium oxide (Shiau and Liang, 1995), with chromium oxide being most efficacious relative to chromium chloride and sodium dichromate (Shiau and Chen, 1993). A minimum dietary chromic oxide level of 204 mg/kg was estimated based on maximum weight gain of hybrid tilapia fed diets containing glucose (Shiau and Shy, 1998). In contrast to the studies with hybrid tilapia, chromic oxide supplementation of diets containing glucose did not alter weight gain, feed efficiency, protein efficiency, or glucose utilization of channel catfish (Ng and Wilson, 1997). In addition, the dietary chromium did not influence whole-body chromium concentrations, indicating the dietary chromium from chromic oxide was not retained by the channel catfish. Hertz et al. (1989) reported improved glucose utilization of common carp fed practical diets supplemented with chromium. Increased intestinal absorption of glucose also was observed in snakehead (*Channa punctatus*) exposed to 1 mmol/L chromium (Sastry and Sunita, 1982). Specific mechanisms by which chromium influences carbohydrate utilization of fish have not been elucidated.

COPPER

Copper functions in hematopoiesis and in numerous copper-dependent enzymes (O'Dell, 1976) including lysyl oxidase, cytochrome *C* oxidase (CCO), ferroxidase, tyrosinase, and superoxide dismutase (SOD). Lysyl oxidase functions in the formation of crosslinks during the synthesis of collagen and elastin. The failure of collagen maturation (crosslinking) in the organic matrix of bone accounts for increased fragility of bones and the associated abnormalities of copper deficiencies (O'Dell, 1976). Failure of collagen and elastin crosslinking and an undefined muscular defect result in enlargement of the heart and cardiac failure in copper-deficient animals.

Copper also is involved in the absorption and metabolism of iron and functions in the formation of hemoglobin in vertebrates. Crustaceans utilize hemocyanin as the oxygen-carrying pigment. This copper-containing pigment has an analogous role to hemoglobin in red-blooded animals (Lovell, 1989). Depledge (1989) estimated that, on a fresh-weight basis, 40% of the whole-body copper load in shrimp is found in hemocyanin. This suggests a considerable increase in the physiological demand for copper by crustaceans above that required by vertebrates.

In addition to the physiological functions of copper, high levels of dietary copper (≥ 500 mg/kg) may be toxic (Julshamn et al., 1988; Berntssen et al., 1999), as is excessive environmental copper (Bryan, 1976). Exposure to waterborne copper does not increase tissue copper accumulation as much as feeding elevated dietary copper (Clearwater et al., 2002; Bielmyer et al., 2005; Hoyle et al., 2007). Copper contamination in the marine environment is generally due to an increase in anthropogenic input and occurs primarily in coastal and estuarine areas, where copper concentrations (up to 0.6 mg Cu/L) far exceed the background copper level in seawater (0.5 µg Cu/L) (Bjerregaard and Vislie, 1986). Due to toxic effects of dissolved copper and other heavy metals, shrimp hatcheries routinely use water that has been treated with ethylenediaminetetraacetic acid to chelate free copper (Lawrence et al., 1981). Although a great deal of research has been conducted on the toxicity of dissolved copper, the dietary essentiality of this nutrient for marine species has received limited attention.

Dietary deficiencies of copper have been documented for several freshwater fish (Ogino and Yang, 1980; Murai et al., 1981; Gatlin and Wilson, 1986a; Julshamn et al., 1988), but fewer marine species (Lin et al., 2008a). Dietary requirements for copper have been established to range from 1.5 to 5 mg Cu/kg diet in species such as common carp, rainbow trout (Ogino and Yang, 1980), channel catfish (Gatlin and Wilson, 1986a), and hybrid tilapia (Shiau and Ning, 2003). Similarly, the copper requirement of grouper was determined to be 4–6 mg Cu/kg diet (Lin et al., 2008a). In addition to growth and feed efficiency, copper-dependent enzymes such as ceruloplasmin, copper- and zinc-dependent SOD, and CCO have been shown to be excellent indicators of copper nutriture (Gatlin and Wilson, 1986a).

Kanazawa et al. (1984) found that the dual deletion of iron and copper had no significant effect on growth and survival of *M. japonicus*. However, in this series of experiments, percentage weight gain was low (40%) and survival was poor (57%); hence, the nutritional stress or the quality of the diet may not have been adequate to induce a dietary deficiency. Davis et al. (1993b) demonstrated a dietary copper deficiency in *L. vannamei* fed semipurified diets containing < 34 mg Cu/kg diet. Deficiency signs included poor growth; reduced copper levels in the carapace, hepatopancreas, and hemolymph; and enlargement of the heart. This response is similar to that of W. Wang et al. (1997), who reported a dietary copper requirement of 25.3 mg Cu/kg diet for *Fennerpenaeus chinensis* based on growth and tissue mineralization. Liu et al. (1990) reported a dietary copper requirement of 53 mg Cu/kg diet for *P. orientalis* based on growth, survival, CCO activity, and tissue mineralization. The dietary copper requirement of *P. monodon* was quantified at 15–21 mg Cu/kg based on weight gain, feed efficiency, and whole-body copper retention (Lee and Shiau, 2002). These results indicate that shrimp cannot meet their physiological needs for copper from seawater and that a dietary source is required for maximum growth and tissue mineralization. It also appears that invertebrate species utilizing copper as a component of their respiratory pigment have an increased copper requirement over vertebrates utilizing iron-based respiratory pigments.

An interaction between dietary copper and zinc has been observed in some terrestrial animals whereby elevated levels of one reduced the bioavailability of the other (NRC, 2005); however, such interactions have not been well established in fish (Knox et al., 1982, 1984; Gatlin et al., 1989). An interaction between copper and selenium was reported in Atlantic salmon in which liver selenium was inversely related to dietary copper (Lorentzen et al., 1998). In addition, elevated dietary copper (20 mg Cu/kg) resulted in increased oxidative stress in grouper, which was relieved by increased dietary selenium (Lin and Shiau, 2007).

IODINE

Iodine is an essential element for a variety of animals. Nearly every cell in the body contains iodine; however, in vertebrates the thyroid gland is the main location of iodine reserves. Thyroid hormones, which contain iodine, are known to have roles in thermoregulation, intermediary metabolism, reproduction, growth and development, hematopoiesis and circulation, as well as neuromuscular functioning (NRC, 2005). Thyroid hyperplasia (goiter) is the most overt sign of iodine deficiency in vertebrates and has been occasionally observed in fish, primarily when goitrogenic compounds such as glucosinolates are present in the diet (Lall, 2002).

One of the first mineral studies with fish evaluated the Chinook salmon's requirement for iodine (Woodall and LaRoche, 1964). Although weight gain of fish was not affected by feeding graded levels of sodium iodide for 24 weeks and an additional 9 months, requirement values of 0.6 mg I/kg diet for fingerlings and 1.1 mg I/kg diet for advanced parr were estimated based on maximal thyroid iodine storage. Levels of 1 to 5 mg I/kg diet have been estimated as adequate for most species (Lall, 2002). Iodine supplementation of a commercial diet was reported to enhance weight gain and reduce stress responses of steelhead trout (Gensic et al., 2004). Another study indicated 4.5 mg I/kg diet protected Atlantic salmon from bacterial kidney disease (Lall, 2002). Iodine supplementation of moist pellets up to 80 mg I/kg increased fillet iodine without impacting health, growth, or plasma thyroid hormone status (Julshamn et al., 2006). The physiological essentiality of iodine has not been evaluated in shrimp.

IRON

Iron is a trace element that is essential for the production and normal functioning of hemoglobin, myoglobin, cytochromes, and many other enzyme systems. In vertebrates, the principal role of iron is as a component of hemoglobin. Red

blood cells are regenerated periodically, and most of the iron is recycled. That which is not recycled is excreted via the bile into the intestine. Like other elements of low solubility, such as zinc and copper, iron is absorbed and transported in the body in a protein-bound form (Lovell, 1989). In vertebrates, mucosal transferrin binds Fe^{2+} in the intestinal lumen and transports it across the mucosal brush border. Within the cell, Fe^{3+} is bound to apoferritin forming ferritin. The amount of apoferritin in the mucosa is regulated by physiological needs for iron. The iron-bound transferrin is then transported in the blood, where the iron is again released at target sites (liver and hematopoietic tissue). Iron and other minerals of low solubility are not easily excreted; thus, mineral excesses are deposited in cells of the digestive system, which are sloughed into the digestive tract for elimination.

In crustaceans, the hepatopancreas has been found to be the organ richest in iron. Storage cells containing iron have been reported in crayfish, *Procambarus clarkii* (Ogura 1959; Miyawaki et al., 1961), and the crab *Cancer irroratus* (Martin, 1973). Iron-transporting proteins have been found in the hemolymph of two species of crabs (Ghidalia et al., 1972; Depledge et al., 1986). These observations indicate the presence of a regulatory mechanism similar to that of vertebrates. In addition to the digestive system, gills appear to play an active role in iron metabolism. In *C. irroratus* iron accumulates by forming a coating around the branchial lamellae during the intermolt cycle, which is then rejected at ecdysis along with the integument (Martin, 1973). Absorption from the water through the gills could provide an additional source of iron.

Iron deficiencies have been documented for several species of fish; however, dietary deficiencies for shrimp have not been observed (Deshimaru and Yone, 1978; Kanazawa et al., 1984; Davis et al., 1992b). Iron deficiency causing hypochromic microcytic anemia has been reported for freshwater fish such as the brook trout (*Salvelinus fontinalis*) (Kawatsu, 1972) and common carp (Sakamoto and Yone, 1978c) and in marine fish such as red sea bream (Sakamoto and Yone, 1976b) and yellowtail (Ikeda et al., 1973). However, growth depression was not observed in these iron-deficient fish. Gatlin and Wilson (1986b) characterized iron deficiency signs of channel catfish and found that fish fed the basal diet (9.6 mg Fe/kg) exhibited suppressed growth and feed efficiency, as well as reduced hemoglobin, hematocrit, plasma iron, transferrin saturation, and erythrocyte-count values. Similar deficiency signs were reported in channel catfish by Lim and Klesius (1997). Additionally, catfish fed an iron-deficient diet experienced earlier mortality when exposed to the bacterial pathogen *Edwardsiella ictaluri* (Lim et al., 2000).

Gatlin and Wilson (1986b) concluded that a minimum of 20 mg supplemental Fe/kg diet (30 mg total Fe/kg) was required by channel catfish for best growth and hematological values. A much higher requirement value of 150 mg Fe/kg from iron chloride was recommended for red sea bream to maintain maximum hematological values (Sakamoto and Yone, 1978d), while 100 mg Fe/kg was reported to maximize weight gain, feed efficiency, and hepatic iron storage in grouper (Ye et al., 2007). Additionally, the dietary iron requirement of hybrid tilapia was estimated to be 85 mg Fe/kg from ferrous sulfate based on weight gain, hepatic iron, and hemoglobin concentrations, whereas approximately twice as much iron from ferric citrate was required due to reduced bioavailability (Shiau and Su, 2003).

Iron has received particular attention with regard to disease resistance because its availability to microorganisms affects their ability to cause infection. A study by Ravndal et al. (1994) indicated that Atlantic salmon families with high levels of serum iron were more susceptible to *Vibrio* infection; however, no relationship was apparent with regard to furunculosis or bacterial kidney disease. Another study with Atlantic salmon reported that supplementation of 400 mg Fe/kg diet to a basal diet containing 160 mg Fe/kg did not alter serum total protein, serum total antibody, hemolytic complement activity, or lysozyme activity in serum, head kidney, or spleen but did increase catalase activity in the head kidney (Andersen et al., 1998). Iron deficiency in channel catfish resulted in increased mortality due to *E. ictaluri* and reduced chemotactic migration of peritoneal macrophages (Sealey et al., 1997; Lim and Klesius, 1997) but antibody production was not affected by dietary iron level (Sealey et al., 1997). Diets containing 60 mg Fe/kg from either iron methionine or iron sulfate provided the highest chemotactic index (Sealey et al., 1997).

In addition to physiological problems caused by iron deficiency, excessive levels of iron may be toxic as well. Excessive iron supplementation appears to have potentially adverse effects on growth of *M. japonicus* (Deshimaru and Yone, 1978; Kanazawa et al., 1984). Additionally, iron-catalyzed lipid oxidation increases with iron supplementation, which in turn may adversely affect feed stability (Desjardins et al., 1987; Sutton et al., 2006). However, Andersen et al. (1998) supplemented extruded fish meal-based diets with 400 mg Fe/kg from iron sulfate and observed no effects on growth, hematology, antioxidant status, or health of Atlantic salmon smolts.

Iron is one of the primary metals involved in lipid oxidation, and ferrous iron is a more potent catalyst of lipid peroxidation than ferric iron (Chvapil et al., 1974; Lee et al., 1981). Ferrous iron catalyzes the formation of hydroperoxides and free radical peroxides by providing a free radical initiator in the presence of unsaturated fatty acids and oxygen. Thus, excessive iron supplementation of marine fish and crustacean diets should be avoided due to the presence of polyunsaturated fats that make these diets particularly susceptible to oxidation. Such oxidation also may reduce the stability of ascorbic acid (Hilton, 1989). Although many practical diets may contain considerable levels of endogenous iron, little is known about its form and availability (Lall, 1989). Hence, a low level of supplementation (≅ 10% of dietary require-

ment) of an available source is often recommended to ensure adequacy of the diet.

MANGANESE

Manganese functions as a cofactor in several enzyme systems, including those involved in urea synthesis from ammonia, amino acid metabolism, fatty acid metabolism, and glucose oxidation (Lall, 2002). Principal signs of manganese deficiency in terrestrial species include reduced growth rate, skeletal abnormalities, convulsions, reduced righting ability, abnormal reproductive function in males and females, and ataxia in the newborn (Lall, 1991).

Dietary deficiencies in fish have resulted in poor growth, skeletal abnormalities, high embryo mortalities, and poor hatch rates (Lall, 2002). A total dietary manganese content of 12 to 13 mg/kg has been recommended for the common carp and rainbow trout (Ogino and Yang, 1980); however, Gatlin and Wilson (1984a) found that 2.4 mg Mn/kg diet was sufficient for normal growth and health of channel catfish. A requirement of 7 mg Mn/kg was estimated for hybrid tilapia based on hepatic Mn superoxide dismutase and whole-body manganese retention (Lin et al., 2008b). Pan et al. (2008) estimated a higher requirement of approximately 14 mg Mn/kg for gibel carp (*Carassius auratus*).

In terms of immunity and disease resistance, deficiencies of manganese along with zinc were reported to decrease leukocyte natural killer cell activity of rainbow trout; however, that activity was restored with supplementation of those minerals (Inoue et al., 1998). However, supplementation of both manganese and zinc at more than 100 mg/kg diet did not enhance resistance of sockeye salmon to bacterial kidney disease nor did it have a significant effect on their production of serum antibodies (Bell et al., 1984).

Even less information on manganese requirements of crustaceans is currently available. Kanazawa et al. (1984) found that supplementation of 10 and 100 mg Mn/kg diet did not improve the growth of *M. japonicus*. However, it should be noted that percentage weight gain during the study was < 70% and the nutritional stress placed on the shrimp would not be considered adequate to reduce body stores enough to induce a deficiency. Because the manganese content of seawater is very low (0.01 mg/L), significant absorption from the water is unlikely. Thus, a dietary source of manganese could be necessary for marine shrimp and fish.

SELENIUM

Selenium is a trace element that functions as a component of the enzyme family called glutathione peroxidase, which converts hydrogen peroxide and lipid hydroperoxides into water and lipid alcohols, respectively. Thus, this enzyme group functions in protecting the cell from deleterious effects of peroxides (Little et al., 1970). Glutathione peroxidase acts along with vitamin E to function as a biological antioxidant to protect polyunsaturated phospholipids in cellular and subcellular membranes from peroxidative damage (Lovell, 1989). The function of this enzyme is complementary to that of vitamin E, which is a lipid-soluble antioxidant. A dietary deficiency of selenium has been generally reported to result in reduced activity of glutathione peroxidase as well as growth reduction. However, a combined deficiency of selenium and the fat-soluble antioxidant vitamin E was required to produce more overt deficiency signs such as nutritional muscular dystrophy and exudative diathesis in channel catfish (Gatlin and Wilson, 1984b; Lall, 2002).

The toxicity of selenium to various animals was established well before its dietary essentiality. Toxicity of dietary selenium has been shown to vary with several factors, including source and duration of exposure. Chronic selenium toxicity has been demonstrated in several fish species at dietary levels of 13 to 15 mg Se/kg from sodium selenite resulting in reduced growth and elevated mortality (Hilton et al., 1980; Gatlin and Wilson, 1984b; NRC, 2005) as well as renal calcinosis (Hilton and Hodson, 1983). More recently, the threshold of selenium toxicity for white sturgeon (*Acipenser transmontanus*) fed graded levels of selenomethionine was estimated to be 10 to 20 mg Se/kg (Tashjian et al., 2006). Cutthroat trout (*Oncorhynchus clarki bouvieri*) were fed diets containing up to 10 mg Se/kg from selenomethionine without showing signs of toxicity (Hardy et al., 2010). A dietary selenium level of 8 mg Se/kg was required for hatchery-reared coho salmon (*Oncorhynchus kisutch*) in freshwater to have eviscerated body selenium concentrations similar to that of their wild counterparts, and this dietary level provided similar seawater survival of the cultured fish as observed in the wild fish (Felton et al., 1996). A subsequent investigation (Halver et al., 2004) reported that the stress of confinement for 30 h during barge transport reduced carcass selenium of Chinook salmon by 20% while liver glutathione peroxidase activity was increased. These studies indicated elevated dietary selenium beneficially affected salmon against the stressful conditions associated with confinement and saltwater transfer by increasing glutathione peroxidase activity.

Fish mortality in natural settings related to waterborne selenium toxicity also has been studied extensively over the past two decades (Hamilton, 2004). Based on these laboratory and field studies, threshold levels for adverse effects of selenium on fish have been estimated at 3–4 mg Se/kg diet and 2–5 μg/L in water (NRC, 2005).

Selenium and vitamin E interrelationships have been investigated in several animal species, and a variety of common and unique deficiency signs have been described (NRC, 1993). Differing responses, especially with respect to gross deficiency signs, were observed when Atlantic salmon (Poston et al., 1976), rainbow trout (Bell et al., 1985), and channel catfish (Gatlin et al., 1986) were fed diets without supplemental selenium, vitamin E, or both nutrients. Mutual sparing of metabolic requirements for either selenium or vitamin E by the other nutrient, as measured by weight

gain and liver lipid oxidation, was recently demonstrated in grouper (Lin and Shiau, 2009). A dietary interaction between dietary selenium and copper also was reported for grouper in that elevated dietary copper induced oxidative stress and reduced the fish's immune response, but selenium at twice the minimum requirement level (1.6 mg Se/kg) reduced the oxidative stress and improved immune responses (Lin and Shiau, 2007). Dietary selenium concentration and supplemental form were reported to influence various immunological responses and resistance of channel catfish to *E. ictaluri* with fish fed 0.20 mg Se/kg from selenomethionine and 0.40 mg Se/kg from selenoyeast or sodium selenite having the greatest resistance to *E. ictaluri* (C. Wang et al., 1997). Antibody production was generally increased with dietary selenium supplementation but was greatest in catfish fed selenoyeast. Macrophage chemotactic response also was enhanced in catfish fed selenoyeast and selenomethionine (C. Wang et al., 1997). Dietary supplementation of organic selenium also has been reported to reduce the harmful effects of waterborne copper toxicity to African catfish (Abdel-Tawwab et al., 2007) and cadmium toxicity to Nile tilapia (Abdel-Tawwab and Wafeek, 2010).

Levels of 0.15 to 0.38 mg Se/kg diet (Hilton et al., 1980) and 0.25 mg Se/kg diet (Gatlin and Wilson, 1984b) were required to provide maximum growth and glutathione peroxidase activity in rainbow trout and channel catfish, respectively. More recently, Lin and Shiau (2005) estimated the minimum selenium requirement of grouper to be 0.7 mg Se/kg based on weight gain and whole-body selenium retention. As indicated previously, elevated dietary selenium from 5 to 8 mg Se/kg increased liver glutathione peroxidase activity in salmon smolts and improved their responses to the stressful conditions associated with confinement and saltwater transfer. The U.S. Food and Drug Administration currently allows selenium supplementation of up to 0.3 mg Se/kg from sodium selenate or sodium selenite in feeds for all animals including aquatic species.[1]

Information on selenium nutrition of crustaceans is rather limited at this time. Davis (1990) found that juvenile *L. vannamei* grew best when fed semipurified diets supplemented with 0.2 to 0.4 mg Se/kg diet. Although a specific dietary level was not quantified, that study indicated shrimp have a dietary requirement for selenium.

ZINC

Zinc is required for normal growth, development, and function in all animal species that have been studied (NRC, 1980). Zinc functions as a cofactor in several enzyme systems and is a component of a large number of metalloenzymes, which include carbonic anhydrase, carboxypeptidases A and B, alcohol dehydrogenase, glutamic dehydrogenase, D-glyceraldehyde-3-phosphate dehydrogenase, lactate dehydrogenase, malic dehydrogenase, alkaline phosphatase, aldolase, SOD, ribonuclease, and DNA polymerase (NRC, 1980; Lall, 2002).

A dietary requirement for zinc has been quantified for a variety of freshwater fish fed semipurified diets based on weight gain, tissue zinc saturation, and/or whole-body zinc retention. These requirement estimates include: 20 mg Zn/kg diet for channel catfish (Gatlin and Wilson, 1983; Scarpa and Gatlin, 1992) and blue tilapia (McClain and Gatlin, 1988), 15–30 mg Zn/kg diet for common carp (Ogino and Yang, 1979), 15–30 mg Zn/kg diet for rainbow trout (Ogino and Yang, 1978), and 26–29 mg Zn/kg for hybrid tilapia (Lin et al., 2008c). The zinc requirement of red drum also has been determined to be 20 mg Zn/kg diet (Gatlin et al., 1991).

The effects of zinc on immunity and disease resistance of fish also have been investigated. One study determined that supplementation of zinc at 200 mg/kg diet did not enhance resistance of nonimmunized channel catfish juveniles to *Aeromonas hydrophila* (Scarpa and Gatlin, 1992). In a more recent study, zinc deficiency caused 100% mortality in channel catfish challenged with *E. ictaluri*, and maximum survival after challenge was achieved with 5 mg Zn/kg provided by zinc methionine or 30 mg Zn/kg provided by zinc sulfate (Paripatananont and Lovell, 1995a). Maximum antibody production in that study was achieved with 15 mg Zn/kg from zinc methionine or 30 mg Zn/kg or greater from zinc sulfate. In a similar study by Lim et al. (1996), channel catfish fed zinc methionine at 20 and 60 mg/kg diet and zinc sulfate at 60 mg/kg diet had higher chemotactic responses of macrophages; however, dietary zinc did not influence phagocytic activity of macrophages for *E. ictaluri*. Additionally, the source or level of dietary zinc did not provide protection against *E. ictaluri* infection.

Zinc nutrition of various crustaceans also has been investigated. Davis et al. (1993c) reported that *L. vannamei* required 33 mg Zn/kg diet to maintain normal tissue mineralization although growth was not affected by graded levels of dietary zinc. A similar requirement value of 32–34 mg Zn/kg was quantified for *P. monodon* by Shiau and Jiang (2006) based on weight gain and whole-body zinc retention.

As previously mentioned in the section on copper, elevated levels of dietary zinc have been shown to reduce the bioavailability of copper in terrestrial animals (NRC, 2005). However, such interactions have not been well established in fish (Knox et al., 1982, 1984; Gatlin et al., 1989).

OTHER MINERALS

There are several other trace minerals established as being essential in terrestrial animals and humans, but for which there is much less information available on aquatic species. Cobalt is one such mineral included in the group that has been studied to only a limited extent in aquatic species. Cobalt is of nutritional significance because it is a component of vitamin B_{12}. Synthesis of vitamin B_{12} by intestinal bacteria

[1]Title 21, Code of Federal Regulations, Part 573.920.

of channel catfish was reduced by removal of cobalt from the diet (Limsuwan and Lovell, 1981). A series of studies conducted in Russian ponds (reviewed by Castell et al., 1986) reported the provision of cobalt in the diet or water increased growth and hemoglobin formation in common carp. Enhanced growth and survival of mullet (*Mugil parsia*) fry was reported by Ghosh (1975) when cobalt chloride at 0.6 to 1 ppm was dissolved in water and dispensed in powdered feed consisting of each portions of rice bran, mustard oil cake and fish meal. Another study (Hertz et al., 1989) investigated the effects of both cobalt and chromium on blood glucose regulation of common carp, and noted improved glucose utilization and increased amino acid incorporation into protein with cobalt supplementation. Molybdenum has been implicated in enhancing growth and survival of carp (George, 1970 cited by Lall, 2002). However, similar studies with other fish species have not been conducted. Intake of dietary fluoride was shown to increase fluoride content of bones in rainbow trout but did not result in any overt responses (Tiews et al., 1982). Other inorganic elements such as arsenic, barium, bromine, cadmium, and strontium are potentially required by the body but their essentiality has been difficult to establish except under highly controlled conditions. Dietary requirements for these elements have not been specifically studied in fish. However, some of these minerals, such as arsenic and mercury, may accumulate in fish tissues and potentially have adverse effects on human consumers. Thus, research on these minerals has primarily focused on bioaccumulation in fish and nutritional quality of seafood products, which is addressed in Chapter 16.

SOURCES AND FORMS

Of the feed ingredients used in practical animal diets, fish meal is the richest source of endogenous minerals. Research on the bioavailability of minerals contained in fish meals has demonstrated that there is considerable variation among fish species (perhaps due to luminal pH) and that the bioavailability of minerals is affected by meal type and ash content. Fish meals, as well as other feedstuffs of animal origin such as meat and bone meal, poultry byproduct meal, and feather meal, are relatively rich in minerals. However, due to low mineral availability and potential inhibitory interactions, prepared diets are routinely supplemented with available sources of copper, phosphorus, manganese, and zinc to prevent dietary deficiencies and maximize fish growth (Watanabe et al., 1988). As the aquatic animal feed industry increases its use of plant feedstuffs, which are generally poor sources of minerals and may contain factors that reduce the bioavailability of minerals, the need for mineral supplements should increase.

Regardless of the form in which calcium and phosphorus are ingested, their absorption is dependent upon solubility at the point of contact with the absorbing membranes; hence, mineral sources that are soluble at luminal pH are potentially more available. The NRC (1983) summarized the relative availability or apparent absorption of various sources of phosphorus for four species of fish. In general, bioavailability of phosphorus (and minerals in general) has been found to be positively correlated with the solubility of the mineral in water. Monobasic phosphates of sodium and potassium are highly available (90 to 95%) sources of phosphorus for channel catfish (Lovell, 1978), common carp (Ogino et al., 1979), red sea bream (Sakamoto and Yone, 1979b), and rainbow trout (Ogino et al., 1979). Di- and tribasic phosphates are highly available to red sea bream (Sakamoto and Yone, 1979b). Dibasic calcium phosphate has an availability of 65% for channel catfish (Lovell, 1989). More recently, phosphorus availability values for monosodium phosphate, monoammonium phosphate, defluorinated rock phosphate, and mono-calcium phosphate to channel catfish were determined to be 88.8, 85.4, 81.7, and 81.2%, respectively (Eya and Lovell, 1997b). Li et al. (1996) also confirmed that dicalcium phosphate and defluorinated phosphates were equally efficacious as phosphorus supplements for channel catfish. Di- and tribasic calcium phosphates have availability values of 71 and 64%, respectively, for rainbow trout (Ogino et al., 1979). Phosphorus availability of inorganic sources to European sea bass decreased in the order of dicalcium phosphate (68%), monocalcium phosphate (56%), and tricalcium phosphate (50%) (Pimentel-Rodrigues and Oliva-Teles, 2007). In yellowtail, apparent phosphorus availability values greater than 92% were reported for monobasic forms of sodium, potassium, and calcium phosphate compared to calcium phosphate di-basic (52.9%) and tribasic (48.8%) (Sarker et al., 2009). For the agastric common carp, the bioavailability of phosphorus from different sources was reported to be more variable with bioavailability values of only 13% for tribasic calcium phosphate, 46% for dibasic calcium phosphate, and 94% for monobasic calcium phosphate (Ogino et al., 1979). In the presence of adequate phosphorus, Nakamuara and Yamada (1980) determined the availability of calcium for common carp to be 58, 37, and 27% from calcium lactate, tri-basic calcium phosphate, and calcium carbonate, respectively. The differences in calcium availability were influenced by the lack of acidic digestion in common carp.

The bioavailability of phosphorus to marine shrimp appears to be intermediate to that of fish with and without true stomachs. Apparent phosphorus availability values for *L. vannamei* include calcium phosphate monobasic, 46%; calcium phosphate dibasic, 19%; calcium phosphate tribasic, 10%; potassium phosphate monobasic, 68%; and sodium phosphate monobasic, 68% (Davis and Arnold, 1994).

In addition to chemical form (solubility) affecting mineral availability, other nutrients affect the availability of certain minerals. For example, lactose may interact with absorptive cells to increase their permeability to calcium ions; large

intakes of iron, aluminum, and magnesium may interfere with the absorption of phosphorus by forming insoluble phosphates; and fats may interact with calcium to form insoluble soaps (Maynard et al., 1979). Hence, availability of calcium and phosphorus may be dependent upon the mineral form (solubility at intestinal pH), and dietary levels of calcium, phosphorus, vitamin D, iron, aluminum, manganese, potassium, magnesium, and fat.

Phosphorus in fish meal exists mainly in the form of insoluble hydroxyapatite originating from hard tissues such as bones. The availability of phosphorus contained in fish meal is fairly low for carp (10–33%) as compared with rainbow trout (60–81%) (Watanabe et al., 1988). Based on unpublished data, Akiyama and Dominy (1989) reported the apparent absorption of phosphorus from fish meal to be 46.5% for *L. vannamei*. Phytate phosphorus, which constitutes approximately 67% of the phosphorus in plant feedstuffs, also is poorly available to fish (Lovell, 1989) and shrimp (Civera et al., 1990; Davis et al., 1993c). Civera et al. (1990) found that the presence of phytate inhibited the availability of dietary calcium and phosphorus to *L. vannamei* and *M. japonicus*, presumably because of the formation of insoluble complexes in the digestive system. The apparent availability of phytate phosphorus was determined to be 47.3% for *M. japonicus* and 8.4% for *L. vannamei* (Civera et al., 1990). Similarly, Davis et al. (1993c) reported that the presence of 1.5% phytate inhibited the availability of dietary phosphorus and zinc to *L. vannamei*. It should be noted that phytate phosphorus can account for a considerable portion of the phosphorus in practical diet formulations. The treatment of plant protein feedstuffs with phytase has been demonstrated to increase the availability of phosphorus to a variety of fish species including rainbow trout (Ketola, 1994; Riche and Brown, 1996; Dalsgaard et al., 2009), Atlantic salmon (Storebakken et al., 1998; Sajjadi and Carter, 2004), channel catfish (Eya and Lovell, 1997b), *Pangasius* spp. catfish (Debnath et al., 2005), striped bass (Hughes and Soares, 1998), and Korean rockfish (*Sebastes schlegeli*) (Yoo et al., 2005). Consequently, phytase supplementation of plant feedstuffs or complete diets may be utilized to increase the availability of phosphorus from this component of the diet. Advancements in plant breeding also have resulted in low-phytic acid varieties of crops such as barley (Overturf et al., 2003; Buentello et al., 2010).

The bioavailability of minerals chelated to organic molecules has generally been reported to be higher than inorganic forms. If an element is chelated by a compound that will release it in ionic form at the site of absorption or will be readily absorbed as the intact chelate, this form may greatly enhance the absorption of the element by preventing its conversion to insoluble chemical compounds in the intestine or by preventing its strong adsorption on insoluble colloids (Scott et al., 1982). Compared with inorganic sources, chelated minerals also are generally less sensitive to the inhibitory action of other compounds (i.e., phytate and fiber) and may have a higher bioavailability in practical diets. Another potential benefit of mineral chelates for aquatic species is reduced solubility in water. However, the higher cost of most mineral chelates relative to inorganic sources has generally limited their use in aquaculture to date.

Several different forms of organic or chelated minerals have been developed for various elements. Amino acid chelates of copper, manganese, and zinc were determined to be more readily available than their inorganic salts based on increased bone and liver deposition in rainbow trout (Apines et al., 2001; Apines-Amar et al., 2004). Copper exchanged montmorillonite is another relatively uncommon form of supplemental copper. When it was included at 1.5 g/kg diet to provide 30 mg Cu/kg, it increased growth of Nile tilapia and protected the intestinal mucosa from invasion by pathogenic bacteria (Hu et al., 2007).

Selenium content and availability have been shown to vary considerably among different feedstuffs. Diets formulated with predominantly plant ingredients may require a selenium supplement. Selenium availability from various inorganic and organic sources has been evaluated in aquatic species. Bell and Cowey (1989) reported that selenium from selenomethionine was most available to Atlantic salmon, and selenium from fish meal was least available. However, based on glutathionine peroxidase activity, selenite and selenocystine were better sources than selenomethionine and fish meal. Lorentzen et al. (1994) determined that dietary selenite yielded higher levels of hepatic selenium in Atlantic salmon compared to selenomethionine, but the latter provided higher muscle and whole-body selenium concentrations. Due to their very toxic nature, sodium selenite or sodium selenate should not be handled in pure form. Selenomethionine was recently determined to be approximately three times more available to hybrid striped bass than sodium selenite (Jaramillo et al., 2009). Likewise, selenomethionine appears to be the most toxic form of selenium at elevated levels (NRC, 2005). A tentative maximum tolerable selenium level for fish in which health or performance would not be impaired was suggested as 2 mg Se/kg diet, although more research to establish appropriate safety factors was recommended (NRC, 2005).

Several different sources of zinc have been evaluated with various fish species. Zinc gluconate was found to be equivalent to zinc sulfate for Atlantic salmon (Maage et al., 2001). The organic chelate zinc methionine was compared to zinc sulfate in both purified and practical diets with channel catfish (Paripatananont and Lovell, 1995b). Zinc methionine was estimated to be three times as potent as zinc sulfate in purified diet, and four to five times more available in soybean-meal-based diets. Zinc picolinate was evaluated by supplementing the diet to provide 30 and 60 mg Zn/kg. This supplement linearly increased serum and whole-body zinc, as well as reduced oxidative stress of rainbow trout (Kucukbay et al., 2006).

INTERACTIONS WITH OTHER DIETARY COMPONENTS

In order to meet an animal's physiological requirements for various minerals, dietary sources must be available. Numerous factors affect the availability of minerals. The most soluble, and consequently the most readily absorbed, forms are the simple state of the element or ionic group of atoms (e.g., Ca^{2+}, Mg^{2+}, Mn^{2+}, PO_4^{3-}). However, in nature, compounds differing in electric charge may bind with minerals forming stable compounds that are less soluble in water. Although such compounds have low solubility in water, the acidic condition of the gastric stomach allows dissociation of the compounds into salts that can be easily absorbed in the intestine. Consequently, in animals without acidic digestive systems, the bioavailability of minerals is generally reduced.

Although the gastric stomach generally increases the availability of minerals, some minerals may interact after being released into the alkaline intestine by forming insoluble precipitates. Excessive levels of calcium and phosphorus react with magnesium and zinc to form insoluble precipitates. Additionally, colloids such as particles of clay, insoluble salts of aluminum, magnesium, iron, and other elements strongly absorb cations. This absorption occurs both through chemical union with highly electronegative areas of the colloidal surface and through attraction of the cation by physical forces (Scott et al., 1982). Consequently, the bioavailability of a given mineral will be dependent upon its dissociation as well as interactions with other dietary components.

The bioavailability of zinc in feedstuffs is generally low, making supplementation essential (Watanabe et al., 1988). The bioavailability of zinc in various fish meals has been found to be inversely related to the tri-calcium phosphate content of the meal. Thus, it is generally lowest in white fish meals, which contain the highest level of tri-calcium phosphate, and slightly higher in brown fish meals (Watanabe et al., 1988). Reduced bioavailability of zinc in response to calcium phosphate supplementation also has been observed in rainbow trout (Ketola, 1979; Hardy and Shearer, 1985; Satoh et al., 1987). Bioavailability of manganese also is reduced when high levels of calcium and phosphorus are in the diet (Watanabe et al., 1997).

Practical diets often contain feedstuffs that are relatively high in phytate, which may also reduce the bioavailability of zinc. The effect of phytate on zinc bioavailability is well established in a variety of terrestrial animals (Oberleas et al., 1962; O'Dell et al., 1964; Savage et al., 1964; Lo et al., 1981), fish (Gatlin and Wilson, 1984c; Richardson et al., 1985; McClain and Gatlin, 1988; Gatlin and Phillips, 1989; Satoh et al., 1989), and shrimp (Davis et al., 1993c). Consequently, practical diets are often supplemented at levels in excess (100 to 150 mg Zn/kg diet) of the established minimum dietary requirement to overcome the effects of inhibitory agents such as phytate. For example, Eid and Ghonim (1994) determined the minimum zinc requirement of Nile tilapia fed purified diets was 30 mg Zn/kg based on weight gain, feed efficiency, serum zinc, and bone zinc. However, Do Carmo e Sa et al. (2004) determined that plant-based diets had to be supplemented with zinc sulfate at 79.5 mg Zn/kg to maximize bone zinc storage of Nile tilapia.

Of all the microminerals, copper, iron, manganese, selenium, and zinc have been demonstrated in some fish species to be most important to supplement in diets due to low levels in practical feedstuffs and/or interactions with other dietary components that may reduce bioavailability (Watanabe et al., 1997). Although supplementation of practical diets with other microminerals has not been shown to be essential in most instances, an inexpensive trace mineral premix containing inorganic forms may be added to most nutritionally complete diets in order to ensure adequacy.

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