Nutrition and Functions of Amino Acids in Aquatic Crustaceans

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Abstract

Crustaceans (e.g., shrimp and crabs) are a good source of protein-rich foods for human consumption. They are the second largest aquaculture species worldwide. Understanding the digestion of dietary protein, as well as the absorption, metabolism and functions of amino acids (AAs) and small peptides is essential to produce cost-effective and sustainable aquafeeds. Hepatopancreas (the midgut gland) is the main site for the digestion of dietary protein as well as the absorption of small peptides and AAs into the hemolymph. Besides serving as the building blocks of protein, AAs (particularly aspartate, glutamate, glutamine and alanine) are the primary metabolic fuels for the gut and extra-hepatopancreas tissues (e.g., kidneys and skeletal muscle) of crustaceans. In addition, AAs are precursors for the syntheses of glucose, lipids, H_2S, and low-molecular-weight molecules (e.g., nitric oxide, glutathione, polyamines, histamine, and hormones) with enormous biological importance, such as physical barrier, immunological and antioxidant defenses. Therefore, both nutritionally essential and nonessential AAs are needed in diets to improve the growth, development, molt rate, survival, and reproduction of crustaceans. There are technical difficulties and challenges in the use of crystalline AAs for research and practical production due to the loss of free AAs during feed processing, the leaching of in-feed free AAs to the surrounding water environment, and asynchronous absorption with peptide-bounded AAs. At present, much knowledge about AA metabolism and functions in crustaceans is based on studies of mammals and fish species. Basic research in this area is necessary to lay a solid foundation for improving the balances and bioavailability of AAs in the diets for optimum growth, health and wellbeing of crustaceans, while preventing and treating their metabolic diseases. This review highlights recent advances in AA nutrition and metabolism in aquatic crustacean species at their different life stages. The new knowledge is expected to guide the development of the next generation of their improved diets.

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Keywords

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Abbreviations

AA	amino acid
EAA	nutritionally essential amino acid
GABA	γ-aminobutyrate
GDH	glutamate dehydrogenase
GOT	glutamate-oxaloacetate transaminase
GPT	glutamate-pyruvate transaminase
mTOR	mechanistic target of rapamycin
NEAA	nutritionally nonessential amino acid
NO	nitric oxide
NRC	National Research Council
ROS	reactive oxygen species

9.1 Introduction

Crustaceans (including shrimp and crabs) are low-fat, good sources of high-quality protein, free amino acids (AAs), small peptides, and poly-unsaturated fatty acids for human consumption (Bhavan et al. 2010; Wu et al. 2016; Wu 2020). Therefore, they are healthy seafoods worldwide. Crustacean farming has been an economically important enterprise in either a marine or a freshwater environment as the second largest aquaculture species (e.g., 7.86 million tons and US$ 57.1 billion in 2016; Tacon 2018). Twenty-seven (27) species of aquatic crustaceans have been reported, which include mainly shrimps, crabs, and crawfish (Tacon 2018).

Crustaceans have particularly high requirements for dietary protein, which ranges from 60% of the diet for some post-larvae to 30–50% of the diet for juvenile shrimp, crabs and lobsters (Unnikrishnan and Paulraj 2010; Jin et al. 2013; Mente 2006). High-protein diets lead to the excretion of a large amount of nitrogen and low water quality. Traditionally, fishmeal has been the major protein source for crustaceans due to its high levels of digestible protein and balanced AA profiles (Unnikrishnan and Paulraj 2010). However, fishmeal is an unsustainable protein source due to its limited source and high price (Hardy 2010). In the culture of crustaceans, the cost of feeds represents more than 50% of the production costs (Mente 2006). Therefore, continued expansion of crustaceans is not unsustainable if fishmeal is their sole or primary protein source. In addition, disease and animal health have been a major limiting factor for the culture of shrimps, crabs, and crawfish (Mente 2006; Stentiford et al. 2012). Knowledge of their optimum requirements for nutrients, particularly AAs, is key to solving this problem, because many AAs regulate key metabolic pathways that are crucial to the maintenance, growth, reproduction, and immune responses of animals (Li et al. 2007, 2009b; Wu 2010; Wu et al. 2014).

Understanding the digestion of dietary protein, as well as the absorption, metabolism and functions of small peptides and AAs are essential to manufacture environmentally-oriented aquafeeds and reduce feed costs in animal production (Li et al. 2009b). Such diets can improve the health and wellbeing of crustaceans, while preventing and treating their metabolic diseases. Although a wide range of dietary AA requirements has been reported for aquatic animals in the literature, our knowledge about AA metabolism and functions in crustaceans is limited. The crustaceans belong to the suborders of the Decapoda with different metabolic, physiological, and immunological characteristics, when compared with other animals such as fish and mammals (NRC 2011; Vazquez et al. 2009). The major objective of this article is to highlight current knowledge about AA nutrition and metabolism in shrimps, crabs, and crawfish at their different stages of lives. This will help to advance the field of protein nutrition and guide the development of future crustacean feeds.

9.2 Protein Digestion and the Absorption of Small Peptides and Free AAs in Crustaceans

The diets of crustaceans contain high concentrations of protein (NRC 2011). The digestive tract of crustaceans is essentially an internal tube and generally divided into three functional segments: foregut (a tubular esophagus and a stomach), midgut (a simple tubule with associated ceca and the hepatopancreas), and hindgut (rectum and anus; Fig. 9.1). The esophagus joins the
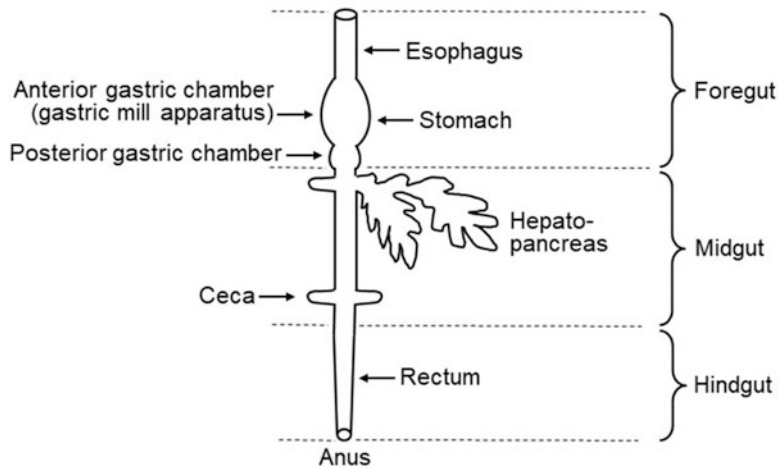
Fig. 9.1 Scheme of the digestive tract of crustaceans. The digestive tract of crustaceans consists of the foregut (a tubular esophagus and a stomach), midgut (a simple tubule with associated ceca and the hepatopancreas), and hindgut (rectum and anus). Cutting and grinding of the ingested foods, as well as their initial digestion (by digestive enzymes from the hepatopancreas) occurs in the anterior chamber of the stomach (the gastric mill apparatus). The food particles enter the anterior midgut and then the joining hepatopancreas. The hepatopancreas secretes digestive enzymes and is the major site for the extracellular digestion of foods and absorption of digestion products or simple nutrients into the hemolymph. Undigested foods and unabsorbed nutrients enter the rectum and exit the gut through the anus

mouth to the stomach [an anterior chamber (the gastric mill apparatus) and a posterior chamber]. The anterior chamber functions in mastication (cutting and grinding) of the ingested food, whereas the posterior chamber keeps food particles from leaving the stomach until the gastric mill has reduced them into a small size (McGaw et al. 2013). Secretion of HCl by gastric epithelial cells results in acidic conditions in stomach fluids (pH = ~ 4 or higher) during digestion. The food particles leave the posterior chamber of the stomach to enter the anterior midgut and then the hepatopancreas (also called the midgut gland or digestive gland; a branching array of blind-ended tubules lined with an epithelium) that connects to the anterior *midgut* via ducts (Ceccaldi and Ceccaldi 1989). The hepatopancreas secretes digestive enzymes that flow into the midgut and then retrograde into the stomach. Much extracellular digestion of foods and absorption of digestion products (free AAs, as well as di- and tri-peptides) or simple nutrients into the hemolymph occur *within* the hepatopancreas (Buarque et al. 2009, 2010; Fernández et al. 1997; Saborowski et al. 2006). The midgut plays a relatively minor role in the digestion and

absorption of nutrients. Di- and tri-peptides (the major products of protein digestion) are taken up by the epithelial cells of the hepatopancreas via the apical-membrane peptide transporter-1, whereas free AAs are taken up by these cells via various sodium-dependent and independent transporters (Wu 2013). Within the absorptive cells, the small peptides are hydrolyzed by peptidases (including proline peptidases) to free AAs. AAs that are not metabolized by the hepatopancreatic cells enter the hemolymph. Undigested food particles and unabsorbed nutrients from the terminal midgut enter the rectum to form feces, which leaves the gut through the anus.

Studies with the southern brown shrimp *Farfantepenaeus subtili*s have shown the highest activity of aminopeptidase in the presence of alanine-, arginine-, lysine- or leucine-β-naphthylamide as a substrate (Buarque et al. 2010). Proteinases and peptidases activities in crustaceans are modulated by several internal and external factors (Saborowski et al. 2006). These enzymes have an optimum pH around 8 (Buarque et al. 2009; Dionysius et al. 1993). Moreover, the enzyme activities are also

influenced by ontogenetic events (Lemos et al. 2000), life stages (Lee et al. 1984), hormones (Gorell and Gilbert 1969; Thomson et al. 1971), the molting cycle (Gimenez et al. 2001, 2002), and diet composition such as protein levels and sources (Lee et al. 1984; Brito et al. 2000; Muhlia-Almazan et al. 2003). All of these results indicate that crustaceans can adapt to changes in their diets and physiological states.

Crustaceans have a high ability to digest a wide range of animal- and plant-source proteins. In whitelegs shrimp, the digestibilities of AAs are greater than 92% (Cruz-Suárez et al. 2009). Proteins from animal resources are better digested than plant proteins in several crustacean species (Forster and Gabbott 1971; Fenucci et al. 1982). A decrease in the digestibility of AAs was observed with an increase in the graded dietary level of rice protein concentrate from 0% to 100% (i.e., 25, 50, 75, and 100%) (Oujifard et al. 2012). The low digestibility of AAs in plant ingredients results from the presence of inhibitors of proteinases and peptidases (Garcia-Carreo et al. 1997; Oujifard et al. 2012). To solve this problem, heating and fermentation are the common ways to remove or reduce these anti-nutritive factors in plant-source feedstuffs (NRC 2011). Moreover, feed additives, such as organic acids and enzymes, can be added to crustacean feeds to improve the utilization of alternative dietary protein sources. In whiteleg shrimp, dietary organic acids can modify the activities of digestive enzymes and the digestibility of dietary protein possibly due to changes in gastric pH and intestinal microbes (Silva et al. 2016). Supplementation with proteases to low fishmeal diets has been reported to improve the growth or feed utilization of some shrimp (Li et al. 2016; Song et al. 2017) and crab (Chowdhury et al. 2018) species.

9.3   The Free AA pool in Crustacean Tissues

Crustaceans have an open circulatory system, where nutrients, oxygen, hormones, and cells are distributed in the hemolymph. Therefore, all of their blood is not contained within vessels, but rather blood is drawn into the heart through holes called the ostia, pumped out again to circulate through tissues, and return to the heart (Wirkner and Stefan 2013). After the hepatopancreas absorb small peptides and free AAs through its single-cell layer of epithelial cells into the hemolymph, AAs participate in metabolic pathways in the whole body as the building blocks of proteins and peptides, substrates for ATP production, and precursors for the syntheses of low-molecular-weight bioactive substances (e.g., NO, neurotransmitters, and thyroid hormones), signaling molecules (Li et al. 2007; Wu 2013). The concentrations of free AAs in most crustacean tissues are higher than those in vertebrate tissues. Table 9.1 shows the concentrations of AAs in the hemolymph of shrimp. The major free AAs in crustaceans are glycine, glutamine, alanine, arginine, and taurine, which may vary among different species (Fig. 9.2; Shinji and Wilder, 2012; Miyagawa et al., 1990). All of these AAs are abundant in animal-source feedstuffs (Li and Wu 2018; Li and Wu 2020a), whereas all plant-source feedstuffs lack taurine and contain low concentrations of glycine (Hou et al. 2019; Li and Wu 2020a; Li et al. 2011a). Of note, arginine phosphate is present in some crustaceans, such as shrimp. Concentrations of free AAs in their tissues are affected by diets and environmental factors, such as salinity (Shinji and Wilder 2012), ammonia levels (Chen et al. 1994), temperature (Rao and Ramachandra 1961), and intracellular protein turnover (Wu 2013). Free AAs in tissues are in dynamic equilibrium with the protein pool. On the molar basis, glycine is the most abundant free AA in the hemolymph (a fluid analogous to the blood in vertebrates) and the whole body of the whiteleg shrimp (*Litopenaeus vannamei*), followed by alanine, taurine, arginine, glutamine and proline in the hemolymph and by arginine, taurine, proline, glutamine, and alanine in the whole body, in descending order (Table 9.1). Of note, in the whole body of the shrimp, most of free AAs represent about 5% (g/g) of their corresponding total AAs (free plus peptide-bound), but free glycine and free arginine account for 30.5% and 23.3% of the total AAs, respectively. In the whole body of the whiteleg shrimp,

Table 9.1 Concentrations of free and peptide-bound amino acids (AAs) in the whole body of whiteleg shrimp[a]

| AA | Free AAs in hemolymph (nmol/ml) | Free AAs in the whole body (mg/g of DM) | Total AAs (free plus peptide-bound) in the whole body[b] | | Ratio of free AAs to total AAs in the whole body |
			mg/g of DM	mg/g of protein AAs	(g/g)
Proteinogenic AAs					
Ala	958 ± 33	5.98 ± 0.26	43.5 ± 0.40	60.3 ± 0.91	0.137 ± 0.003
Arg[b]	576 ± 21	11.0 ± 0.48	47.0 ± 0.22	65.1 ± 0.43	0.233 ± 0.006
Asn	189 ± 11	1.21 ± 0.05	31.4 ± 0.46	43.5 ± 0.88	0.038 ± 0.001
Asp	80.1 ± 4.2	1.62 ± 0.06	37.7 ± 0.43	52.3 ± 0.95	0.043 ± 0.001
Cys	152 ± 11	1.10 ± 0.05	11.6 ± 0.25	16.1 ± 0.55	0.095 ± 0.002
Gln	562 ± 12	6.25 ± 0.29	40.6 ± 0.58	56.3 ± 0.97	0.154 ± 0.003
Glu	95.3 ± 6.8	2.78 ± 0.13	65.9 ± 0.44	91.3 ± 0.76	0.042 ± 0.001
Gly	1024 ± 63	15.3 ± 0.66	55.6 ± 0.32	77.1 ± 0.71	0.275 ± 0.005
His	101 ± 5.5	1.14 ± 0.05	15.0 ± 0.24	20.8 ± 0.49	0.076 ± 0.002
Ile	116 ± 7.4	1.55 ± 0.06	29.8 ± 0.29	41.3 ± 0.63	0.052 ± 0.001
Leu	162 ± 13	2.70 ± 0.11	49.8 ± 0.41	69.0 ± 0.88	0.054 ± 0.001
Lys	257 ± 15	3.92 ± 0.12	50.5 ± 0.45	70.0 ± 0.94	0.078 ± 0.002
Met	32.7 ± 1.6	0.90 ± 0.04	15.2 ± 0.23	21.1 ± 0.44	0.053 ± 0.001
Phe	70.5 ± 8.3	1.53 ± 0.05	33.1 ± 0.57	45.9 ± 0.96	0.046 ± 0.001
Pro	308 ± 19	6.60 ± 0.31	49.2 ± 0.55	68.2 ± 1.2	0.134 ± 0.004
OH-Pro	45.2 ± 3.6	0.031 ± 0.001	8.90 ± 0.26	12.3 ± 0.43	0.0035 ± 0.0002
Ser	254 ± 15	1.64 ± 0.06	37.8 ± 0.39	52.4 ± 0.86	0.043 ± 0.001
Thr	162 ± 6.9	1.89 ± 0.07	29.4 ± 0.34	40.7 ± 0.70	0.064 ± 0.001
Trp	28.3 ± 1.4	1.12 ± 0.04	8.80 ± 0.18	12.2 ± 0.40	0.127 ± 0.003
Tyr	30.5 ± 1.8	2.43 ± 0.08	27.1 ± 0.32	37.6 ± 0.68	0.090 ± 0.002
Val	224 ± 9.7	2.17 ± 0.09	33.6 ± 0.37	46.6 ± 0.75	0.065 ± 0.001
Total AAs	5428 ± 76	72.7 ± 1.0	721.5 ± 9.2	1000	–
Non-proteinogenic AAs					
β-Alanine	25.2 ± 3.8	0.009 ± 0.0003	–	–	–
Cit	0.24 ± 0.02	Trace amount[c]	–	–	–
Orn	148 ± 8.6	0.16 ± 0.01	–	–	–
P-Arg	37.4 ± 2.8	10.9 ± 0.32	–	–	–
Taurine	717 ± 55	9.06 ± 0.09	–	–	–

Cit citrulline, *DM* dry matter, *OH-Pro* 4-hydroxyproline, *Orn* ornithine, *P-Arg* phosphoarginine
[a]Values are means ± SEM, n = 8. Whiteleg shrimp (*Litopenaeus vannamei*) were fed a diet consisting of the following (as-fed basis): 20% fishmeal, 10% soybean meal, 15% wheat flour, 35% poultry by-product, 1.5% soybean oil, 1% soy lecithin, 1% cholesterol, 0.1% vitamin C, 0.13% choline chloride, 4.6% K_2HPO_4, 0.7% $MgCl_2$, 0.1% astaxanthin (5%), 0.5% vitamin-mineral premix, and 10.37% cellulose (Li and Wu 2020b). The composition of the vitamin-mineral premix (g/kg premix) was: vitamin A, 0.4; vitamin D_3, 0.04; vitamin E, 40; vitamin K_3, 2.40; vitamin B_5, 21.74; inositol, 30; vitamin B_3, 28; vitamin B_1, 6.53; biotin, 0.3; folic acid, 1.2; vitamin B_{12}, 0.04; KI, 1.06; $CuSO_4.5H_2O$, 1.10; $MnSO_4$.H_2O, 1.25; $ZnSO_4.7H_2O$, 13.68; and cellulose, 840.19. The crude-protein content of the diet was 43.0% (dry matter basis). The shrimp were raised in water (25 °C and 3–5 ppt salinity). Hemolymph (0.1 ml; a fluid that is analogous to the blood in vertebrates) was obtained from the shrimp (15 g/shrimp) at 24 h after the last feeding. Hemolymph is a fluid that is analogous to the blood in vertebrates. Free and peptide-bound AAs in the whole shrimp were analyzed as described by Li and Wu (2020a). The amounts of amino acids in the whole body were calculated on the basis of their intact molecular weights. The content of dry matter in the whole body of the shrimp was 24.2%. The true protein (calculated on the basis of the molecular weights of amino acid residues; i.e., intact molecular weight – 18) in the whole body of the shrimp was 60.9% of dry matter, whereas collagen represents 10.0% of the total true protein in the whole body. Cys is the sum of cysteine plus 1/2 cystine.
[b]Excluding phosphoarginine. The content of arginine as phosphoarginine in the whole body of the shrimp was 7.47 mg/g of dry matter, as analyzed by high-performance liquid chromatography (Wu and Meininger 2008)
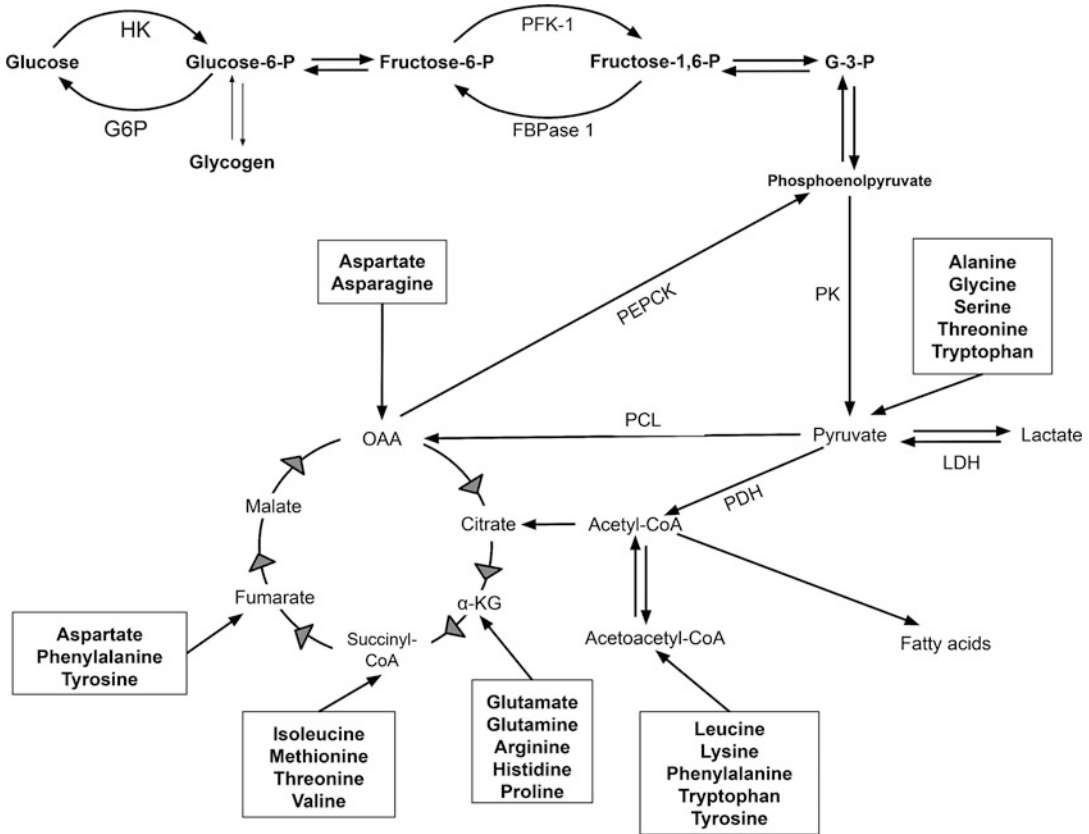[c]The value was 0.18 ± 0.01 μg/g of dry matter

Fig. 9.2 Different metabolic pathways for the catabolism of amino acids converge to common intermediates that feed into the Krebs cycle, lipogenesis, and glucogenesis pathways in crustaceans. *G6Pase* glucose-6-phosphatase, *PCL* pyruvate carboxylase, *PEPCK* phosphoenolpyruvate carboxykinase, *PDH* pyruvate dehydrogenase, *PK* pyruvate kinase, *LDH* lactate dehydrogenase, *HK* hexokinase, *PFK-1* phospho-fructokinase-1, *G-3-P* glyceradehyde-3-phosphate, *α-KG* α-ketoglutarate, *OAA* oxaloacetate

the ratio of total free proteinogenic AAs (72.7 mg/g of dry weight) to the total proteinogenic AAs (721.5 mg/g of dry matter) is 1.0:10.0 (Table 9.1). The high abundance of free AAs is consistent with their important role in the maintenance of osmolality and metabolism in shrimp.

9.4 Protein Synthesis in Crustacean Tissues

The process of protein synthesis in both crustaceans and other animals include five steps: (1) gene transcription; (2) initiation of translation; (3) peptide elongation; (4) termination, and (5) posttranslational modifications (Wu 2013).

In crustaceans, the rate of protein synthesis is generally higher in the hepatopancreas, followed by the heart, gill, tail and claw muscle in descending order (Houlihan et al. 1990; Mente et al. 2011). Among these tissues, protein synthesis in skeletal muscle is crucial for shrimp growth and production. A postecdysial increase in muscle fiber length and the associated increase in the sarcomere number are accompanied by an increase in muscle protein synthesis (Carter and Mente 2014). The rate of muscle protein synthesis (Ks, the percentage of the protein mass synthesized per day) is 1.26%/day at 27 °C in whitelegs shrimp (Mente et al. 2002), 1.15%/day at 15 °C for shore crabs (*Carcinus maenas*; El Haj and Houlihan 1987), and 0.9–1.4%/day at 30 °C

in brown tiger prawn (*Penaeus esculentus*; Hewitt 1992). For comparison, the rate of protein synthesis is lower at 0.3–0.4%/day in the claw, leg and abdominal muscles of the American lobster (*Homarus Americanus*, Haj et al. 1996). The rate of muscle protein synthesis also varies with muscle fiber type and muscle type. Slow-type tonic muscle fibers have a rate of protein synthesis that is 2.1 times greater than fast-type phasic fibers (El Haj and Houlihan 1987). Protein synthesis plays a vital role in the growth, development, health and survival of animals (Carter and Mente 2014; Li et al. 2020c). For example, vitellogenesis (synthesis of v*itellogenin* as a precursor protein of egg yolk in the blood or hemolymph of females) occurs in the ovary and hepatopancreas to support reproduction (Tseng et al. 2001). Increases in protein synthesis in the midgut gland after feeding enhance the secretions of digestive enzymes for the digestion of dietary nutrients (Houlihan et al. 1990).

The growth of crustaceans depends on ecdysis (also known as molt), which refers to the replacement of their rigid carapace with a new and larger one generated underneath the former exoskeleton that consists primarily of chitosan (Comeau and Savoie 2001). Therefore, protein synthesis is highly related to the molt cycle. The highest rate of protein synthesis occurs during the premolt stages in shore crabs (El Haj and Houlihan 1987). Moreover, protein synthesis is also influenced by several abiotic and biotic factors, such as hormones (Carter and Mente 2014), starvation and re-feeding (Pellegrino et al. 2013), dietary composition, hypoxia, hyperoxia, temperature, salinity, and other environmental factors (Intanai et al. 2009; Mente et al. 2002, 2003). For example, the rates of protein synthesis, survival, and specific growth are higher in shrimp fed diets with high quality proteins than in shrimp fed low quality proteins (Mente et al. 2002). Of note, muscle protein synthesis is substantially higher in brown tiger prawn (*Penaeus esculentus*) fed a 50%-protein diet than a 30%- or 40%-protein diet (Hewitt 1992). Similar to other animals, protein synthesis requires a large amount of energy in crustaceans and accounts 20% to 37% of oxygen consumption in the shore crab

(Houlihan et al. 1990). Therefore, starch and lipids are often included in artificial diets for crustacean as an energy source to spare protein and improve protein deposition. The protein-sparing effect of dietary digestible carbohydrate has been reported in *Litopenaeus vannamei* (Wang et al. 2015). In crabs fed a high-digestible carbohydrate diet, the rate of muscle protein synthesis measured with ^{14}C-leucine has been reported to be 2.3-fold greater than that in crabs fed a high protein diet (Pellegrino et al. 2013). This conclusion, however, may not be valid because leucine is extensively catabolized by skeletal muscle and therefore, is not an appropriate tracer for the measurement of its protein synthesis (Wu 2013).

Substantial amounts of collagens are present in tissues of crustaceans, including the shell (consisting of 22–24% dry matter) and skeletal muscles of shrimp. For example, shrimp shell consists of the following (dry matter basis): 25–40% protein, 15–20% chitin, 45–50% calcium carbonate, and 15–40 mg astaxanthin/kg, with the protein comprising of 60–75% collagen, 4–5% elastine, and 20–35% keratine (Immaculada et al. 2009). Kimura and Tanaka (1986) reported that the collagen content in the skeletal muscles of three species of crustaceans (giant river prawn, fleshy prawn and spiny lobster) was 2.4% to 2.6% of total protein. The content of collagen as the percentage of total protein in the muscles of crustaceans is as follows: 1.1–2.2% in the shrimp (*Trachypenaeus curvirostris*, *Palaemon paucidens*, and *Pandalus borealis*), 2.6–2.9% in prawn (*Penaeus japonicas*), 2.5–2.7% in lobster (*Panulirus Iongipes*), 0.2–0.8% in crabs (*Charybdis japonica*, *Portunus trituberculatus*, *Chionoecetes opilio* ♂, *Chionoecetes opilio* ♀, and *Erimacrus isenbeckii*), 3.4% in crayfish (*Procambarus clarkia*), and 5.9–6.2% in squilla (*quilla Oratosquilla oratoria*) (Yoshinaka et al. 1989). For comparison, collagen represents 2% of total protein in beef skeletal muscle (Wu et al. 2016). The AA composition and solubility of the major collagen in the crustacean muscles are similar to those of Type V collagen in vertebrate skeletal muscles (Yoshinaka et al. 1989). As a major

constituent of the connective tissue, collagen supports the structure, locomotion, mechanical strength of the muscles, bones and fin in crustaceans. Based on the content of 4-hydroxyproline in the whole body of shrimp (Table 9.1), the abundance of collagen in the whole body of shrimp appears to be 66% lower than that in vertebrates (Wu 2013).

9.5   Catabolism of Energy Substrates for ATP Production in Crustacean Tissues

The requirement of crustaceans for dietary protein has been reported to be 30–60%, depending on their species, developmental stage, and production conditions (Halver and Hardy 2002; Cuzon et al. 2004; Unnikrishnan and Paulraj 2010; Jin et al. 2013; Mente 2006). However, the rate of retention of dietary nitrogen is only about 17–30%, which is even lower than that for some fish species (Bulbul et al. 2016; Panini et al. 2017; Qiu et al. 2017). In addition, the oxygen:nitrogen ratio (the ratio of oxygen consumed to nitrogen excreted; O/N, mol/mol) is often employed in energetic studies as an indicator for the use of organic substrates (i.e., lipids, carbohydrates or proteins) as metabolic fuels. An oxygen:nitrogen ratio in shrimp is < 40 (Coelho et al. 2019; Comoglio et al. 2004; Zhang et al. 2019), indicating AAs may be their predominant energy substrates. The limited utilization of glucose by penaeid shrimp has been reported in some studies, and the recommended levels of digestible carbohydrates starch in diets are generally less than 20% (Guo et al. 2006). Rosas et al. (2002) have suggested that shrimp (*Litopenaeus vannamei*) are well adapted to dietary protein as a source of energy because of its limited ability to use high carbohydrate. In crabs (*Neohelice granulate*), dietary proteins have been suggested as an important source of energy (Pellegrino et al. 2013). AAs (especially alanine) are important substrates in the gill tissue of the blue crab, and appears to play a role in both short-term cell volume regulation and long-term osmoregulatory processes (Pressley and Graves 1983).

In all animals, individual AAs have their own catabolic pathways because of their different structures (Wu 2013). However, the catabolism of many AAs shares a number of common steps to generate pyruvate, oxaloacetate (OAA), α-Ketoglutarate (α-KG), fumarate, succinyl-CoA, and acetyl-CoA (Fig. 9.2). For example, the carbon backbones of some AAs are converted to α-KG by glutamate dehydrogenase (GDH) and transaminases. Aminotransferases have been reported in the skeletal muscle, gill and hepatopancreas of crabs (*Carcinus maenas;* Chaplin et al. 1967). The catabolism of glutamine involves its deamination by phosphate-activated glutaminase to produce glutamate and ammonia. The major end product of AA metabolism in crustaceans is ammonia, which represents more than 50% of their nitrogenous wastes (Regnault 1987). Free AAs are the second most important nitrogenous waste since they account for 10–25% of the total excreted nitrogen in different species (Regnault 1987). Urea and uric acid are nitrogenous end-products but are usually excreted by crustaceans in small amounts (< 10%).

To generate ATP, the carbon backbone of glutamate, alanine, and aspartate are converted into α-KG, pyruvate, and oxaloacetate by GDH, glutamate-pyruvate transaminase (GPT), and glutamate-oxaloacetate transaminase (GOT), respectively (Wu 2013; Richard et al. 2010; Lu et al. 2015). We found that in both whiteleg shrimp (*Litopenaeus vannamei*) and blue crabs (*Callinectes sapidus*), AAs, such as aspartate, glutamine and glutamate, provide the bulk of energy but the oxidation of glucose for ATP production is very limited in their skeletal muscle and ovaries (Table 9.2 and Fig. 9.3). In both animal species, aspartate is the predominant metabolic fuel among the AAs (Fig. 9.3). Similarly, both GPT and GOT are present in different tissues (hemolymph, hepatopancreas, gills and skeletal muscle) of shrimp (*Fenneropenaeus indicus*), with the activity of GOT being 2–3 times higher than that of GPT in the same tissue (Mohankumar

**Table 9.2** Rates of oxidation of energy substrates in the intestines of whiteleg shrimp and blue crabs[1]

	D-[U-14C] Glucose (5 mM)	[U-14C] Palmitate (2 mM)	L-[U-14C] Glutamate (2 mM)	L-[U-14C] Glutamine (2 mM)	L-[U-14C] Alanine (2 mM)	L-[U-14C] Aspartate (2 mM)	L-[U-14C] Leucine (2 mM)
Oxidation of an energy substrate in tissues of whiteleg shrimp (nmol CO_2/mg tissue per 2 h)							
Midgut (n = 6)	3.89 ± 0.55^d	0.97 ± 0.07^f	7.72 ± 0.27^b	6.44 ± 0.28^c	4.39 ± 0.29^d	12.3 ± 0.44^a	1.71 ± 0.11^e
HP (n = 8)	1.36 ± 0.07^b	0.45 ± 0.01^f	0.82 ± 0.02^d	1.23 ± 0.09^b	0.99 ± 0.04^c	1.83 ± 0.07^a	0.64 ± 0.03^e
Gill (n = 8)	2.52 ± 0.13^a	0.49 ± 0.03^d	1.72 ± 0.09^c	1.94 ± 0.16^{bc}	0.81 ± 0.06^d	2.19 ± 0.11^{ab}	0.31 ± 0.03^e
Skeletal Muscle (n = 8)	0.19 ± 0.02^c	0.02 ± 0.00^e	0.44 ± 0.02^b	0.45 ± 0.02^b	0.09 ± 0.01^d	1.03 ± 0.07^a	0.03 ± 0.00^e
Oxidation of an energy substrate in tissues of blue crabs (nmol CO_2/mg tissue per 2 h)							
Midgut (n = 6)	4.38 ± 0.13^c	7.88 ± 0.36^a	2.21 ± 0.15^d	0.85 ± 0.07^e	0.84 ± 0.06^e	5.77 ± 0.29^b	0.27 ± 0.02^f
HP (n = 12)	1.74 ± 0.11^b	5.75 ± 0.27^a	0.41 ± 0.05^e	0.08 ± 0.01^f	0.90 ± 0.06^d	1.33 ± 0.13^c	0.32 ± 0.04^e
Gill (n = 9)	8.05 ± 0.31^a	5.35 ± 0.38^b	2.13 ± 0.19^c	2.45 ± 0.42^c	1.22 ± 0.10^d	5.00 ± 0.18^b	0.33 ± 0.03^e
Skeletal Muscle (n = 12)	0.04 ± 0.00^f	0.16 ± 0.01^d	1.75 ± 0.18^b	0.10 ± 0.01^e	0.20 ± 0.01^c	2.19 ± 0.16^a	0.06 ± 0.01^f
Ovary (n = 12)	0.08 ± 0.01^{ef}	0.74 ± 0.06^c	2.09 ± 0.18^b	0.06 ± 0.01^f	0.23 ± 0.02^d	5.62 ± 0.33^a	0.10 ± 0.01^e

HP Hepatopancreas

[1]Rates of oxidation of an energy substrate by the 13-g shrimp (*Litopenaeus vannamei*) or the 150-g blue crab (*Callinectes sapidus*) tissue were determined with the use of its [U-14C]-labeled tracer, as described by Jia et al. (2017). A tissue was incubated in 1 ml of oxygenated Krebs-Henseleit bicarbonate buffer (pH 7.4) at 26 °C for 2 h. The incubation medium also contained a mixture of energy substrates (5 mM D-glucose, 2 mM palmitate, 2 mM L-glutamate, 2 mM L-glutamine, 2 mM L-alanine, and 2 mM L-leucine) plus either D-[U-14C]glucose, [U-14C]palmitate, L-[U-14C]glutamate, L-[U-14C]glutamine, L-[U-14C]alanine, L-[U-14C]aspartate, or L-[U-14C]leucine. The specific radioactivity of each tracer was 100 dpm/nmol. At the end of the 2-h incubation, $^{14}CO_2$ produced by a tissue was collected into 0.2 ml of Soluene to calculate the rate of CO_2 production from a substrate. Results were analyzed by one-way analysis of variance and the Student-Newman-Keuls multiple comparison, as described by Assaad et al. (2014)

a–f: Within a row for shrimp or blue crabs, means not sharing the same superscript letter differ ($P < 0.05$)

Fig. 9.3 ATP production from the oxidation of individual substrates in tissues (the midgut, hepatopancreas, gill plus skeletal muscle) of the 15-g whiteleg shrimp *Litopenaeus vannamei* (**Panel A**) and the 150-g swimming crab *Portunus trituberculatus* (**Panel B**). The rates of ATP production were calculated from the data in Table 9.1, as described by Li et al. (2020b).[1] The contribution of an individual substrate to total ATP production in tissues incubated in the presence of a mixture of substrates. *Glc* glucose, *Pa* palmitate, *Glu* glutamate, *Gln* glutamine, *Ala* alanine, *Asp* aspartate, *Leu* leucine
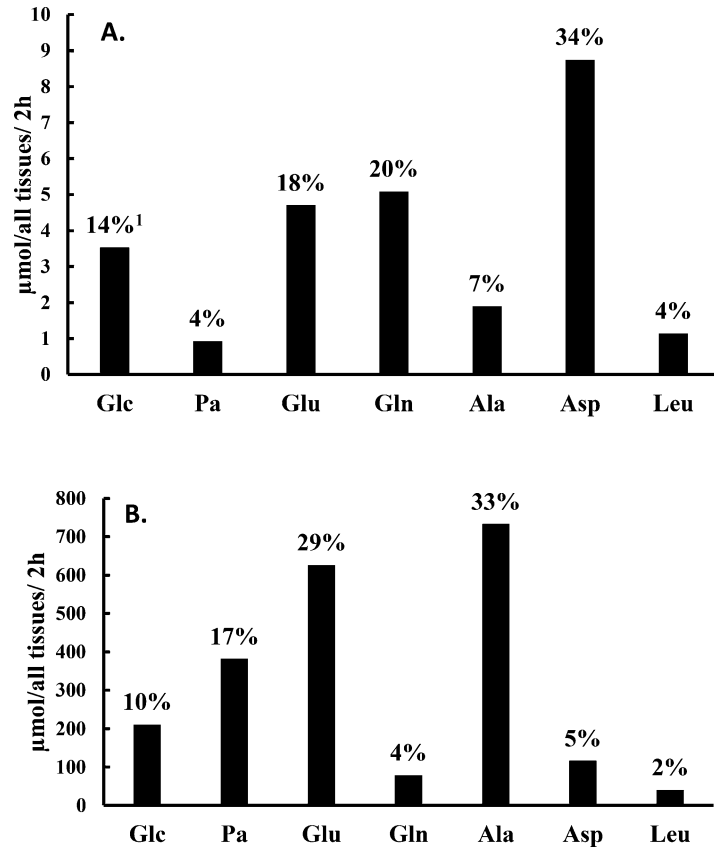
and Ramasamy 2006). GDH is largely responsible for the production of ammonia from AAs in crustaceans (Fernández-Urruzola et al. 2011). In whiteleg shrimp (*Litopenaeus vannamei*, the activity of GDH increases with increasing the dietary protein level from 25% to 50% (Li et al. 2011b). The measurement of GDH activity in the crude homogenates of the shrimp (*Crangon crangon*) suggests that the oxidative deamination of glutamate by GDH may account for all the ammonia excretion by this species (Batrel and Regnault, 1985). GDH transcripts are detected in most tissues of Chinese mitten crabs (*Eriocheir sinensis*; Wang et al. 2012), freshwater prawn (*Macrobrachium rosenbergii*; Chakrapani et al. 2017), whiteleg shrimp (*Litopenaeus vannamei*; Li et al. 2009a), and mud crabs (*Scylla paramamosain*; Lu et al. 2015).

Although AAs are the major energy sources for crustaceans, the rates of their oxidation to CO_2 vary among different tissues and species. For example, the specific activity of GPT in the skeletal muscle and gills of black tiger shrimp (*Penaeus monodon*) is about 3-times the value measured in the hepatopancreas (Richard et al. 2010). The activity of GDH is also relatively low in the hepatopancreas of black tiger shrimp, suggesting a minor role of this tissue in glutamate catabolism (Richard et al. 2010). Likewise, although GDH is expressed in the skeletal muscle, epithelium, eyestalk, hepatopancreas, and gill of Pacific white shrimp, its enzymatic activity in the hepatopancreas is much lower than that in the other four tissues (Li et al. 2009a). Similarly, the rate of CO_2 production from aspartate is 3–6 times higher than that from glucose in the

intestine and skeletal muscle of whiteleg shrimp, but the rate of oxidation of these two substrates is quantitatively comparable in the hepatopancreas (Table 9.2). Of particular note, in blue crabs, palmitate is the primary energy source for the midgut and hepatopancreas, with the rate of its oxidation being substantially higher than that of any AA substrates (Table 9.2). In both whiteleg shrimp and blue crabs, AAs are the most important energy substrates for ATP production in skeletal muscle. Richard et al. (2010) also reported that skeletal muscle has high activities of GPT and GDH for glutamate catabolism in black tiger shrimp.

Phosphate-activated glutaminase may be quantitatively the major enzyme for initiating glutamine catabolism in crustaceans. For example, in a fresh-water crab (*Paratelphusa hydrodrom*us), a high correlation between glutaminase activity and ammonia excretion rate has been observed at various salinity levels (Krishnamoorthy and Srihari, 1973). In whiteleg shrimp, the rate of the oxidation of glutamine is similar to or even higher than that of glutamate in various tissues (Table 9.2). However, the organs (except for the gill) of blue crabs oxidize much more glutamate than glutamine, which may be attributed to the low glutaminase activity. The gill of blue crabs oxidized both glutamate and glutamine at relatively high rates. This is in agreement with a previous report that glutaminase activity is most active in the gills of three crab species, indicating that this organ is an active site of glutamine hydrolysis and glutamate degradation (King et al. 1985). Skeletal muscles of crabs have a high activity of glutamine synthetase and may be the major site for glutamine synthesis in the body (King et al. 1985). Interestingly, the activities of GDH and glutaminase are undetectable or very low in the hepatopancreas of the three crab species studied (King et al. 1985). Similarly, our results indicated that glutamine and other AAs are not the primary energy substrates in the hepatopancreas of blue crabs. To date, our knowledge of AA catabolism in crustaceans is very limited (Table 9.3).

9.6 Glucogenesis and Lipogenesis in Crustaceans

AAs can be the precursors for glucose and lipid syntheses to provide the body with glucose and lipids (Fig. 9.2). Gluconeogenesis and its related key enzymes [e.g., phosphoenolpyruvate carboxykinase (PEPCK)] have been demonstrated in different tissues of crustacean species, such as the skeletal muscle, hepatopancreas, and gill (Reyes-Ramos et al. 2018; Vinagre and Da Silva 2002; Schein et al. 2004). The conversion of ^{14}C-alanine and ^{14}C-glycine into glucose occurred in the hepatopancreas, gill and skeletal muscle of crabs (*Chasmagnathus granulate*; Oliveira et al. 1997; 2004; Vinagre and Da Silva, 2002; Martins et al. 2011). The in vitro experiments also showed that these tissues were able to incorporate ^{14}C-glycine to lipids (Vinagre and Da Silva 2002; Martins et al. 2011). The presence of gluconeogenesis from AAs in the skeletal muscle of crabs is interesting, because such a biochemical pathway is absent from terrestrial mammals and birds (Wu 2018).

Glucose and lipids are important energy sources for crustaceans under certain physiological conditions or stresses (Reyes-Ramos et al. 2018). For example, intramuscular lipids are used for ATP production in crabs in the fall and winter (Kucharski and Da Silva 1991). Dietary AAs are converted into lipids in skeletal muscle when crabs (*N. granulate*) are fed diets with high protein content, and the intramuscular lipids serve as an important energy reserve for the animals during osmoregulation and in the winter (Pellegrino et al. 2013). Moreover, gluconeogenesis and lipogenesis contribute to the adjustment of the intracellular concentration of nitrogenous compounds to withstand changes in the salinity of the surrounding water (Martins et al. 2011). Therefore, both gluconeogenesis and lipogenesis from AAs are important for the growth and health of crustaceans exposed to different levels of salinity. Previous experiments indicated that the incorporation of [^{14}C]alanine into glucose in the jaw muscles of crabs submitted to a

Table 9.3 Nutritional and physiological functions of amino acids and their metabolites in crustaceans

Metabolites	Amino acids	Reported functions in crustaceans	References
NO, polyamines	Arginine	Improves antioxidant and immune systems in shrimps *Fenneropenaeus chinensis* and *Marsupenaeus japonicus*	Jiang et al. (2006)
Phosphoarginine	Arginine	Storage of biological energy, controlling osmoregulation in crustaceans, such as the shrimp (*Litopenaeus vannamei*), the blue crab (*Callinectes sapidus*), and the common littoral crab (*Carcinus maenas*)	Holt and Kinsey (2002) and Kotlyar et al. (2000)
NO, polyamines, taurine, phosphoarginine	Arginine, ornithine, and methionine	Regulation of osmotic and ionic homeostasis in crustances, such as blue crabs (*Callinectes sapidus* Rathbun)	Lovett and Watts (1995)
Nucleotides, ATP	Glutamine, glycine and aspartate	Improves the growth of black tiger shrimp (*Penaeus monodon*)	Do Huu et al. (2012), (2013)
Carnitine, hydroxylysine, taurine, polyamines	Lysine and methionine	Improves immune functions, antioxidant defense systems, and energy metabolism in whilteleg shrimp (*Litopenaeus vannamei*) and in narrow clawed crayfish (*Astacus leptodactylus leptodactylus* Eschscholtz, 1823)	Safari et al. (2015) and Zhou et al. (2017)
Glucosamine, glutamate, ATP	Glutamine	Serves as a substrate for glycoprotein synthesis and as a female signal (i.e. contact sex pheromone) in mate recognition [e.g., in caridean shrimp (*Palaemonetes pugio*)]; improves wound healing, pathogen encapsulation, and maintenance of normal crustacean connective tissues in custaceans	Caskey et al. (2009) and Martin et al. (2003).
Catecholamines, melanin	Phenylalanine and tyrosine	As components of primary stress responses in whilteleg shrimp (*Litopenaeus vannamei*)	Aparicio-Simón et al. (2010)
Glutathione	Cysteine, glutamate and glycine	Improves growth, antioxidant system and stress resistance in whilteleg shrimp (*Litopenaeus vannamei*)	Xia et al. (2018)
Glutathione	Glycine	Improves growth, antioxidant and immune system in whilteleg shrimp (*Litopenaeus vannamei*)	Xie et al. (2014)
Pyrroline-5-carboxylate	Proline	Improves growth, antioxidant system and stress resistance in whilteleg shrimp (*Litopenaeus vannamei*)	Xie et al. (2015a)
GABA, ATP	Glutamate	Improves growth, antioxidant system and stress resistance in whilteleg shrimp (*Litopenaeus vannamei*)	Xie et al. (2015b)
Serotonin, melatonin	Tryptophan	Regulator of growth, reproductive function and agonistic behavior in the black tiger shrimp (*Penaeus monodon*) and the mud crab (*Scylla serrata*)	Wongprasert et al. (2006)and Laranjia et al. (2010)

GABA γ-aminobutyric acid, *NO* nitric oxide

hyperosmotic shock increased by 77% over the control group (Schein et al. 2004). In the posterior gills of *N. granulata* subjected to hyper- and hypo-osmotic stresses, the formation of ^{14}C-lipids from ^{14}C-glycine increased at 72 h after the treatment, but the activity of PEPCK (a rate-controlling enzyme for glucose synthesis) decreased (Martins et al. 2011). Similarly, the rate of lipid synthesis in shrimp exposed to both hypo- or hyper-osmotic conditions was slightly enhanced with an increase in FAS activity, when compared with a normo-osmotic condition (Chen et al. 2014). Thus, the partition of AAs toward the synthesis of either lipids or glucose in crustaceans, depending on nutritional, physiological and environmental factors.

9.7   Syntheses of Bioactive Metabolites in Crustaceans

In addition to the syntheses of proteins, lipids and glucose, AAs are the precursors of many low-molecular-weight substances with important and diverse biological roles in animals (Wu 2013, 2018). These products of AAs include NO, billirubin, carnosine and related dipeptides, carnitine, catecholamines, neurotransmitters, creatine, glucosamine, glutathione, heme, histamine, polyamines (putrescine, spermidine and spermine), purines, and pyrimidines, and are produced in a tissue-specific manner (He and Wu 2020; Wu 2013). Polyamines, which are synthesized from methionine and arginine, play vital roles in chromatin structure, gene transcription and translation, DNA stabilization, signal transduction, cell growth, and proliferation in animals. Polyamines are also involved in the regulation of osmotic and ionic homeostasis by interacting directly with the Na^+, K^+-ATPase enzyme in crabs (Lovett and Watts 1995). GSH is formed from cysteine, glutamate, and glycine via two ATP-dependent enzymes in the cytosol: γ-glutamyl-cysteine synthetase and glutathione synthetase (Wu 2013). Glutathione exerts both growth-promoting and immunostimulatory effects in *Litopenaeus vannamei* (Xia and Wu 2018). L-Phosphoarginine (arginine phosphate), which is generated from arginine and ATP by arginase kinase, exists in skeletal muscles from various invertebrate animals. Of particular note, concentrations of L-phosphoarginine in the skeletal muscles of some crustacean species (e.g., crayfish) can be up to 83 to 100 mM (Ennor et al. 1956; Marcus and Morrison 1964). We found that the concentration of phosphoarginine in the hemolymph of *Litopenaeus vannamei* was about 40 nmol/ml. The main function of phosphoarginine is to store biological energy like phosphocreatine in animals (Wu 2013). Phosphoarginine also plays a role in the metabolic support of the gill's function to regulate osmoregulation in crustaceans (Holt and Kinsey 2002; Kotlyar et al. 2000). However, knowledge about the metabolism and functions of these AA metabolites in most crustacean species is limited.

9.8   Functions of AAs in the Culture of Crustacean Species

9.8.1   Molt and Survival

The growth of crustaceans occurs through the shedding of an old exoskeleton (shell) and the formation of a new exoskeleton, and is greatly influenced by the extended intermolt period (molt frequency) and the molt increment (carapace and body weight growth at molt). Moreover, the survival of some crustacean species is highly dependent on the molting processes. For example, many deaths are due to the presence of calcium deposits embedded on and in the inner surface of the exuvial exoskeleton, which is known as the molt death syndrome (Bowser and Renée 1981; Wang et al. 2016). The molting process is under the control of several regulatory hormones, environmental factors (Hosamani et al. 2017), and diets (Kibria 1993; Millikin 1980). The cumulative molts in crabs are strongly affected by voluntary feed and protein intakes, indicating that AAs are required for tissue growth especially during the postmolt period (Nguyen et al. 2014). AAs have been suggested as important factors for molting processes through energy provision for ecdysis, osmoregulation, collagen synthesis, and the removal of the exoskeleton (Dooley et al. 2002), as well as the regulation of hormone release (Qi et al. 2019). For example, free proline and glycine may be used as metabolic fuels during ecdysis (Claybrook 1983) and substrates for the synthesis of the new exoskeleton in the later premoult (Yamaoka and Skinner 1976). Concentrations of a molt hormone, ecdysterone, are increased in the serum of crab (*Eriocheir sinensis*) receiving dietary supplementation with arginine (Qi et al. 2019). The same species have higher survival rates and molt frequency when fed diets containing adequate lysine and arginine (Jiang et al. 2005; Qi et al. 2019). More details about the functions of AAs in the molting of shrimp are presented in Table 9.4.

Osmoregulation is an essential physiological process for the majority of aquatic crustaceans since many of them have been widely farmed in

Table 9.4 Main functions of amino acids at different stages of the molt cycle in shrimp

	Stage	Duration	Exoskeleton	Feeding	Main functions of AAs
Postmolt	A	1–2 h	Soft exoskeleton	None	Osmoregulation regulation, protein synthesis for tissue growth
	B	2–5 h	Little hardened exoskeleton	Weak	
Intermolt	C	8–10 days	Hard exoskeleton	Maximal	Energy sources; and protein and peptide syntheses
	D0	1–2 days	Epidermis starts apolysis	Maximal	
	D1	1–2 days	No new cuticle	Decrease	
Premolt	D2	2 days	New cuticle appears	Decrease	
	D3	1 day	Interval between the old and the new cuticle	Decrease	Collagen synthesis, osmoregulation regulation, and hormone release
	D4	1 day	Water absorption and old exoskeleton splits	None	
Molt	E	15 min	Old cuticle is shed, body expanded		Energy source and osmoregulation regulation

The molt stage is adapted from (Rao et al. 2008)

inland and oceans with different environmental conditions (Romano and Zeng 2012). As a result, the crustaceans usually are faced with numerous stresses such as low or high salinity, high density, and hot or cold temperatures. Free AAs in the hemolymph appear to play important roles in ATP production (Pressley and Graves 1983). Their levels generally increase in the hemolymph under various stress conditions (Shinji and Wilder 2012). Of particular note, some free AAs (e.g., glutamate, proline, glycine, alanine, taurine and arginine) are known to be involved in the active adjustment of intracellular osmoregulation in marine invertebrates (Tan et al. 1981; Chen and Chen 2000; Liu et al. 2012; 2018; Chakrapani et al. 2017). A recent review has indicated that an increase in protein levels in the diet of *Litopenaeus vannamei* is a practical method of nutritional modulation to increase their production at extreme high and low salinities (Li et al. 2015). After an acute salinity change, the survival of whiteleg shrimp is increased with increasing the dietary glycine level from 2.26% to 2.70% (Xie et al. 2014).

AAs play an important role in controlling osmoregulation in crustaceans because their metabolic enzymes such as transaminase (Koyama et al. 2018), GDH (Lu et al. 2015) and arginine kinase (Holt and Kinsey, 2002; Kotlyar et al. 2000) are regulated by salinity levels. In the abdominal muscle of the kuruma shrimp, the concentrations of alanine and glutamine are elevated in response to increased salinity in association with a decrease in GPT gene expression and an increase in GDH gene expression (Koyama et al. 2018). Acute salinity stress increases GDH expression, as well as the syntheses of glutamate, proline and alanine in the muscle of the Chinese mitten Crab (*Eriocheir Sinensis*) to meet the demand for osmoregulation at hyperosmotic conditions (Wang et al. 2012). Consistent with this finding, a reduction in ^{14}C-alanine oxidation appears to be one of the mechanisms responsible for the increase of the free AA pool in the hepatopancreas of crabs (*Chasmagnathus granulate*; Schein et al. 2005) during hyperosmotic stress. A hyperosmotic stimulus also induces proline synthesis from glutamate in *Tigriopus californicus* (Burton 1991).

Much evidence shows that AAs play a central adaptive role in crustaceans during exposure to cold, starvation and ammonia (Chen et al. 1994, 2000; Zhou et al. 2011). For example, the accumulation of proline and alanine in the hepatopancreas seems to be a common response to cold stress in some invertebrates (Hanzal and Jegorov 1991; Fields et al. 1998; Liu et al. 2018). Increasing the content of proline from 2.02% to 2.6% in low (15%) fishmeal diets improved the tolerance of *Litopenaeus vannamei* to ammonia stress (Xie et al. 2015a, b). Moreover, shrimp fed diets with a deficiency of lysine had the greatest incidence and severity of neural lesions when they were challenged with subsequent stress exercises

(Katzen et al. 1984). Clearly, it is imperative to study the functions of specific AAs in crustaceans exposed to different stresses.

9.8.2   Growth and skeletal muscle development

AAs have been traditionally classified as essential (EAAs) or nonessential (NEAAs) for animals, including crustaceans. The diets of crustacean species must contain ten EAAs for survival and growth: arginine, methionine, valine, threonine, isoleucine, leucine, lysine, histidine, phenylalanine, and tryptophan, all of which are not synthesized de novo by eukaryotic cells (NRC, 2011). These AAs are considered as limiting nutrients in commercial feed formulas and are indispensable for the growth, development and survival of the animals. If one of the EAAs is deficient, it will limit the use of all AAs for intracellular protein synthesis, therefore increasing their oxidation to CO_2. For example, a low rate of retention of dietary protein in the *Litopenaeus vannamei* results from a deficiency of lysine (Xie et al. 2012) or threonine (Zhou et al. 2013) in their diets. Purified or semi-purified diets have been employed to determine both qualitative and quantitative requirements of crustaceans for dietary EAAs. Lysine, arginine, and methionine are regarded as the most limiting factors for whole-body growth. Most of these studies were based on the growth performance of select crustaceans as shown in Table 9.5. To date, NEAAs have been recommended to be included in the diets of all animals (Wu 2013). This revises the classical "ideal protein" concept to formulate balanced diets for improving protein accretion, feed efficiency, and health in animals (Wu 2018). A recent study indicated that weight gains and specific growth rates were increased in juvenile Pacific white shrimp receiving dietary supplementation with glycine (Xie et al. 2014). Many factors, such as feeding regime, stocking density, water quality, and other rearing conditions, may affect the requirements of aquatic organisms for dietary AAs (Façanha et al. 2016; Zhang et al. 2018).

AAs can promote muscle development and protein synthesis by either providing the building blocks or stimulating signaling pathways. In mammals, dietary supplements with branched-chain amino acid (BCAAs) alone elicits an anabolic response (e.g., muscle protein synthesis; Wolfe 2017; Wu 2013). An evolutionarily conserved protein kinase, mechanistic target of rapamycin (mTOR), is the master regulator of protein synthesis and cytoskeleton remodeling, as well as intracellular protein degradation via autophagy (Wu 2013). AAs, such as leucine, arginine, glutamine, glycine, tryptophan and valine, activate the mTOR cell signaling to initiate protein synthesis in skeletal muscle and intestine (Li et al. 2011c; Wu 2018). The mTOR plays an important role in the regulation of growth, molting, cell differentiation, and nutrient metabolism in crustacean species (Abuhagr et al. 2014; Shyamal et al. 2018; Wu et al. 2019). In the Chinese white shrimp (*Fenneropenaeus chinensis*), intraperitoneal administration of leucine and arginine stimulated the expression of fch-TOR and activated the mTOR signaling pathway in skeletal muscle (Sun et al. 2015a). Functional AAs are expected to enhance the growth, survival, and productivity of crustaceans, as reported for terrestrial mammals and birds (Wu 2018).

9.8.3   Release of Hormones

Similar to terrestrial animals, hormones in crustaceans are messengers that help to regulate their physiological states and functions, such as temperature, satiety, nutrient and energy metabolism, growth, development, and reproduction. For example, AAs regulate muscle growth not only through direct actions on myogenic regulatory factors and mTOR signaling, but also indirectly via the growth hormone/insulin-like growth factor (IGF) axis. Growth hormone in serum and the expression of IGF2 in the hepatopancreas of the Chinese mitten crab *(Eriocheir sinensis)* were significantly enhanced by dietary supplementation with arginine (Qi et al. 2019). In addition, the concentrations of insulin and neuropeptide Y in the blood of *Litopenaeus vannamei* were increased in response to dietary supplementation

Table 9.5 Reported requirements of crustacean species for dietary lysine, arginine and methionine

Species	Initial body weight	Dietary crude protein, % (sources)	Isonitrogenous control	Requirement (% of the diet, model)	Variables	References
Lysine requirements						
Whiteleg shrimp (*Litopenaeus vannamei*)	0.52 g	40 (FM and WGM)	Asp/Gly (1:1)	1.64 (BL)	SGR	Xie et al. (2012)
	3.62 g	38 (FM and CGM)	Arg[a]	2.11 (Anova)	WG, SGR	Feng et al. (2013)
Giant tiger prawn (*Penaeus monodon*)	21 mg	40 (casein and gelatin)	Asp and Glu	2.08 (BL)	WG	Millamena et al. (1998)
Atlantic ditch shrimp (*Palaemonetes varians*)	17 mg	45 (FM and WGM)	AAs mix[b]	2.42 (BL), 2.63 (EX)	WG	Palma et al. (2015)
Swimming crab (*Portunus trituberculatus*)	7.86 g	50 (FM and SBM)	Asp/Gly (1:1)	2.17 (BL)	SGR	Jin et al. (2015a)
Chinese mitten crab (*Eriocheir sinensis*)	2.05 g	38 (casein, FM, SBM)	Glu	2.34 (Qua)	WG	Ye et al. (2010)
	6.86 mg	60 (casein and gelatin)	AA mix[c]	2.55	ML, survival	Jiang et al. (2005)
Arginine requirements						
Whiteleg shrimp (*Litopenaeus vannamei*)	0.5 g	41 (FM and WGM)	Asp/Gly (1:1)	1.96 (BL)	SGR	Zhou et al. (2012)
	3.62 g	38 (FM and CGM)	Lys[a]	1.80 (ANOVA)	WG, SGR	Feng et al. (2013)
Giant tiger prawn (*Penaeus monodon*)	21 mg	35 (casein and gelatin)	Asp and Glu	1.85 (BL)	WG	Millamena et al. (1998)
	1.19 g	45 (casein)	Casein	2.5 (BL)	WG	Chen et al. (1992)
Kuruma shrimp (*Marsupenaeus japonicus*)	0.25 g	50 (casein and gelatin)	Glu	2.66 (BL)	WG	Alam et al. (2004a)
Atlantic ditch shrimp (*Palaemonetes varians*)	17 mg	45 (FM and WGM)	AAs mix[b]	2.05 (BL), 2.39 (EX)	WG	Palma et al. (2015)
Chinese mitten crab (*Eriocheir sinensis*)	2.03 g	38 (casein, FM, SBM)	Glu	3.62 (Qua)	WG	Ye et al. (2010)
	6.86 mg	60 (casein and gelatin)	AA mix[c]	2.0 (Anova)	ML, survival	Jiang et al. (2005)
Swimming crab (*Portunus trituberculatus*)	4.72 g	50 (FM and SBM)	Asp/Gly (1:1)	2.77 (BL)	SGR	Jin et al. (2016)
Methionine requirements						
Whiteleg shrimp (*Litopenaeus vannamei*)	0.55 g	40 (FM, SBM)	Asp/Gly (1:1)	0.91 (Qua)	WG	Lin et al. (2015)

(continued)

Table 9.5 (continued)

Species	Initial body weight	Dietary crude protein, % (sources)	Isonitrogenous control	Requirement (% of the diet, model)	Variables	References
	4.18 g	38 (FM, SBM)	Asp/Gly (1:1)	0.67 (Qua)	WG	Lin et al. (2015)
	9.77 g	34 (FM, SBM)	Asp/Gly (1:1)	0.66 (BL)	WG	Lin et al. (2015)
Atlantic ditch shrimp (*Palaemonetes varians*)	17 mg	45 (FM and WGM)	AAs mix[b]	0.96 (BL), 0.99 (EX)	WG	Palma et al. (2015)
Chinese mitten crab (*Eriocheir sinensis*)	2.05 g	38 (Casein, FM, SBM)	Glu	1.12 (Qua)	WG	Ye et al. (2010)
Giant tiger prawn (*Penaeus monodon*)	21 mg	37 (casein and gelatin)	Asp and Glu	0.89 (BL)	WG	Millamena et al. (1996)
Swimming crab (*Portunus trituberculatus*)	11.3 g	50 (FM and SBM)	Asp/Gly (1:1)	0.96 (BL)	SGR	Jin et al. (2015b)

Regression model: *BL* broken line, *Qua* quadratic, *EX* exponential
Parameters: *IGR* instantaneous growth coefficient, *WG* weight gain, *SGR* specific growth rate, *ML* Molt
Protein sources: *FM* fishmeal, *SBM* soybean meal, *SPC* soybean protein concentrate, *WGM* wheat gluten meal, *CGM* corn gluten meal
[a]The study is about the optimal ratio and content of lysine to arginine in diet for shrimp
[b]Premix of amino acids (g/100 g): cystine, 5; tryptophan, 3; threonine, 11; isoleucine, 9; histidine, 12; valine, 12; leucine, 15; phenylalanine, 20; tyrosine, 13
[c]Amino acid mixture (g/100 g): leucine, 8.53; isoleucine, 5.01; lysine, 7.06; methionine, 1.27; phenylalanine, 5.17; threonine, 5.21; tryptophan, 2.76; valine, 2.37; histidine, 2.29; aspartic acid, 11.31; serine, 4.74; glutamic acid, 16.74; proline, 5.62; glycine, 7.25; alanine, 7.07; tyrosine, 6.96; cysteine, 0.63

with GABA (Xie et al. 2015b). Tryptophan is the precursor of the monoaminergic neurotransmitter serotonin (5-hydroxytryptamine). In mud crabs, tryptophan supplementation contributed to a significant increase of serotonin in the hemolymph, thus suppressing the agonistic behavior of mud crabs during aggressive encounters and improving their survival (Laranjia et al. 2010). In the Chinese mitten crab (*Eriocheir sinensis*), dietary supplementation of tryptophan can promote limb regeneration by regulating regeneration-related gene expression and the digestion of foods within the hepatopancreas, which may be related to the enhanced levels of melatonin and the binding of serotonin and dopamine to their corresponding receptors (Zhang et al. 2019). In the juvenile *Litopenaeus vannamei*, dietary supplementation with tryptophan was beneficial to improve its growth performance possibly by mediating serotonin and GABA signaling pathways (Sun et al. 2015b).

9.8.4   Immune and Antioxidant Responses

Proper nutrition is critical not only to achieve optimal growth rates but also to maintain the health of cultured aquatic animals (Pohlenz and Gatlin 2014). AAs are essential components of the cells and tissues of the immune system, and play a vital role in the immunity of mammals, fish and crustacean species (Trichet 2010; Li et al. 2007). Like other invertebrates, crustaceans lack adaptive immune systems and depend solely on the innate immune system to defend against infectious pathogens (Vazquez et al. 2009). The prophenoloxidase activating system (the proPO-system) and associated factors are important mediators of immunity in crustaceans. The proPO is activated by substances of microbial origins (e.g., β-1,3-glucans, lipopolysaccharides, and peptidoglycans) to stimulate the circulating

hemocytes (large granular hemocytes, small granular hemocytes, and hyaline cells). These cells play important roles not only through direct sequestration and killing of infectious agents but also by synthesis and exocytosis of a battery of bioactive molecules (Söderhäll and Cerenius 1992). Along with hemocytes, crustaceans possess plasma proteins or humoral factors, such as lectin, α-2 macroglobulin responsible for clotting, lipopolysaccharide-binding protein, β-glucan-binding protein, antimicrobial peptides, and lysosomes (Trichet, 2010; Vazquez et al. 2009). As the nitrogenous precursor for NO, arginine has a beneficial effect on tissue oxygenation and immune function for animals (Wu et al. 2009), including crustaceans (Qi et al. 2019; Zhu et al. 2009). Thus, increasing the dietary arginine content from 1.72% to 3.72% improved the growth, feed efficiency survival, immunity, and disease resistance to *Aeromonas hydrophila* in the juvenile Chinese mitten crab (Qi et al. 2019).

Similarly, dietary supplementation with tryptophan to Chinese mitten crabs increases their survival after a challenge with pathogens (Yang et al. 2019).

Reactive oxygen species (ROS) are highly reactive molecules that may contribute to radiation-induced cytotoxicity (e.g., chromosome aberrations, protein oxidation, and muscle injury), as well as metabolic and morphologic changes (e.g., increased muscle proteolysis and dysfunction of the central nervous system) in animals (Fang et al. 2002). Endogenous antioxidant defenses are crucial for the control of ROS production and the prevention of oxidative damage in cells. The principal defense systems against oxygen free radicals are superoxide dismutase, glutathione, glutathione peroxidases, glutathione reductase, catalase (a heme-containing enzyme), and antioxidant nutrients (Fig. 9.4). AAs and their derivatives are important antioxidant nutrients for crustacean species, as for
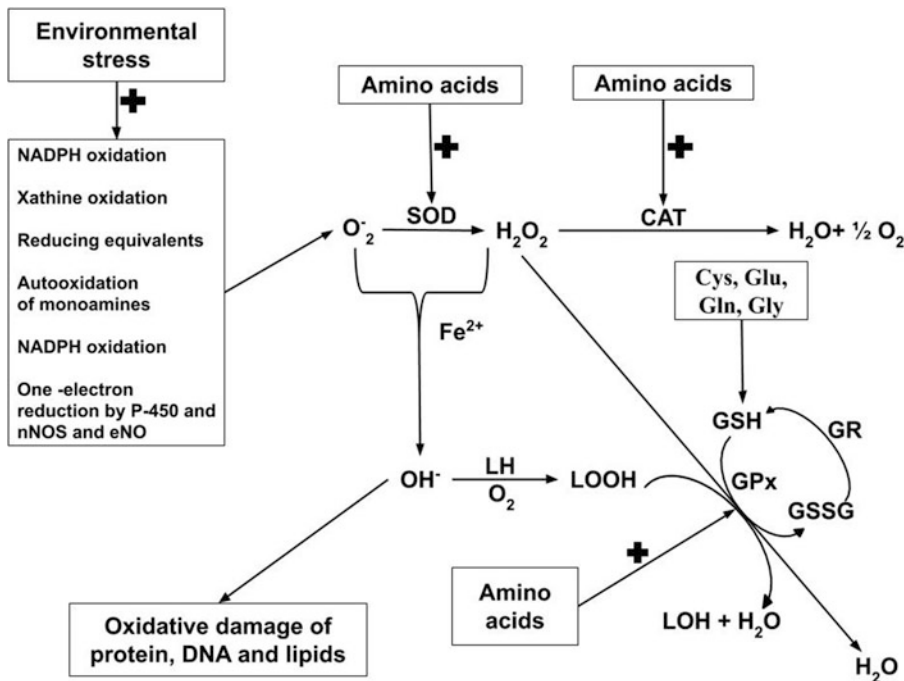

Fig. 9.4 Roles of amino acids and their metabolites as antioxidants in crustaceans. *Cys* cystine, *CAT* catalase, *Gln* glutamine, *Glu* glutamate, *Gly* glycine, *GPx* glutathione peroxidase, *GR* glutathione reductase, *GSH* glutathione, *GSSG* oxidized glutathione, *Lys* lysine, *Leu* leucine, *LH* lipids (unsaturated fatty acids), *LOOH* lipid hydroperoxide, *Met* methionine, *SOD* superoxide dismutase. (Adapted from Fang et al. 2002)

terrestrial animals (Fang et al. 2002). For example, glutathione, which is the most abundant thiol-containing substance of low molecular weight in cells, is synthesized from glutamate, cysteine, and glycine. Dietary supplementation with glutathione to *Litopenaeus vannamei* enhances immunity and antioxidant defenses (Xia and Wu 2018). Glycine supplementation also improves the resistance of the shrimp to acute salinity challenge (Xie et al. 2014). More details about the functions of AAs and their derivatives in immunity and antioxidant responses are summarized in Table 9.6. Adequate AA nutrition plays a crucial role in protecting crustaceans from infectious and metabolic diseases, such as the white spot syndrome caused by viral infection (Corteel 2013), bacterial infection (Zhang et al. 2018), and oxidant-induced tissue damage (Dong et al. 2018; Li et al. 2020a,c).

9.8.5 Spawning and Larval Development in Crustaceans

Most crustaceans have separate sexes. The weight of the gonad of maturing shrimp or crabs increase during their reproductive development, which prepares sufficient nutrients needed for the formation of egg yolk or spermatogenesis. This process is important to sustain the normal development of the embryos and the production of pre-feeding larvae in crustacean species (Islam et al. 2010). Optimum development of ovaries is necessary for maximum crab production as they are a popular edible tissue (Wu et al. 2020). Vitellogenesis is the process of yolk formation, which plays the central role in ovarian development and reproduction (Subramoniam 2011). Vitellogenin is an egg yolk precursor protein and is synthesized in the hepatopancreas and gonad tissues in decapod crustaceans (Tsukimura 2001). Its synthesis is under the control of estradiol-17β and other neuropeptidic precursors from the nervous system (Fig. 9.5). Furthermore, the hepatopancreas is an important site for the syntheses of vitellogenin and sex steroid hormones. Therefore, the crustacean hepatopancreas is crucial for maximum growth and optimum maturation of ovaries. An unbalanced or incomplete diet causes poor reproductive performance or may even stop animals from reproducing (Woulters et al. 2001). As noted previously, the release of some hormones can be influenced by dietary AA intake. By augmenting the syntheses of egg yolk proteins, hormone peptides and enzymes during maturation and reproduction, AAs are also essential to ovarian development. Indeed, we found that AAs, particularly aspartate and glutamate, are important metabolic fuels in the ovaries of blue crabs (Table 9.2). Thus, increasing dietary provision of AAs (particularly aspartate and glutamate) may beneficially improve reproduction in crustaceans.

Protein and AAs are the main components of dry matter in invertebrate eggs, and support embryonic survival, growth and development (Heras et al. 2000; Xu et al. 2013). Moreover, broodstock nutrition can significantly affect the biochemical profiles of embryos and, therefore,

Table 9.6 Primary roles of amino acids during the life cycle of shrimp and crab species

Life cycle			
Shrimp	Crab	Feeding	The main functions of AAs
Fertilized egg	Fertilized egg	–	Improves the quality of fertilized eggs
Nauplius	–	Yolk reserves	Improves survival and development
Zoae	Zoae	Microalgae	
Mysis	Megalopa	Algae and zooplankton	
Post-larvae	–	Zooplankton and micro-diets	
Juvenile	Juvenile	Pellet diets	Improves survival and growth
Adult	Adult	Pellet diets	Improves the development of gonads and sperm; enhances egg production

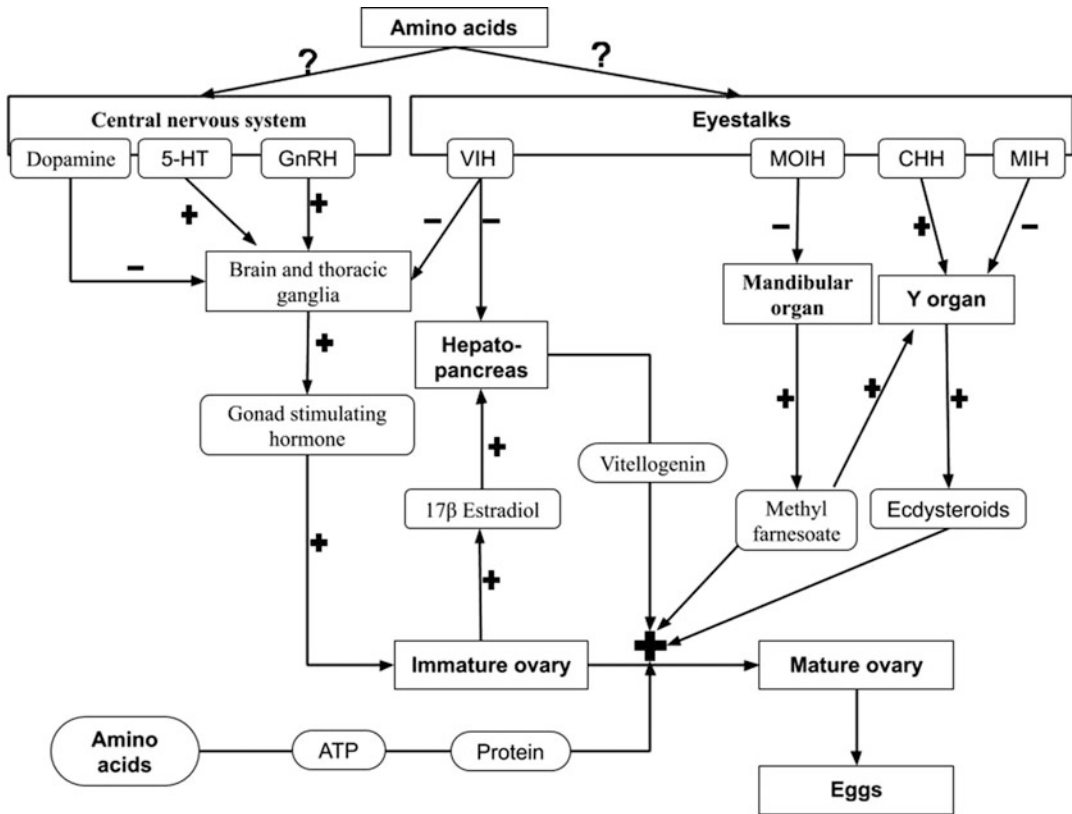The life cycle is adapted from Tuan (2016) and Mcleady et al. (2015)

Fig. 9.5 Roles of internal and external factors in the regulation of reproduction in female crustaceans. *DA* Dopamine, *5-HT* 5-hydroxytryptamine (serotonin), *VIH* vitellogenesis-inhibiting hormone, *MOIH* Mandibular organ-inhibiting hormone, *CHH* crustacean hyperglycemic hormone, *MIH* molt-inhibiting hormone, *B & TG* brain and thoracic ganglia, *GOSH* gonad stimulating hormone, *MO* mandibular organ, *MF* methyl farnesoate, *E2*, 17β-estradiol, *Vg* vitellogenin '+' and '-' denotes activation and inhibition, respectively. (Adapted from Pamuru (2019) and Subramoniam (2011), (2017))

embryogenesis, the quality of larvae and post-larvae (Calado et al. 2005; Harrison 1990). Shrimp (*Litopenaeus setiferus*) fed a 35%-protein diet had a lower sperm quality than shrimp fed a 45%-protein diet, indicating that dietary AAs are important for its reproductive performance (Gonimier et al. 2006). There are suggestions that a deficiency of dietary protein or certain AAs can induce *Daphnia pulex* to enter a resting, non-reproductive state (Koch et al. 2009, 2011). Arginine and histidine can enhance not only the number of eggs, but also the development of subitaneous eggs in *Daphnia pulex* (Fink et al. 2011).

Crabs and shrimp must initiate exogenous feeding after yolk nutrients are no longer sufficient to support the metabolic demand of their larvae. The diet for the larvae relies on either live food (algae and zooplankton) or artificial micro-diets, depending on the life stage. Free AAs are important for the metamorphosis of crustacean larvae by providing them with energy, enhancing protein synthesis in their tissues, and promoting their rapid growth (Bahabadi et al. 2018; Rønnestad et al. 2000). For example, feeding *Litopenaeus vannamei* larvae taurine-enriched rotifers improved their survival and development (Jusadi et al. 2011). Likewise, the enrichment of *Artemia* with lysine increased the survival, growth performance, and stress resistance capacity of *Litopenaeus vannamei* post-larvae (Bahabadi et al. 2018).

9.9 Conclusion and Perspectives

Both EAAs and NEAAs play vital roles in the production of aquatic crustacean species. AAs are substrates for ATP production, as well as the syntheses of lipids, glucose, protein and other bioactive molecules (e.g., NO, creatine, polyamines, GABA, catecholamines, and glutathione). In addition, AAs increase the ability of crustaceans to resist various adverse factors (such as hyperosmotic, ammonia, hot and cold stresses), improve their immune and antioxidant defense systems, and regulate their hormone release, metabolic pathways and osmotic homeostasis. Thus, dietary AAs are vital to the growth, development, reproduction, health, and survival of these aquatic animals. Dietary protein and AAs may also play important roles in spawning and larval development, although traditional studies have focused on the nutrition of lipids.

Based on the recent advances in our understanding of AA metabolism and nutrition in shrimp and crabs, considerations should be given on the use of crystalline AAs (particularly aspartate, glutamate, glutamine, leucine, and glycine) and their alternative sources to feed crustaceans for enhancing their survival and productivity (Huo et al. 2017; Wu 2018). At present, there are several technical difficulties and challenges in the use of crystalline AAs to formulate diets for crustacean species. We would also like to propose solutions to solve the problems.

First, although heating can increase the digestibility of native proteins in plants by unfolding the polypeptide chains and removing the intrinsic protease inhibitors, overheated meals or feeds are undesirable because they have reduced biological values in animals (Wu 2018). The Maillard reaction during the feedstuff heating process damages protein and AAs, leading to reductions in the digestibility of dietary protein and the bioavailability of AAs in feeds (Deng et al. 2005). Animal-source feedstuffs, which contain large amounts and proper balances of AAs (Li and Wu 2020a), can be used as the major source of dietary AAs to reduce the inclusion of plant-source ingredients and fishmeal in the diets of crustaceans.

Second, leaching can lead to the loss of nutrients (including protein and AAs) from the diets fed to crustacean species such as shrimp and crabs, particularly because most of them are slow and continuous eaters. These animals can pick up a feed pellet, cradle it with their maxillipeds (an appendage modified for feeding in crustaceans that is situated in pairs behind the maxillae), and begin to tear and crush the end of a pellet with their mandibles (Obaldo et al. 2006). Therefore, nutritional studies with shrimp and crabs have met with the difficulties of enhancing feeding efficiencies due to the leaching of nutrients before feed pellets are consumed by the animals. If crustaceans are fed an experimental diet with a high leaching rate, their estimated requirements for dietary AAs may be inaccurate. To optimize the utilization of crystalline AAs, a possible approach is to coat AAs with lipids (Alam et al. 2004b; Gu et al. 2013).

Third, crystalline AAs in diets enter the systemic circulation of crustaceans more rapidly than the protein-bound AAs, possibly resulting in the asynchronous absorption of dietary AAs and a suboptimal efficiency of utilization of dietary AAs (Lovell 1991; Guo et al. 2020). For example, there are higher percentages of AAs lost in the urine (e.g., 13.6% for His; 17.6% for phenylalanine; and 8–10% for isoleucine, leucine, lysine and valine) when shrimp fed diets with crystalline AAs in comparison with diets with proteins (Liou et al. 2005). Similarly, a previous study showed that shrimp fed diets with coated crystalline methionine grew more rapidly than those fed diets with uncoated crystalline methionine (Chi et al. 2011). Therefore, it is necessary to systematically evaluate the efficiency of utilization of different free AAs (either coated vs crystalline) to define an appropriate replacement level of protein-bound AAs by crystalline AAs. Some studies with pigs (Gahl et al. 1994) and rainbow trout (Tran et al. 2007) demonstrated that the efficiencies of utilization of supplemented crystalline AAs varied with diets, depending on protein sources especially at suboptimal dietary levels of AAs. This

means that AAs with the same quantity and quality may yield different effects on the growth of animals when they are supplemented to diets with various feedstuff ingredients.

Fourth, there are no standardized diets or AAs as isonitrogenous controls for nutritional research in crustaceans. Due to the inadequate understanding of NEAAs in the past decades, glutamate, glycine and aspartate have long been used as an isonitrogenous control in nutritional experiments. This is inappropriate based on recent studies with terrestrial animals (Hou and Wu 2018; Wu 2018), fish (Li et al. 2020a), and crustaceans (Xie et al. 2014, Xie et al. 2015a, b) indicating that these AAs have nutritional or physiological effects in the animals. We suggest that L-alanine be used as the isonitrogenous control in nutritional studies with crustaceans where it is not a test AA.

Fifth, there is limited knowledge about the cell- and tissue-specific metabolism of AAs in different aquatic crustaceans (e.g. crabs and shrimp). For example, GPT and GOT are abundant in both the mitochondria and the cytoplasm of hepatocytes of many animal species (Wu 2013). Thus, the activities of these two enzymes in serum are often determined to assess hepatic integrity in human medicine. Similarly, both enzymes in the hemolymph of giant tiger prawn and Pacific white shrimp have been regarded as important indicators of the hepatopancreatic injury (Pan et al. 2003; Liu et al. 2019). This, however, it may be not valid for all species of shrimp and crabs. For example, the activity of GPT and GDH in the hepatopancreas of black tiger shrimp (*Penaeus monodon*) is either very low or undetectable (Richard et al. 2010). Furthermore, in blue crabs, the hepatopancreas is not a main site for the catabolism of AAs (Table 9.2).

Finally, although there has been active research to determine the dietary requirements of crustaceans for crude protein over the past 50 years (Table 9.5), much emphasis should be directed to studies of the dietary requirements of these animals for NEAAs. Nutritionists should move away from the traditional concept of crude protein toward all AAs with nutritional and physiological functions in the animals. The composition of AAs in the diets with various protein sources for crustaceans may differ substantially even though the diets have the same crude-protein level. Dietary requirements of crustaceans for all AAs (including AAs that are synthesized in animal cells, such as glutamate, glutamine and glycine) should be defined to optimize dietary formulations for both health and growth performance. Research on the metabolism and functions of AAs is fundamental to achieve this goal so as to manufacture future environment-friendly aquafeeds and reduce feed costs in crustacean production. The new nutritional concepts of "dietary requirements of animals for NEAAs" and "functional AAs", which were originally proposed on the basis of basic and applied studies with terrestrial animals (Wu 2010), are expected to transform nutritional studies with shrimp and crabs, as well as feeding practices in the global crustacean production (Xie et al. 2014, 2015a,b).

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