4

Dietary Energy Utilization and Metabolic Integration

Nutrients are required by animals to sustain life processes and allow activity, growth, and reproduction. Nutrients serve as precursors for the biosynthesis of structural or storage molecules, enzymes, metabolic intermediates, and a plethora of other molecules. A proportion of the nutrients consumed is catabolized to harness chemical (free) energy, which is required for use in anabolic and other life-sustaining processes (Blaxter, 1989; Mayes, 2000). Animals do not simply metabolize energy *per se*. Instead they metabolize specific nutrients, each with specific roles and metabolic fates (Van Milgen, 2002). Several nutrients or metabolic intermediates derived from nutrients are used simultaneously in the same process, and interactions between nutrients are numerous. A large number of endogenous (genetics, sex, physiological state, nutritional history, etc.) and exogenous (temperature, stressors) factors also affect the fate of nutrients in animals (Blaxter, 1989). To quantitatively examine the utilization of all dietary components in a detailed and integrative fashion is highly desirable. However, it also is an extremely complex undertaking. Numerous frameworks have been developed to describe and predict the utilization of dietary nutrients by animals in a practical fashion (Dumas et al., 2008). Bioenergetics or biochemical thermodynamics, the study of the energy changes accompanying biochemical reactions in biological systems (Patton, 1965; Mayes, 2000), has been the foundation of several of the more popular frameworks. Life processes (e.g., anabolic reactions, muscular contraction, active transport) obtain energy by chemical linkage with some energy being transferred to synthetic reaction and some energy lost as heat. According to the first law of thermodynamics, the partition of energy-yielding components between catabolism as fuels and anabolism as storage in tissues can be tracked by the study of the balance between dietary energy intake and expenditure.

Ege and Krogh (1914) were possibly the first to apply the principles of bioenergetics to fish. Since then, hundreds of reports on studies of energy utilization and expenditure for a range of species of fish have been produced. Numerous reviews have also been written on nutritional energetics (bioenergetics applied in a nutritional context), including those of Phillips (1972), Brett and Groves (1979), Cho et al. (1982), Elliott (1982), Cho and Kaushik (1985, 1990), Tytler and Calow (1985), Smith (1989), Jobling (1994), Kaushik and Médale (1994), Cho and Bureau (1995), Cui and Xie (1999), Médale and Guillaume (1999), and Bureau et al. (2002).

Nutritional energetic frameworks have progressively evolved over the past five decades to include some considerations for the types of macronutrients consumed and/or body tissue components deposited (e.g., body protein and body lipids) or more or less explicit representations of the digestion of feed components, metabolism of absorbed nutrients, and partition of nutrients among tissues and functions within the animal (Kielanowski 1965; Baldwin and Bywater, 1984; Emmans and Fisher, 1986; Emmans, 1994; Noblet et al., 1994; DeLange, 1997; Lupatsch et al., 1998, 2003; Birkett and de Lange, 2001; Van Milgen, 2002). This chapter's objective is to present key principles of nutritional energetics and their underlying metabolic and physiological mechanisms, and review estimates of energy expenditure in different fish and shrimp species. This chapter also aims to identify the gaps in knowledge, highlights some of the limitations of common nutritional energetics frameworks, and fosters a reflection about the need for aquaculture nutritionists to examine growth and nutrient utilizations in a more explicit, mechanistic and integrative fashion in the future.

STANDARD ENERGY PARTITIONING SCHEME—NRC 1981 NOMENCLATURE

Different systems of nomenclatures that describe the partitioning of energy in animals have been used. This is especially apparent in fish biology where the nomenclatures and modes of expression of energy transaction used are extremely diverse. In 1981, a subcommittee of the Committee on Animal Nutrition of the National Research Council was appointed to develop a systematic terminology for descrip-

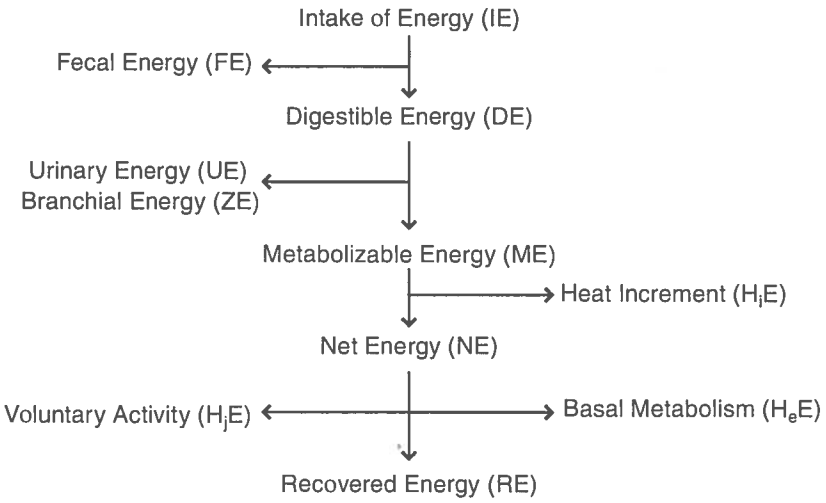

FIGURE 4-1 Schematic representation of the energy flow through an animal (NRC, 1981).

tion of energy utilization by domestic animals, including fish (NRC, 1981). This system is presented schematically in Figure 4-1 and has been widely adopted by animal nutritionists around the world, sometimes with some modifications. This pragmatic nomenclature (Table 4-1) has also been adopted by a number of fish nutrition researchers. This nomenclature was adopted in this document with minor modifications and additions.

Classically, animal nutritionists have expressed all measurements of energy transactions in terms of calories. The calorie used in nutrition is termed the 15°C calorie (cal), which is the energy required to raise the temperature of 1 g water from 14.5 to 15.5°C. One thousand calories is a kilocalorie (kcal). The kcal is a common unit of expressing dietary energy in animal nutrition. The joule (J) was adopted in the Système International des Unités (SIU, International System of Units) as the unit for expression of electrical, mechanical, and chemical energy. One J is defined as 1 kg m^2/sec^2 or 10^7 erg. One 15°C cal is equivalent to 4.184 J. The joule, like most other SIU units, has gained in popularity as the unit for expressing dietary energy in scientific literature. However, it is the National Research Council (NRC) policy to use the kcal as the unit of reference in nutritional energetics. Values in this document are presented in both units where feasible and practical.

GROSS ENERGY AND INTAKE OF ENERGY

Gross energy (GE) is the commonly used term for enthalpy (ΔH) of combustion in nutrition. However, as opposed to enthalpy, GE is generally represented by a + sign (Mayes, 2000). GE content of a substance is usually measured by its combustion in a heavily walled metal container (bomb) under an atmosphere of compressed oxygen. The method of determination is referred to as bomb calorimetry. Under these conditions, the carbon and hydrogen are fully oxidized to carbon dioxide and water, as they are in vivo. However, the nitrogen is converted to oxides, which is not the case in vivo. The oxides of nitrogen interact with water to produce strong acids, an endergonic reaction. These acids can be estimated by titration, allowing a correction to be applied for the difference between combustion in an atmosphere of oxygen and catabolism in vivo (Blaxter, 1989).

The GE content of an ingredient or a compounded diet

TABLE 4-1 Terminology of Types of Dietary Energy and Energy Budget Components

Dietary Energy Types	Abbreviation
Gross energy	GE
Digestible energy	DE
Metabolizable energy	ME
Net energy	NE
Energy Budget Components/Terms	
Intake of energy	IE
Fecal energy losses	FE
Digestible energy intake	DEI
Urinary and branchial (nonfecal) energy losses	UE + ZE
Metabolizable energy intake	MEI
Surface losses	SE
Heat losses (heat production)	HE
Basal metabolism	H_eE
Fasting heat losses	HE_f
Maintenance energy	HE_m
Voluntary activity energy losses	H_jE
Heat increment of feeding	H_iE
Heat of digestion and absorption processes	H_dE
Heat of formation and excretion of metabolic wastes	H_wE
Heat of transformation and retention of substrates	H_rE
Recovered energy	RE

depends upon its chemical composition. The mean values of GE of carbohydrates, proteins, and lipids are on average 4.11, 5.64, and 9.44 kcal/g (17.2, 23.6, and 39.5 kJ/g), respectively (Blaxter, 1989). Intake of energy (IE) is the notation adopted by NRC (1981) for the intake of GE of an animal (Figure 4-1). Intake of energy is simply the product of feed consumption (g) and GE (kcal/g).

FECAL ENERGY LOSSES—DIGESTIBLE ENERGY

Before the feed components can serve as fuels for animals, they must be digested and absorbed. Some feed components resist digestion and pass through the digestive tract to be voided as fecal material. Egestion (excretion as feces) of components containing GE is referred to as fecal energy losses (FE). The difference between the GE and FE of a unit quantity of this diet is termed the digestible energy (DE). Digestible energy intake (DEI) was adopted by NRC (1981) to represent intake of digestible energy, the product of feed intake (g/fish) and DE (kcal/g) of the feed or IE minus FE (Figure 4-1, Table 4-1).

Variation in the digestibility of dietary components is generally a major factor affecting the variation in their usefulness as energy sources to the animal. The FE often represents about 15–30% of IE for fish and shrimp fed practical diets and is a significant loss of energy. The DE values are better estimates of levels of "available" energy to the animal than are GE values of feeds and ingredients (Cho and Kaushik, 1990). Consequently, formulation on a DE (and digestible nutrients) basis is more practical and logical than formulating on GE or crude nutrients (e.g., crude protein) basis. Formulation based on a DE basis has gained popularity in fish and crustacean nutrition over the past 30 years. Methods for determining digestibility and the factors that affect the digestibility of nutrients and energy are reviewed later in this document (Chapter 12).

Digestible energy content is thought to be one of the major factors controlling feed intake in fish (Lee and Putnam, 1972; Jobling and Wandsvik, 1983; Kentouri et al., 1995; Paspatis and Boujard, 1996; Lupatsch et al., 2001a). This assumption is derived from evidence in the literature showing that when offered diets with various DE levels, fish appear to adjust their feed intake to maintain a particular (daily) energy intake (Jobling and Wandsvik, 1983; Boujard and Médale, 1994; Kaushik and Médale, 1994; Yamamoto et al., 2000; Lupatsch et al., 2001b; Yamamoto et al., 2002, 2005). The capacity of fish to adjust to diets of different DE density is believed to be determined by the physical capacity of the digestive tract (Lupatsch et al., 2001b). Nevertheless, the expected feed intake adjustments of the fish to dietary DE was not observed in several other studies (Alanärä, 1994; Alanärä and Kiessling, 1996; Helland and Grisdale-Helland, 1998; Koskela et al., 1998; Peres and Oliva-Tele, 1999; Encarnação et al., 2004; Geurden et al., 2006). Feeding trials with lipid-rich diets did not indicate a negative feedback from extra dietary DE on feed intake in rainbow trout (Geurden et al., 2006). There is increasing evidence that feed intake of animals is regulated in part by the lean growth potential of animals (Encarnação et al., 2004; Geurden et al., 2006; Dumas et al., 2007). Animals will seek to eat a sufficient amount of a nutritional adequate diet to allow them to achieve their target or preferred performance unless limited by constraints or overridden by an externally managed intervention (Oldham et al., 1997). The differences in feed intake of fish fed diets with different DE levels observed in several studies is likely a reflection of the animals trying to adjust their feed intakes to consume sufficient amounts of different digestible nutrients to enable them to meet their growth and/or body composition targets, not simply a response to DE level of the diet per se. The DE density of the diet in itself has limited effect on feed intake regulation of fish. However, it represents a practical and valuable mode of expression of the digestible nutrient density of the diet.

NONFECAL LOSSES—METABOLIZABLE ENERGY

Catabolism of certain nutrients results in the production of metabolic wastes (e.g., ammonia) that must be excreted by the animal. Fish and shrimp excrete metabolic wastes through their gills and in urine. Excess of some nutrients, such as water-soluble vitamins, glucose, and amino acids, and some metabolites are also excreted in the urine as the result of glomerular filtration, which is present in most fish species (Dantzler, 1989). The excretion of ammonia and other types of combustible materials, such as urea, creatinine, glucose, amino acids, trimethylamine (TMA), and trimethylamine oxide (TMAO), through the gills and in urine results in energy losses that must be accounted for in an energy budget. Excretion of combustible products through the gills is termed branchial energy loss (ZE) and through the urine, urinary energy loss (UE). Subtracting these nonfecal losses from DE results in an estimate of the metabolizable energy (ME) value of the diet:

$$ME = IE - (FE + UE + ZE)$$

Direct determination of the ME values of diets for fish and shrimp is very difficult. Smith (1971) developed a metabolic chamber and experimental procedure that collect combustible products excreted from the gills and urine and quantify UE + ZE and allow estimation of ME of feedstuffs. However, this method requires restraint of the fish in a sealed vessel with a diaphragm separating the front from the rear portion of the body. This is a source of considerable stress in most fish species. Fish do not feed freely under such conditions and need to be force fed. These force-fed fish frequently vomit. Due to the stress, the animal generally exhibits much lower, and often negative, nitrogen balance than a free-swimming

animal feeding normally. As a result, the estimates of UE + ZE obtained with this method are much greater than would be the case for unrestrained fish feeding normally, and the estimates of ME of diets and feed ingredients is much lower than expected (Cho and Kaushik, 1990).

Branchial (ZN) and urinary (UN) nitrogenous wastes represent the bulk of the nonfecal energy losses of fish and crustaceans. Ammonia consists of approximately 85% of the nitrogenous wastes excreted by fish, whereas urea usually consists of less than 15% (Kaushik and Cowey, 1991). Monitoring production of N wastes in water of the rearing environment is an approach that has been commonly used (Brett and Zala, 1975; Kaushik, 1980a,b; Kaushik et al., 1982; Dosdat et al., 1996; Chakraborty and Chakraborty, 1998), but it requires an elaborate sampling protocol and considerable care.

Given the limitations of direct measurement of UE + ZE and UN + ZN, the use of an indirect method to estimate UE + ZE has been recommended as a means of obtaining realistic estimates (Cho and Kaushik, 1985). Cho and Kaushik (1990) proposed that the branchial and urinary excretion of 1 g of nitrogen by fish under normal conditions could be equated to an energy loss of 5.95 kcal (24.9 kJ), based on an energy of combustion value of ammonia (82.3% N by weight) of 4.90 kcal/g (20.5 kJ/g) (Bradfield and Llewellyn, 1982). Using this approach, the sum of branchial and urinary N excretion (ZN + UN) can be estimated by the difference between digestible nitrogen intake (DNI) and recovered nitrogen (RN) as follows:

$$ZN + UN = DNI - RN,$$
$$ZE + UE = (ZN + UN)\ 5.95\ \text{kcal/g N},$$
$$ME = DE - (ZE + UE)$$

Estimates of nonfecal losses are variable, but their contribution to the energy budget of fish is commonly no more than 3–6% of ME (Kaushik, 1998; Bureau et al., 2002). The main factors affecting nonfecal energy losses are those that influence the retention of protein/amino acids by the body and hence govern the loss of nitrogenous endproducts through the gills or in the urine.

Excretion of other combustible compounds may occasionally contribute significantly to UE + ZE of animals. Estimates of UE + ZE based on nitrogenous waste compounds excretion may occasionally underestimate actual nonfecal energy losses (Bureau et al., 1998). For example, excretion of glucose in the urine (Yokote, 1970; Furuichi, 1988; Kakuta and Namba, 1989; Bureau et al., 1998; Deng et al., 2000), as well as through the gills (Hemre and Kahrs, 1997), has been detected in fish made hyperglycemic by feeding a diet containing high levels of digestible carbohydrate or injected with glucose. The energy lost as urinary glucose is, nonetheless, relatively small and has since been estimated to be less than 5% of the ME intake of the animal (Bureau, 1997).

SURFACE ENERGY LOSSES

Shedding of combustible components through losses of mucus, scales, and epithelial cells represents loss of energy that is termed surface energy loss (SE). These losses are difficult to quantify in fish and are probably small. However, molting is an essential part of the growth processes of crustaceans. The exuvia produced by crustaceans results in the loss of combustible material and can also be classified as SE. Limited information is available on the SE losses of crustaceans. Based on a brief review of available information from unpublished information from past trials, Bureau et al. (2000) estimated SE associated with molting in growing penaeid shrimp to be small, approximately equivalent to 3% of ME intake.

HEAT LOSSES

Combustion of organic molecules results in the release of heat. For example, the combustion of one mole of glucose in a bomb calorimeter results in the liberation of 670 kcal (2,803 kJ) as heat (Blaxter, 1989). When oxidation of glucose occurs in the tissues, some of the energy is not lost immediately as heat but is captured in high-energy phosphate bonds through coupling reactions. Under aerobic conditions, glucose is completely oxidized to CO_2 and water, and the equivalent of 36 high-energy phosphate bonds are generated per molecule. The total energy captured in ATP per mole of glucose oxidized is 334 kcal (1,398 kJ), or the equivalent to approximately 50% of the enthalpy of combustion (or GE) (Blaxter, 1989). The remainder is dissipated as heat. In turn, when ATP generated by the catabolism of glucose is hydrolyzed during coupling with endergonic reaction, only a fraction of the free energy may be retained in the synthesized compounds and the rest is liberated as heat. Therefore, ultimately free energy liberated by exergonic reactions that is not captured in the products of anabolism (e.g., protein, lipids, carbohydrates, and nucleic acids) is liberated as heat by biological organisms.

The first law of thermodynamics states that heat produced by a chemical reaction is always the same, regardless of whether the process occurred directly or proceeded through a number of intermediate steps (Blaxter, 1989). Therefore, the amount of heat liberated depends on the chemical nature (energy content) of the compounds catabolized and of the overall reaction rather than the chemical reactions pathways by which this catabolism occurred.

According to the NRC (1981) nomenclature, HE is the total heat losses of an animal. It is also commonly designated as "metabolic rate" (Kleiber, 1975), which actually represents a much broader term. The HE is an indication of the intensity of ongoing metabolic reactions in the animal. A relatively large number of reviews have discussed at length the merits of various methodological approaches for

measurement of HE in fish (Cho and Kaushik, 1985; Tytler and Calow, 1985; Cho and Kaushik,1990; Cho and Bureau, 1995; Bureau et al., 2002).

Three components of animal metabolism lead to the release of energy as heat. Heat liberated by animals as a consequence of the need to sustain the structure and function of the body tissues is termed basal metabolism (H_eE) according to NRC (1981) nomenclature or minimal metabolism according to the nomenclature of Blaxter (1989). Physical activity also increases metabolic rate because of work done, and it is termed heat of voluntary activity (H_jE). The ingestion of feed increases the metabolic rate as a consequence of the extra work needed to ingest, digest, and metabolically utilize the components of the diet. This increase is termed the "heat increment of feeding" (H_iE). Standard dynamic action (SDA) is another term commonly used in the literature for this type of heat loss.

BASAL/MINIMAL METABOLISM

Animals require a continuous supply of free energy for those functions of the body immediately necessary for maintaining life regardless of whether or not food or feed is consumed. Basal/minimal metabolism (H_eE) represents use of energy for such things as the circulation of the blood, pulmonary ventilation, repair and replacement of cells, homeostasis, transport of ions (especially of sodium and potassium), and muscle tone. In fish and shrimp, H_eE is clearly related to temperature because environmental temperature has a determinant effect on the internal temperature, rate of biochemical reactions, and metabolic rate of the animal.

Meaningful assessment of H_eE requires the conditions by which standardized measurements are made. This objective is achieved by attempting to measure a minimum rate of heat production free of any controlling factors known to increase it. Such factors include exercise (voluntary movement), the consumption of feed and its subsequent metabolism, and the physical environment. The object of standardization is to ensure comparability of estimates rather than to establish some absolute minimum value of metabolism that is compatible with life. A number of terms have thus arisen to describe these presumably standardized measurements of "minimal metabolism." With domesticated animals, and hence fish under aquaculture conditions, what is usually measured is the fasting heat production (HE_f) (Blaxter, 1989). HE_f is also known as standard metabolism in the fish biology literature (Elliott, 1982). HE_f of different species of fish measured under various conditions has been reported in a very large number of studies. Unfortunately, significant variability in the estimates of HE_f or H_eE of fish reported in the literature exists and is probably mostly due to very significant differences in the methodological approaches and experimental conditions (Cho and Bureau, 1995).

It is difficult to ensure that the fish are in a state of muscular repose (complete rest) because they need to maintain their orientation in the water, which requires some muscular activity. It has been suggested that H_eE could be estimated using fasted fish swimming at different rates by extrapolation to zero activity (Smith, 1989). However, fish of many species spend considerable periods resting on the bottom of tanks or maintaining their position in quiet water with minimal activity. Consequently, HE_f of free-swimming animals has been regarded as a close approximation of minimal or basal metabolism (Cho and Kaushik, 1990). Oxygen consumption of free-swimming fish fasted for 3 to 7 days to eliminate the effect of the feed consumed, and its subsequent metabolism is the most common approach for measuring HE_f (Kaushik and Médale, 1994; Cho and Bureau, 1995; Glencross and Felsing, 2006). Measuring carcass energy losses during fasting is another common method of estimating HE_f and, consequently, H_eE (Cho and Kaushik, 1985; Lupatsch et al., 1998, 2003; Glencross et al., 2010). Oxygen consumption of fasting fish and carcass energy losses during fasting have been shown to result in similar HE_f estimates for rainbow trout (Bureau, 1997) and Asian sea bass (Glencross and Felsing, 2006; Glencross, 2008).

Available data on the HE_f of fish species show that, for a given weight, the rates are 1/5 to 1/20 of terrestrial vertebrates. Data from Kaushik and Gomes (1988), Cho (1991), and Bureau (1997) suggest HE_f of approximately 7.17–9.56 kcal/kg $BW^{0.80}$ (30–40 kJ/kg $BW^{0.80}$) per day for rainbow trout between 15 and 18°C. Based on carcass energy loss during starvation, Kaushik et al. (1995) calculated that Nile tilapia (*Oreochromis niloticus*) lost 16.73 kcal/kg $BW^{0.80}$ (70 kJ/kg $BW^{0.80}$) at water temperatures of 28°C. Using the same approach, Lupatsch et al. (2003) estimated the HE_f of gilthead sea bream (*Sparus aurata*) to be 10.04 kcal/kg $BW^{0.82}$ (42 kJ/kg $sBW^{0.82}$), that of European sea bass (*Dicentrarchus labrax*) to be about 8.37 kcal/kg $BW^{0.80}$ (35 kJ/kg $BW^{0.80}$), and that of white grouper (*Epinephelus aeneus*) to be 5.98 kcal/kg $BW^{0.79}$ (25 kJ/kg $BW^{0.79}$) at a water temperature of 23°C, while Glencross (2008) estimated the HE_f of Asian sea bass (*Lates calcarifer*) to be 10.28 kcal/kg $BW^{0.80}$ (43 kJ/kg $BW^{0.80}$) at 30°C. By comparison, HE_f has been reported to vary between 40.6 to 141.0 kcal/kg $BW^{0.75}$ (170 to 590 kJ/kg $BW^{0.75}$) per day in homeothermic domestic animals (Blaxter, 1989). The low HE_f of fish compared to homeotherms can be attributed to the lack of expenditure for thermoregulation, lower sodium pump activity, their buoyancy, and the mode of nitrogen excretion (ammoniotelism).

EFFECT OF BODY WEIGHT ON BASAL METABOLISM

In poikilotherms as well as in homeotherms, H_eE in absolute terms (kcal/animal per day) increases as the mass of the animal increases. The relationship of body weight to metabolic rate in animals can be described by the general equation $Y = aW^b$, where Y is the metabolic rate, W is the body weight, and "a" is a coefficient dependent on species and temperature.

The logarithm of H_eE increases linearly with the logarithm of the body mass (Blaxter, 1989). However, the slope of this relation is lower than 1, which means that in all species, animals of smaller size spend more energy per unit of mass than animals of larger size. The value of the exponent for fish has been described as ranging from 0.50 to 1.00. Hepher (1988), who reviewed experimental data from the literature, concluded that the exponent 0.8 describes, with reasonable accuracy, the change in metabolic rate with body mass of several fish species. Detailed observations by Brett and Groves (1979), Hogendoorn (1983) for African catfish (*Clarias gariepinus*), Cui and Liu (1990) for six different teleost species (*Cyprinus carpio*, *Oreochromis mossambicus*, *Pseudobagrus fulvidraco*, *Carassium auratus*, *Macropodus chinensis*, and *Pseudorasbora parva*), Cho (1991) for rainbow trout, Sanchez et al. (1993) for turbot (*Scophthalmus maximus*), Lupatsch et al. (1998) for gilthead sea bream, Liu et al. (2000) for mandarin fish (*Siniperca chuatsi*) and Chinese snakehead (*Channa argus*), Lupatsch et al. (2003) for European sea bass and white grouper, and Glencross (2008) for barramundi (also known as Asian seabass) suggest that across species the exponent is greater than 0.7 and less than 1.0. Thus it appears reasonable to assume metabolic body weight (MBW) can in practice be calculated using $kg^{0.8}$ for most fish species. However, recent evidence suggests that for penaeid shrimp, the appropriate scaling coefficient may to be closer to 1.0 (Lupatsch et al., 2008).

EFFECT OF TEMPERATURE ON BASAL METABOLISM

Water temperature is the major factor determining the metabolic rate and energy expenditure of poikilothermic animals, such as teleosts and crustaceans.

Based on mathematical analysis of oxygen consumption data of fasting rainbow trout reared at water temperatures ranging from 5 to 16°C, Cho and Kaushik (1990) concluded that H_eE as a function of water temperature could be described as:

$$H_eE = ((-1.04 + 3.26(T) - 0.05(T)^2) / (BW^{0.824})) / d$$

where: H_eE is basal metabolism (kilojoules), T is water temperature (°C), and BW is body weight (kg).

Glencross (2008) developed the following equation to estimate the H_eE of barramundi (Asian sea bass):

$$H_eE = (0.4462426 - 0.0848448(T) + 0.0048282(T)^2 - 0.0000750(T)^3) \times BW^{0.80}$$

where: BW is body weight (g).

Within species and certain temperature ranges, increasing water temperature results in a curvilinear (almost linear) increase of H_eE (Figure 4-2). Studies with Asian sea bass (barramundi) have reported a significant increase in H_eE above thermal optimum for this species (Bermudes et al., 2010). Increases in temperature above thermal optima result in metabolic disorders that can affect H_eE. As temperature continues to be elevated, fish reduce feeding activity and metabolic perturbations lead to death (upper lethal temperature). The upper lethal temperature varies, and the effect of temperature on H_eE may vary with species and strains within a species (Jonsson and Jonsson, 2009). Conversely, the metabolic rate of fish is reduced when water temperature and consequently body temperature of the fish is reduced. This reduction continues until the lower lethal limit is reached and the fish dies. This lower limit differs with species and for some species, such as some Antarctic fish species, can be slightly below 0°C (Clarke and Johnson, 1999).

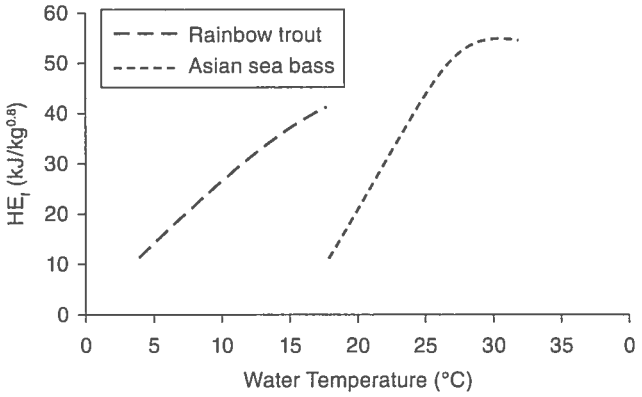

FIGURE 4-2 Fasting heat losses of rainbow trout, *Oncorhynchus mykiss*, and Asian sea bass, *Lates calcarifer* (expressed as HE_f, kJ per $kg^{0.8}$ per day and as a function of water temperature).

Studies with temperate and tropical species show no clear relationship between preferred environmental temperature and H_eE across species (Médale and Guillaume, 1999). However, Clarke and Johnson (1999) observed a curvilinear relationship between metabolic rate and temperature based on analysis of data from 69 teleost fish species. These different conclusions may be related to the fact that the analysis of Clarke and Johnson (1999) was based on a survey of published data from 69 species with only one temperature per species, defined as the "experimental temperature most representative of that experienced in the wild." Using this approach, a statistically significant curvilinear relationship is seen but is mostly the results of low metabolic rate for polar species (water temperature < 5°C) and higher metabolic rate for certain fish species between 35 and 40°C (Bureau et al., 2002). At their optimal growth temperature, H_eE of salmonids (Cho and Kaushik, 1990), mandarin fish (Liu et al., 2000), Chinese snakehead (Liu et al., 2000), gilthead sea bream (Lupatsch et al., 1998), European sea bass (Kaushik, 1998; Lupatsch et al., 2003), grouper (Lupatsch et al., 2003) and barramundi (Glencross, 2008) appear to be fairly similar, at about 5.98–11.95 kcal/kg $BW^{0.80}$ (25–50 kJ/kg $BW^{0.80}$) per day.

BASAL METABOLISM OF SHRIMP

The fasting oxygen consumption of the 30 g crayfish *Cherax tenuimanus* (Smith) at 22°C was estimated to be about 0.04 mg O_2 per minute (Villarreal, 1990), corresponding to H_eE of about 26 kJ/kg BW per day. Tchung (1995) observed that the HE_f of blue shrimp (*Penaeus stylirostris*) weighing between 20–28 g at 28°C was about 14.82 kcal/kg $BW^{0.66}$ (62 kJ/kg $BW^{0.66}$) per day during the intermolt period. Data from Gauquelin (1996) suggest that fasting oxygen consumption of *Penaeus stylirostris* weighing between 20–30 g was 3.3 g O_2/kg BW per day, which corresponds to HE_f of 10.76 kcal/kg BW (45 kJ/kg BW) per day. Lupatsch et al. (2008) estimated the HE_f (based on carcass energy losses during fasting) of Pacific white shrimp, *Litopenaeus vannamei* (1–35 g) kept at 28°C to be about 32 cal/g $BW^{0.95}$ (134 J/g $BW^{0.95}$), which is equivalent to 11.7 kcal/kg $BW^{0.8}$ (49 kJ/kg $BW^{0.8}$) per day. Oxygen consumption data from Maldonaldo et al. (2009) suggest an HE_f of about 2.4–8.4 kcal/kg $BW^{0.8}$ (10–35 kJ/kg $BW^{0.8}$) per day of Pacific white shrimp weighing between 0.2 and 6.0 g live weight reared at 28–32°C. Available data suggest that at their respective optimal temperatures, H_eE of different shrimp and other crustacean species is similar to that of fish species.

The effect of temperature on fasting oxygen consumption has been studied by Ocampo (1998) with *Penaeus californiensis* in the intermolt stage. Fasting oxygen consumption increased from 0.19 to 0.35 to 0.43 mg/g per hour when temperature increased from 19 to 23 to 27°C, respectively. Taken together, these results suggest that the effect of temperature on H_eE in shrimp is similar to that seen in fish. Results of several studies on oxygen consumption of fasting crustaceans are scattered throughout the literature. There is a need to review and analyze the available experimental evidence using the approaches that have been applied to higher vertebrates.

MAINTENANCE ENERGY REQUIREMENT

Although frequently confused, maintenance energy requirement (HE_m) and basal metabolism (H_eE) are two closely related but distinct concepts. Figure 4-3 contrasts the concepts of maintenance and basal metabolism. HE_m is generally defined as the amount of ME required for an animal to maintain zero energy balance (zero energy gain, RE = 0). The most commonly used method for estimating HE_m consists in feeding fish at different levels and using regression of the results of RE as a function of ME intake and extrapolating to zero carcass energy gain (i.e., RE = 0) (Figure 4-3). In theory, HE_m should be equal to H_eE plus the H_iE associated with feeding a maintenance ration. Consequently, HE_m values would be expected to be 20–60% greater than H_eE. Estimates of HE_m obtained across studies with the same species often have relatively large variances. Several factors, such as methodological approach, scaling factor used to calculate metabolic weight, regression model, and composition of the diet used, may have significant impacts on the estimate of HE_m. Evidence from a large number of

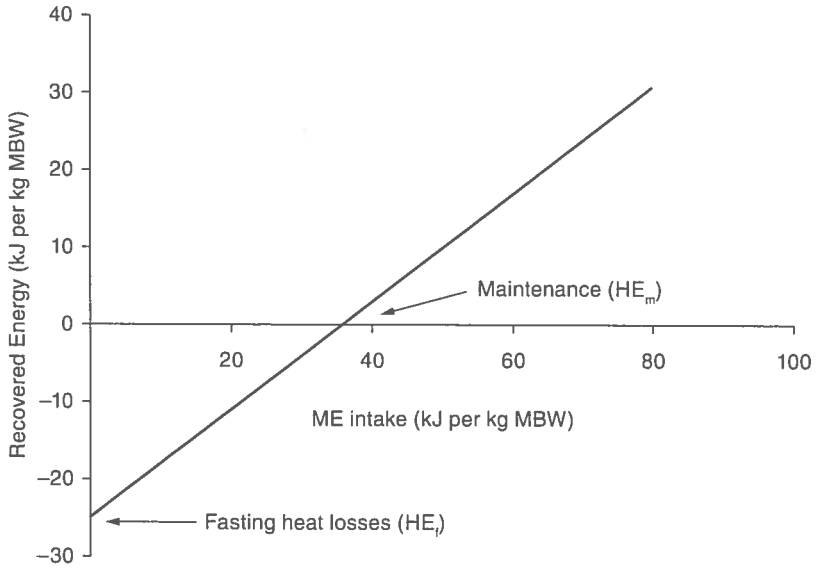

FIGURE 4-3 Illustration of the concept of maintenance and fasting heat losses (recovered energy [RE] as a function of metabolizable energy [ME] intake of fish and illustration of the concept of maintenance [HE_m] and fasting heat losses [HE_f] [an estimate of basal metabolism (H_eE)]).

published studies suggests that HE_m of fish reared at their optimum growth temperature is approximately 9.6–19.1 kcal/kg $BW^{0.8}$ (40–80 kJ ME / ($BW^{0.8}$)) per day. A summary of the results of a number of these studies is presented in Table 4-2. Due to the difficulty associated with measuring ME and because UE + ZE is generally small, it is increasingly common (although theoretically incorrect) to estimate HE_m on a DE basis for fish and shrimp.

It is worth noting that at zero carcass energy gain (RE = 0), fish fed a nutritionally adequate diet still deposit body protein (positive "protein-energy" gain) and mobilize body lipids (negative "nonprotein" energy gain) and still gain live weight (Figure 4-4). This phenomenon is found in all young animals fed a maintenance ration that is adequate in protein (Blaxter, 1989). Many have argued that the concept of maintenance is an irrational concept for growing animals and accordingly should be phased out. Others have argued that the concept of maintenance, although far from perfect, is still very useful in practice (Baldwin and Bywater, 1984).

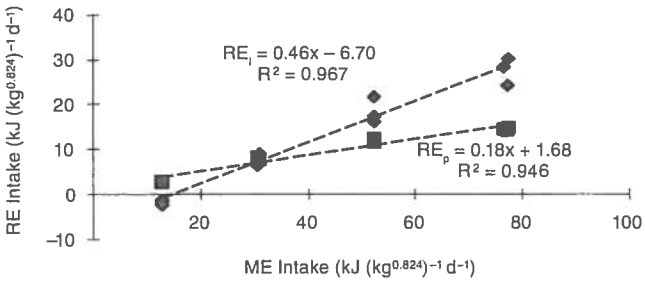

FIGURE 4-4 Recovered energy and metabolizable energy in rainbow trout, *Oncorhynchus mykiss*. (Recovered energy [RE] as protein [RE_p, squares] and lipid [RE_l, diamonds] as a function of metabolizable energy [ME] intake of rainbow trout reared at 8.5°C [data from Bureau et al., 2006]).

TABLE 4-2 Estimate of Maintenance[a] Energy Requirement of Different Fish and Shrimp Species Obtained Through Feeding Trials

Species	Weight (g/fish)	Temperature (°C)	HE_m (kcal ME/$kg^{0.80}$ per day)	HE_m (kJ ME/$kg^{0.80}$ per day)	Reference
Atlantic salmon (*Salmo salar*)	5	15	4.06	17	Bureau et al. (1999)
Asian sea bass/Barramundi (*Lates calcarifer*)	15	30	8.37[b]	35[b]	Glencross (2008)
	410	30	11.00[b]	46[b]	Glencross (2008)
Channel catfish (*Ictalurus punctatus*)	8–10	27	5.98	25	Gatlin et al. (1986)
Chinese sucker (*Myxocyprinus asiaticus*)	12	27	9.08	38	Yuan et al. (2009)
European sea bass (*Dicentrarchus labrax*)	15–140	24	10.76[b]	45[b]	Lupatsch et al. (2001a, 2003)
Gilthead sea bream (*Sparus aurata*)	30–160	24	11.47[b]	48[b]	Lupatsch et al. (1998, 2003)
Pacific white shrimp (*Litopenaeus vannamei*)	1.5–7.5	28	26.29[b]	110[b]	Lupatsch et al. (2008)
Rainbow trout (*Oncorhynchus mykiss*)	150	18	13.86–17.93	58–75	Kaushik and Gomes (1988)
	158	9	4.54	19	Bureau et al. (2006)
	300	15	7.89	33	Storebakken et al. (1991)
	96	14	10.04[b]	42[b]	Glencross (2008)
	55	16	10.52[b]	44[b]	Glencross (2009)
Red drum (*Sciaenops ocellatus*)	5.5	25	11.47	48	McGoogan and Gatlin (1998)
Nile tilapia (*Oreochromis niloticus*)	330	28	14.34	60	Lupatsch et al. (2010b)
Tra catfish (*Pangasianodon hypothalamus*)	40	32	9.56[b]	40[b]	Glencross et al. (2010)
Yellowtail (*Seriola quinqueradiata*)	750	15	18.40	77	Watanabe et al. (2000)

[a]HE_m.
[b]Expressed on a digestible energy (DE) basis.

HEAT LOSSES FOR VOLUNTARY ACTIVITY

Fish have an efficient mode of transportation. Their bodies are supported by water, and they do not need to expend energy against gravity like terrestrial animals. A streamlined body moving through the water is one of the most efficient forms of transportation. A large number of studies have focused on the metabolic cost of swimming for fish (Brett and Groves, 1979). Krohn and Boisclair (1994) suggested that the metabolic costs of turning and accelerating may be substantially more than the cost of swimming at constant speed in a straight line. Estimation of the energetic cost of activity may be very significant for wild fish due to their need to expend considerable amounts of energy to acquire food and escape predators. H_jE associated from activity is difficult to estimate separately from H_eE because there is always a certain amount of voluntary activity in any group of fish (Cho et al., 1982). It has been assumed that when constructing an energy budget of free-swimming fish under normal aquaculture conditions, the cost of activity is rather negligible and is already included in the estimate of H_eE (Bureau et al., 2002). This assumption may be an oversimplification of reality. Cooke et al. (2000) used electromyogram telemetry to observe a 60% increase in voluntary swimming activity and a 26% increase in oxygen consumption in rainbow trout held at high stocking density compared to those held at low stocking density. These authors hypothesized that differences in feed efficiency observed in fish held at different stocking densities may be related to increase in energy losses due to activity. Conversely, a recent study with European sea bass (Lupatsch et al., 2010a) found that oxygen consumption as well as HE_m of fish were higher at low stocking density, but no difference was found in feed efficiency or growth rate, apart from a slightly reduced body lipid content of fish kept at the low stocking density. It may be concluded that H_jE may be a significant contribution to HE of fish under certain conditions but that there are likely significant differences between species, life stages, rearing environments, and environmental conditions. More work needs to be carried out to quantify H_jE of the numerous fish species cultured under a great variety of rearing environments. The broad range of technologies available today (e.g., mesocosms, underwater camera, sonar, radio transmitters, global positioning system [GPS], internal temperature loggers, electromyogram telemetry, image analysis software) combined with traditional techniques (e.g., respirometry, comparative carcass analysis) could enable accurate quantification of H_jE of fish reared under practical conditions.

HEAT INCREMENT OF FEEDING

Ingestion of feed by an animal that has been fasting results in an increase in the animal's HE. This expenditure of energy due to feeding is referred to as heat increment of feeding (H_iE). This component of the energy budget is also referred to as extra heat, specific dynamic action (SDA), calorigenic effect, and dietary thermogenesis in the literature. The factors that contribute to H_iE have traditionally been separated into three categories: (1) digestion and absorption processes (H_dE), (2) formation and excretion of metabolic wastes (H_wE), and (3) transformation and interconversion of the substrates and their retention in tissues (H_rE). For crustaceans, H_iE should also include cost of molting (H_xE).

ESTIMATES OF HEAT INCREMENT OF FEEDING

The length of time for which consumption of diet influences HE depends on many factors; chief among these factors are the quantity and quality of the diet, the water temperature, and growth (nutrient deposition) of the animal. The rise in oxygen uptake corresponds more or less to the rate of transit of feedstuffs through the digestive tract (Kaushik and Dabrowski, 1983). The H_iE principally depends on the balance of dietary nutrients and the plane of nutrition (Brody, 1945). Therefore, attempts to measure the H_iE of individual feed ingredients that are a nutritionally unbalanced diet (Smith et al., 1978; Tandler and Beamish, 1979) or measurements of effect of fish size (Beamish, 1974) and fish density (Medland and Beamish, 1985) performed under forced activity conditions have very doubtful meaning. Similarly, the estimation of H_iE of an animal without reference to its growth and nutrient deposition (energy or protein and lipid deposition) (e.g., Ross et al., 1992) is also inadequate.

Many studies have shown highly significant linear (or largely linear) relationships between ME intake and RE (Figure 4-5). The slope is often identified as the "efficiency of metabolizable utilization for production," K_g or K_{pf}, and has been reported to vary between 0.26–0.70 for various fish species fed practical diets (Meyer-Burgorff et al., 1989; Cui and Liu, 1990; Azevedo et al., 1998; Lupatsch et al., 1998; Médale et al., 1998; Ohta and Watanabe, 1998; Rodehutscord and Pfeffer, 1999; Peres and Oliva-Teles, 2000; Lupatsch et al., 2003; Bureau et al., 2006; Glencross, 2008; Yuan et al., 2009). Consequently, in most of the species studied so far, H_iE appears to be equivalent to 30–75% of ME intake in fish fed nutritionally adequate diets. Although significant interspecific differences exist, a large proportion of the variability in the estimates of H_iE among studies can likely be attributed to differences in diet composition and composition of weight gain (protein vs. lipid deposition), as well as a variety of methodological issues (such as experimental protocol, range of data, stress conditions, assessment of feed intake and nutrient digestibility, and statistical model used). Estimate of H_iE may only be applicable to a certain set of conditions (same species, life stage, and diet composition). However, for a given diet and species, H_iE expressed as a proportion of ME intake, DE intake, or RE does not appear to significantly vary with water temperature (Azevedo et al., 1998; Lupatsch et al., 1998; Rodehutscord and Pfeffer, 1999; Lupatsch et al., 2003; Lupatsch and Kissil, 2005), at

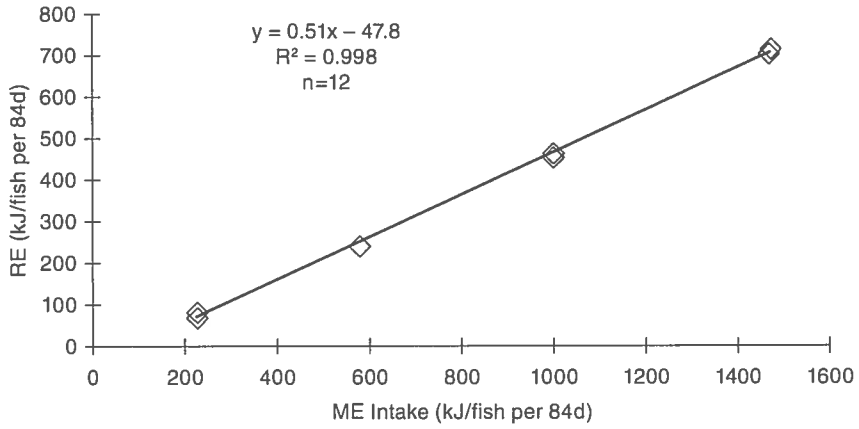

FIGURE 4-5 Recovered energy and metabolizable energy in Atlantic salmon, *Salmo salar* (recovered energy [RE] as a function of metabolizable energy [ME] intake in Atlantic salmon fed at different feeding levels. The slope indicates the "efficiency of ME utilization for production [K_{pf}]" and 1-slope is an estimate of the heat increment of feeding [H_iE] of the animal. [Source of data: Bureau et al., 1999, n = 12]).

least within a certain range of temperatures (which depends on species, strains, and rearing environment). Outside this thermal range metabolic perturbations occur, and these negatively affect efficiency of ME and DE utilization (Bermudes et al., 2010). The effect of feeding levels is less consistent across studies. Some studies have shown no effect of feeding level on efficiency of ME utilization (K_{pf}) (Azevedo et al., 1998; Lupatsch et al., 2001a,b; Bureau et al., 2006; see also Figure 4-5). However, results from other studies (Glencross, 2008; Lupatsch et al., 2008; Helland et al., 2010) suggest that at high feeding levels, efficiency of energy utilization tends to decrease, and H_iE, as a proportion of ME or RE, appears to increase significantly. This decrease in efficiency of energy utilization or increase in H_iE may be attributable to the curvilinear response in protein deposition that is observed at high feeding levels as animals approach their so-called maximal protein deposition rate or PD_{max} (Bureau et al., 2006; Dumas et al., 2007). The relationship between ME and RE and H_iE are the results of complex metabolic processes, and properly contrasting results of studies requires a more comprehensive analysis of nutrient utilization and metabolism, as opposed to a simple relation of ME intake and energy retention.

There are limited data on H_iE in shrimp. Interpretation of data from Warukamkul et al. (2000) suggest K_{pf} of the black tiger shrimp (*Penaeus monodon*) can be estimated at about 0.5; therefore, for every 0.12 kcal (0.5 kJ) of RE or H_eE, 0.12 kcal (0.5 kJ) is expended as H_iE. Lupatsch et al. (2008) observed that the efficiency of DE utilization by Pacific white shrimp was only about 30%, indicating that for every 0.07 kcal (0.3 kJ) of RE or H_eE, 0.17 kcal (0.7 kJ) is expended as H_iE. Estimation of H_iE in crustaceans is complicated by the difficulty in properly estimating ME intake of the animal and by contribution of molting processes to energy losses. Estimates of cost of molting (H_xE) are very scarce. Read and Caulton (1980) estimated that as much as 25% of RE accumulated in intermolt may be expended due to molting. This high estimate is very difficult to corroborate, and more work is needed to estimate heat losses in shrimp throughout their growth cycle.

DIGESTION AND ABSORPTION PROCESSES

Digestion and absorption processes (H_dE) refer to the heat losses related to biochemical and "mechanical" aspects of feeding and digestion in fish. Early studies using either "sham feeding" or feeding nondigestible materials such as kaolin or cellulose indicated that "mechanical SDA" approached 10–30% of total H_iE (Tandler and Beamish, 1979). However, other studies found that neither sham feeding nor kaolin feeding significantly increased the metabolic rate of the fish (Jobling and Davies, 1980). Emmans (1994) estimated the heat losses associated with egesting indigestible material to be about 0.91 kcal (3.8 kJ) per g of fecal organic matter (FOM) in terrestrial animals. If this value is applicable to fish, H_dE would probably represent less than 10% of H_iE of fish fed high-quality practical diets.

Heat losses associated to the enzymatic hydrolyses of lipids, polysaccharides, and proteins in the lumen of the gut have been estimated, in theory, to be about 0.1–0.2% of the GE of the substrate hydrolyzed (Blaxter, 1989). The absorption of certain products of digestion, such as amino acids, peptides, and glucose by the intestinal mucosa often occurs through an energy-dependent transport system known as active transport. Carrier proteins simultaneously transport the target molecule and a cotransported ion. The maintenance of a sodium gradient across the membrane is achieved by an ATP-dependent sodium transporter working in the opposite direction. This transporter hydrolyses one ATP molecule per every three sodium ions extruded. Theoretical cost of transport of glucose through active transport is one-third of an

ATP, which is equivalent to less than 1% of the GE of glucose or about 1% of the potential amount of ATP generated by the aerobic metabolism of glucose (i.e., 36 ATP). Absorption of lipid digestion products differ significantly: triacylglycerides (TAG) are hydrolyzed to free fatty acids (FFA) and monoacylglycerol (MAG) in the lumen. The FFA and MAG are absorbed passively, and TAG are resynthesized in the mucosa and exported as chylomicron to the circulation. Synthesis of TAG and chylomicrons requires a certain amount of energy, but again, this amount represents only a small proportion of the GE content of these molecules (Blaxter, 1989).

Heat losses arising from anaerobic fermentation in the gut is another factor contributing to H_dE. However, fermentation in most fish species is very limited (Leenhouwers et al., 2008), perhaps with the exception of certain marine herbivorous fish species (Clement, 1996). Very few quantitative studies on fate of volatile fatty acids and energy lost in the fermentation process have been conducted for fish. However, available estimates of fermentation suggest that heat losses associated with fermentation are small (Leenhouwers et al., 2008).

Overall, the heat losses associated with diet ingestion and digestion (H_dE) are probably small compared to that associated with metabolic work ($H_rE + H_wE$) (Brody, 1945). The physiological basis of this increased heat production is the postabsorptive processes related to ingested diet. These processes are primarily the metabolic work required for the synthesis of proteins and lipids in the tissues from the newly absorbed, metabolized amino acids, fatty acids, and glucose.

FORMATION AND EXCRETION OF METABOLIC WASTE

Deamination and catabolism of amino acids lead to ammonia production. As ammonia is toxic and cannot be rapidly eliminated by mammals and birds; these animals synthesize urea and uric acid, which are less toxic. The energy cost of synthesis for these products is 3.11 and 2.39 kcal/g N (13 and 10 kJ/g N), respectively, for urea and uric acid (Martin and Blaxter, 1965). The concentration of urea and uric acid for further excretion by the kidneys in terrestrial animals requires additional expenditure of energy. In contrast, ammonia is the primary waste product of protein catabolism in fish (Kaushik and Cowey, 1991). Urea is mainly the product of degradation of purines and arginine catabolism. As ammonia is efficiently excreted by the gills, fish generally do not require energy to detoxify or concentrate this waste. As a result, heat of formation and excretion of metabolic waste (H_wE) should represent only a very small fraction of H_iE of fish.

TRANSFORMATION OF SUBSTRATES AND RETENTION IN TISSUES

The heat losses associated with transformation of the substrates and their retention in tissues (H_rE) should represent a very large proportion of H_iE in animals. Much evidence suggests that the efficiency of utilization of ME varies with the chemical nature of the energy-yielding nutrients absorbed (Blaxter, 1989). When a fasting animal is refed, nutrients absorbed by the animal replace body constituents as the source of energy. The efficiency of utilization of ME is in proportion to the ATP yield of the nutrients absorbed (Blaxter, 1989; Van Milgen, 2002).

Growing animals accrete new tissues where part of the energy supplied is stored mainly as protein, lipid, and glycogen. Theoretical efficiency of transformation to or retention of substrates in tissue has been calculated for higher vertebrates (Blaxter, 1989; Flatt, 1992; van Milgen, 2002), and these theoretical costs are also valid for fish given the great similarity of the intermediate metabolism of fish and higher vertebrates. According to the calculations of Blaxter (1989) and van Milgen (2002), converting glucose into glycogen costs 5% of the energy of glucose as H_iE whereas converting glucose into lipids entails an increase of H_iE equal to about 30% of its GE. Conversion of dietary lipids into body lipids is, in theory, about 96%; therefore 4% of GE of lipids is dissipated as H_iE. The maximum theoretical efficiency of the conversion of dietary amino acids into body proteins is 85% efficient, entailing an H_iE of 15% of the GE value of proteins (Blaxter, 1989; van Milgen, 2002). Conversion of amino acids into body lipids is, in theory, only 66% efficient so approximately 34% energy would be lost as H_iE.

Protein and lipid deposition is the result of both synthesis and degradation rates of either protein or lipid, respectively, i.e., their turnover rates. Energy is lost as heat in the biochemical reactions that lead to protein synthesis and degradation, lipogenesis, and lipolysis, and in regulating and integrating the various cellular metabolic activities involved in protein and lipid deposition (van Milgen, 2002). Calculation of the theoretical costs of protein and lipid deposition is extremely complicated and fraught with uncertainties. Alternatively, these costs can be estimated in an empirical manner based on statistical analysis of energy expenditure and protein and lipid depositions.

PRACTICAL NET ENERGY SYSTEMS

Many studies have attempted to relate ME to RE (or H_iE) and then tried to delineate the various determinants of H_iE. The most popular approach is a factorial one and was first proposed by Kielanowski (1965). Factorial approaches have been at the foundation of popular energy requirement systems for pigs (NRC, 1998), chickens (NRC, 1994), beef cattle (NRC, 2000), and dairy cattle (NRC, 2001; Kebreab et al., 2003).

In the classic factorial approach, the partial energy costs for protein and lipid deposition are determined through a multiple regression approach using ME intake as the independent variable and protein and lipid energy deposition rates as the dependent variables to determine (Reeds, 1991).

The energy cost for lipid and protein deposition is simply defined as ME required to promote a defined increment in body protein or lipid. The partial efficiency of ME utilization for whole body growth (K_{pf}), protein deposition (K_p), and lipid deposition (K_f) is the ratio of net energy retained to the corresponding ME intake components:

$$ME = HE_m + RE_p / K_p + RE_f / K_f$$

Using this type of approach, Emmans (1994) concluded that the net energy cost for protein retention in terrestrial livestock species was 2.54 kcal per kcal of protein retained (that is, 1.54 kcal of heat expended for each 1 kcal of protein deposited) equivalent to a K_p of 40%. The calculated energy cost for lipid retention was 1.4 kcal and 1.1 kcal per kcal lipid deposited (i.e., heat losses of 0.4 or 0.1 kcal per each 1 kcal lipid deposited) when deposited from nonlipid or lipid, respectively. These are equivalent to a K_f = 90% when deposited from lipid and K_f = 70% when deposited from nonfat substrates.

An increasing number of studies have used the factorial approach to estimate HE_m, K_p, and K_f of fish. Results from these studies are summarized in Table 4-3. The estimates of K_p ranged between 0.49 and 0.81 and those of K_f between 0.66–0.91. These values appear to be similar to that observed with mammals and birds.

The factorial approach of Kielanowski (1965) has been criticized because there is, in general, a strong correlation between protein and lipid depositions and that it is much easier to control ME intake than it is to control protein and lipid depositions (Emmans, 1995). If multicolinearity is present to a harmful degree in physiological data, multiple linear regressions often yield nonsensical results (Slinker and Glantz, 1985; Birkett and de Lange, 2001; Azevedo et al., 2005). To overcome some of the limitations of the factorial approach, a multivariate approach has been proposed by van Milgen and Noblet (1999). In this multivariate approach, protein deposition (PD) and lipid deposition (LD) (dependent variables) are considered a function of ME intake. Azevedo et al. (2005) investigated the utilization of ME for growth vs. maintenance in rainbow trout and Atlantic salmon using both the factorial and multivariate approaches. The estimates

TABLE 4-3 Estimates of Maintenance[a], Cost of Protein[b] and Lipid Deposition[c] Determined Using the Factorial Approach[d]

Species	Temp. (°C)	HE_m (per day)	HE_m (per day)	K_p	K_f	Reference
Common carp (*Cyprinus carpio*)	18	10.04 kcal/kg$^{0.75}$	42 kJ/kg$^{0.75}$	0.56	0.72	Schwartz and Kirchgessner (1995)
European sea bass (*Dicentrarchus labrax*)	23	9.56 kcal/kg$^{0.79}$	40 kJ/kg$^{0.79}$	0.53	0.90	Lupatsch et al. (2003)
Gilthead sea bream (*Sparus aurata*)	23	10.28 kcal/kg$^{0.83}$	43 kJ/kg$^{0.83}$	0.53	0.76	Lupatsch et al. (2003)
Grouper (*Epinephelus* spp.)	23	7.41 kcal/kg$^{0.83}$	31 kJ/kg$^{0.83}$	0.56	0.91	Lupatch et al. (2003)
Rainbow trout (*Oncorhynchus mykiss*)	15	0.33 kcal/g$^{0.39}$	1.37 kJ/g$^{0.39}$	0.54	0.90[e]	Rodehutscord and Pfeffer (1999)
Rainbow trout (*O. mykiss*)	8.5	8.37 kcal/kg$^{0.80}$	35 kJ/kg$^{0.80}$	0.53	0.90[e]	Azevedo et al. (2005)
Rainbow trout[f] (*O. mykiss*)	8.5	4.78 kcal/kg$^{0.80}$	20 kJ/kg$^{0.80}$	0.43	0.81	Azevedo et al. (2005)
Rainbow trout (*O. mykiss*)	8.5	4.54 kcal/kg$^{0.80}$	19 kJ/kg$^{0.80}$	0.63	0.72	Bureau et al. (2006)
Atlantic salmon (*Salmo salar*)	8.5	8.37 kcal/kg$^{0.80}$	35 kJ/kg$^{0.80}$	0.81	0.90[e]	Azevedo et al. (2005)
Atlantic salmon[f] (*S. salar*)	8.5	4.78 kcal/kg$^{0.80}$	20 kJ/kg$^{0.80}$	0.52	0.81	Azevedo et al. (2005)
Mulloway (*Argyrosomus japonicus*)	20–26	10.76 kcal/kg$^{0.80}$	45 kJ/kg$^{0.80}$	0.49	0.75	Pirozzi et al. (2010)

[a]HE_m.
[b]K_p.
[c]K_f.
[d]Using the method of Kielanowski (1965).
[e]Fixed.
[f]Multivariate analysis.

obtained with the factorial and multivariate approaches were different (Table 4-3) but highly dependent on the model and corresponding assumptions (Azevedo et al., 2005).

Significant differences in the energy cost of protein deposition exist between theoretical assumptions (86% efficient) and that calculated using the factorial and multivariate approaches (43–81% efficient). Both the factorial and multivariate approaches are based on statistical relationships between inputs and outputs, without a true representation of the underlying biological principles. Results obtained with the factorial and multivariate approaches may be statistical artifacts. Protein and lipid depositions are not merely the deposition of energy but rather the results of a highly complicated array of metabolic processes. Rates and efficiencies (hence turnover) of deposition are governed by dietary factors (nutrient balance and utilization) and biological factors (e.g., genetics, physiological state of the animal, and types of tissue made).

In growing animals, the rates of protein synthesis greatly exceed those of protein deposition (Reeds et al., 1981). The efficiency of retention of protein deposition in fish ranges from 30 to 70% (Langar and Guillaume, 1994). Therefore, changes in protein turnover are a possible explanation for the variable energy cost of protein deposition (Reeds et al., 1985; Milligan and Summers, 1986). On the other hand, when problems associated with structural and kinetic heterogeneity of amino acid pools are involved (Watt et al., 1991), in addition to the difficulty of measuring the synthesis of rapidly turning over proteins (Wheatley et al., 1988), one is led to conclude that current estimates of protein synthesis in vivo may underestimate actual rates. Consequently, the theoretical energy cost for protein deposition will also be underestimated. The nutrient composition of the diet may also contribute to K_p. Catabolism of amino acids due to amino acid excess or imbalances in the diet or low nonprotein energy content will result in waste of energy (H_iE associated with catabolism of amino acid higher than that of lipid) and a decrease in K_p. Diets with unbalanced amino acid composition will result in lower retained energy as protein and consequently result in K_p values lower than that achievable if the diet were perfectly balanced. The type of amino acid catabolized also plays a role in loss of energy (van Milgen et al., 2002).

Nonetheless, heat losses associated with lipid deposition estimated using the factorial and multivariate approaches appear to be close to the theoretical chemical cost. Since K_f differs depending on the origin of the lipid deposited, the composition of the diet will also affect the efficiency of lipid deposition. Dietary intake of preformed lipids will lead to a high efficiency of utilization for lipid deposition, whereas de novo synthesis of lipid from dietary carbohydrates will lead to a slightly lower efficiency. The pathways of lipogenesis in fish are qualitatively similar to those in other vertebrates. A review of the literature reveals significant differences amongst species and studies on the same species in terms of de novo lipogenesis. Some studies have indicated that de novo fatty acid synthesis is generally limited in fish fed high-lipid diets (Brauge, 1994; Dias et al., 1998). Consequently, in fish fed high-lipid diets, where almost all the lipid deposited is of dietary origin (Brauge, 1994), the cost of lipid deposition is apparently very low (Table 4-3).

The early studies of Cho et al. (1976) showed that an increase in dietary fat levels led to a decrease in H_iE. LeGrow and Beamish (1986) confirmed that the increase in oxygen uptake as dietary protein levels increased was consistent, regardless of the dietary lipid level, thus highlighting the importance of dietary digestible protein/digestible energy (DP/DE) ratios. In addition, the effectiveness of the energy derived from the catabolism of different amino acids by the fish is unclear (Encarnação et al., 2006). It has been suggested that digestible carbohydrates are possibly also significant contributors to H_iE (Beamish et al., 1986; Hilton et al., 1987). Beamish et al. (1986) observed that fish fed a diet with high glucose content consumed more oxygen than fish fed a diet with the same protein level but rich in lipid, suggesting that an increase in digestible carbohydrate intake results in an increase in H_iE. The fish fed the diet high in glucose had a lower nitrogen retention efficiency (N gain:N intake) than the fish fed the diet high in lipid (Beamish et al., 1986). These data suggest that the effect on H_iE observed was in fact more related to the variation in dietary lipid and the sparing of protein than to the digestible carbohydrate itself. However, Helland and Grisdale-Helland (1998) observed that, at low intake levels, an increase in digestible starch at the expense of digestible protein resulted in an increase in oxygen consumption of Atlantic salmon but no change in the N gain:N intake. Bureau et al. (1998) observed a very poor retention of ME of digestible starch fed to rainbow trout, suggesting that at high levels of intakes, the utilization of digestible carbohydrates can be associated with very significant H_iE. However, these authors observed no significant increase in oxygen consumption in fish fed diets with very high digestible starch levels. They speculated that anaerobic catabolism of glucose may be a source of energy loss for fish fed high carbohydrate diets. This hypothesis should be investigated.

The discussion above illustrates the value of using net energy (NE) systems (factorial and multivariate approaches) as simple and practical means of estimating the cost of protein and lipid depositions in animals. However, more accurate and detailed delineation of the cost of growth requires detailed analysis of digestion, metabolism, and retention of individual nutrients, and thus, the use of framework based on nutrient utilization as opposed to energy utilization.

RECOVERED ENERGY

Protein and lipids are the main energy-yielding components of the bodies of animals. Glycogen, another energy-yielding body component, generally represents only a small proportion of the body of the animal (< 1%). Based on detailed allometric analysis, Shearer (1994) and Dumas et al.

(2007) concluded that the protein concentration in the whole body of growing salmonid was relatively constant, but that their lipid concentration was highly variable and affected by both endogenous (fish size, growth rate) and exogenous (dietary, environmental) factors. Studies with numerous other species also support this conclusion (Lupatsch et al., 2003; Glencross, 2008; Glencross et al., 2010).

Studies have shown that the composition of biomass gain includes more lipids and less water in a large fish than in a small fish (Shul'man, 1974; Dumas et al., 2007). Consequently, more energy is contained in one unit of biomass gain for a large fish (e.g., 2.39 kcal/g BW or 10 kJ/g BW) than for a small fish (e.g., 0.96–1.20 kcal/g BW or 4–5 kJ/g BW). The protein of the whole body appears to vary little with growth of a given species of fish, whereas whole-body GE content varies considerably over time. There are about 4 g of water associated with each gram of protein tissue deposited (Dumas et al., 2007). On a wet-weight basis, tissue contains about 16% protein. Since protein has a GE of 5.64 kcal/g (23.6 kJ/g), protein contributes about 0.88 kcal GE/g (3.7 kJ GE/g) of wet tissue. Lipids are stored in tissues generally substituting water (Dumas et al., 2007), although experimental evidence suggests that lipid deposits can significantly contribute to live weight gain of fish. Differences exist in the lipid deposition dynamic amongst species, strains, life stages, and animals with different nutritional histories. Whole-body GE content increase seen in fish of increasing weight is mainly due to increasing lipid content.

The difference between the enthalpy of combustion (i.e., GE) of the body at the beginning and at the end of a period of time is referred to as RE. The most direct way of estimating RE is to determine GE (by bomb calorimetry) of representative whole-body samples from a group of experimental animals at the beginning and at the end of a growth assay. This method of determining RE is termed "comparative carcass analysis" or "slaughter technique." Alternatively, RE can be estimated by difference between IE and FE, UE + ZE, and HE (Blaxter, 1989) and is also known as the "energy balance technique." The RE can either be positive or negative and represents the enthalpy of combustion of organic compounds stored or lost by the body of the animal.

Protein and lipid deposition are two distinct biological processes regulated by different mechanisms. The relative importance of protein and lipid deposition depends upon a great number of nutritional factors. Severe feed restriction results in significant alteration of the protein to lipid deposition ratio in fish. As mentioned earlier, fish fed a maintenance ration (ration resulting in RE = 0) can still deposit body protein (positive protein-energy gain) while mobilizing body lipids (Figure 4-4). There is clearly evidence that live weight gain is largely driven by protein deposition (Dumas et al., 2007). Studies on the effect of feeding levels on fish have shown that protein and lipid deposition increase linearly with feed allocation, but that they have different slopes and intercepts (Figure 4-4). At severe feed restriction, protein deposition greatly exceeds lipid deposition. However, energy deposition as lipids often exceeds that as protein at moderate to high feeding levels (Lupatsch et al., 2001b; Dumas et al., 2007). A number of studies have also shown that protein deposition tends to plateau off at high feeding levels (Lupatsch et al., 2003; Glencross, 2008; Glencross et al., 2010), whereas lipid deposition does not appear to level off (increases linearly). A decreasing protein to lipid deposition ratio can be observed at a feeding level approaching maximum protein deposition (Dumas et al., 2007). Differences in the GE content of the carcass and RE of fish are largely determined by variations in lipid depositions.

High DE intake and feeding diets with improper balance of DP to DE and protein sources with poorer essential amino acid profile generally result in the deposition of a larger proportion of RE as lipid. Seasonal changes in body composition, in relation to specific physiological stages or endocrine status are also known to occur. There are also considerable interspecific differences in lipid deposition and tissue distribution. Nutrient deposition and temporal changes in body composition of fish and effects of all the factors mentioned above need to be investigated more systematically than has been the case in the past (Dumas et al., 2010).

REPRODUCTION AND GONADS—OVUM ENERGY

Reproduction is a demanding period of life for many organisms. Resources need to be diverted from somatic growth into processes necessary for successful breeding (Thorpe, 1992; Hendry and Berg, 1999). The chemical composition and energy content of gonads and gametes have been fairly well characterized for a number of fish species (Kaushik and Médale, 1994). The energy content of the eggs, termed ovum energy (OE) in the nomenclature proposed by NRC (1981), of rainbow trout has been estimated to be about 6.45 kcal/g (27 kJ/g) dry matter, irrespective of the size of the eggs. The average energy content in eggs, measured in about 50 teleosts, was 5.62 kcal/g (23.5 kJ/g) dry matter whatever the size of eggs. Lupatsch et al. (2010b) determine that eggs of tilapia had a GE of 2.51 kcal/g (10.5 kJ/g) wet weight. The total amount of energy stored in the eggs would represent 8–15% of gross energy of the whole body, very much correlated to the gonado-somatic index (Kaushik and Médale, 1994). For the majority of the species, the male gonads with maturity represent only one small proportion of the body mass. On the other hand, the ovaries with maturity can represent up to 30% of the body mass of certain species. In some multiple spawners, such as the gilthead seabream, total egg production over a single season can even reach 100% of body mass (Tandler et al., 1995). Lupatsch et al. (2010b) determined that adult female tilapia produced about 1 g of egg per kg BW per day.

There are few studies on the actual cost of gonad formation (e.g., H_iE associated with nutrient deposition in gonads). Efficiency of DE utilization (K_{pf}) for both gonad formation and somatic growth in adult female tilapia was recently esti-

mated to be 0.63, which is very similar to estimates of K_{pf} for somatic growth in the same species (Lupatsch et al., 2010b).

The total cost of reproduction exceeds that of only production of the gametes. The development of secondary sexual characters, the production of mucus, nuptial behaviors and activities, migration, and other aspects are all processes that involve expenditure of energy. Under aquaculture conditions, expenses associated with reproduction may certainly not be as dramatic as those incurred in wild fish. However, reproduction is a critical part of the production cycle for farmed species and involves fairly dramatic changes in the energy partitioning by the animal. Reproduction involves the synthesis and temporary storage of new tissues that are formed almost regardless of the level of dietary energy intake, the necessary energy being withdrawn from other body tissues if the dietary supply is insufficient (Jonsson et al., 1991, 1997). Consequently the redistribution of tissue energy that takes place in the breeding season can complicate measurements of energy balance and estimation of the cost of growth (Lupatsch et al., 2010b).

CALCULATION OF ENERGY REQUIREMENT FOR GROWTH

The essential thrust of studies on bioenergetics of animals is to provide a convenient and accurate system to predict feed requirements or efficiency of feed utilization based on body weight, growth rate, sex, activity, physiological state, environment, and the composition of feed provided to the animal (Baldwin and Bywater, 1984). A large number of bioenergetics models of different formats have been developed to predict growth, feed ration, FCR, and waste outputs of various fish species under a variety of conditions (Cho, 1992; Cho and Bureau, 1998; Kaushik, 1998; Lupatsch and Kissil, 1998; Cui and Xie, 1999; Bureau et al., 2002, 2003; Zhou et al., 2005; Glencross, 2008; Lupatsch et al., 2008; Glencross et al., 2010; Pirozzi et al., 2010).

Fish growth has usually been predicted using two different approaches in bioenergetics models. One approach assumes that IE drives weight gain. This assumption is encountered mostly in fisheries and ecology studies because the availability of food in natural ecosystems often limits fish growth (Elliott, 1976; Kitchell et al., 1977; From and Rasmussen, 1989). An alternative approach considers genetic or potential/desired growth rate rather than nutrition as the factor limiting animal growth (Hubbell, 1971; Calow, 1973; Oldham et al., 1997). Here, IE is a function of the requirements of the individual to achieve a given growth potential or growth target. This approach was suggested by Winberg (1956) and is mostly used in aquaculture where fish are generally fed to satiation with nutritionally complete diets (Cho, 1990, 1992; Lupatsch, et al., 2001b; Zhou et al., 2005; Glencross, 2008).

In several bioenergetics models, FE, UE + ZE, H_iE, and H_eE, as well as GE content of the carcass, are considered to be fixed fractions of IE, regardless of the composition of the feed and performance of the fish (e.g., Hanson et al., 1997). Basic understanding of nutrition should indicate that these are unreasonable assumptions. It is also common to observe energy requirement expressed as absolute amount of DE required per kg body weight per day for "maximal production" or energy expenditure and deposition expressed as a proportion of "maximal feed consumption" (C_{max}) in numerous fish bioenergetics studies (e.g., Gatlin et al., 1986; McGoogan and Gatlin, 1998; Ohta and Watanabe, 1998; Cui and Xie, 1999; Elliott and Hurley, 1999; Watanabe et al., 2000). Maximal production or C_{max} of an animal are factors that are highly dependent on genetics, diet composition, environmental conditions (e.g., temperature), husbandry practices, health status, and other variables. Maximum production and C_{max} are, therefore, highly variable parameters. Consequently, the energy requirement for maximum production calculated in some studies (i.e., energy requirement expressed in absolute term such as kcal per fish per day) can only be valid for the extremely specific conditions (e.g., diet composition, strain, temperature, and culture conditions) encountered in the study. Fish growing at different rates will deposit nutrients at different rates and, consequently, have different energy and feed requirements. Energy requirement should therefore be calculated for explicitly expressed levels of performance (e.g., expected or achievable level of performance), feed composition, and life stage (Cho, 1991, 1992; Cho and Bureau, 1998; Kaushik, 1998; Guillaume et al., 1999). This is at the basis of a large number of factorial models that have been developed in recent years for different aquaculture species (Cho and Bureau, 1998; Lupatsch et al., 1998, 2003, 2008; Bureau et al., 2003; Glencross, 2008). These factorial models divide energy requirement into its different components or fractions.

Cho (1991) proposed factorial models to determine energy requirement of fish based on expected level of performance, diet composition, and expected body composition. The approach of Cho (1991) was slightly modified by Cho (1992), Cho et al. (1994), Cho and Bureau (1998), and Bureau et al. (2002, 2003) and used to estimate feed requirement and waste outputs of different salmonid fish species reared under commercial-like conditions. Lupatsch et al. (1998) proposed a similar approach and subsequently used this approach to estimate energy, protein, and feed requirements of a variety of fish species (Lupatsch et al., 1998, 2001a, 2003, 2008).

Using the approach of Cho (1991), estimation of DE requirements and, consequently, feed requirements (or allocation) of fish can be determined as follows:

1. Characterization of diet (including DE content)
2. Calculation of expected live weight gain and RE
3. Allocation of H_eE based on fish size and water temperature
4. Allocation of H_iE for maintenance and energy deposition
5. Allocation of UE + ZE

6. Calculation of minimum DE requirement
7. Calculation of feed requirement.

Using the approach similar to that of Cho (1991), energy, oxygen, and feed requirements and expected feed efficiency of fish of different sizes reared under different conditions or rearing periods can be calculated (Cho and Bureau, 1998). Table 4-4 presents energy and oxygen requirements and theoretical feed efficiency of rainbow trout reared at 12°C and fed a practical diet (44% digestible protein and 4.54 Mcal [19 MJ] DE) at different weights or growing from 1 g to 1,000 g. The DE requirements to produce 1 kg biomass of rainbow trout were estimated to vary from about 2.51 Mcal (10.5 MJ) for 1 g fish to 6.41 (26.8 MJ) for 1 kg fish.

Table 4-5 similarly presents estimates of energy and oxygen requirements and theoretical feed efficiency of European sea bass but calculated using the approach of Lupatsch et al. (1998). Glencross (2008) used a very similar approach for Asian sea bass (Table 4-6). It is of utmost importance to understand that these estimates are only valid for the

TABLE 4-4 Energy and Oxygen Requirements[a] and Expected Feed Efficiency of Rainbow Trout[b] (*Oncorhynchus mykiss*)

Live Weight (g/fish)	RE[c]	H_eE[d]	H_iE[e]	UE + ZE[f]	DE[g]	Oxygen[h] (g/kg weight gain)	Feed Efficiency[i]
	Mcal/kg (MJ/kg) Weight Gain						
1	1.34 (5.6)	0.24 (1.0)	0.81 (3.4)	0.12 (0.5)	2.51 (10.5)	320	1.8
5	1.34 (5.6)	0.33 (1.4)	0.81 (3.4)	0.12 (0.5)	2.63 (11.0)	350	1.7
10	1.36 (5.7)	0.41 (1.7)	0.81 (3.4)	0.12 (0.5)	2.68 (11.2)	370	1.7
50	1.43 (6.0)	0.53 (2.2)	0.86 (3.6)	0.14 (0.6)	2.99 (12.5)	430	1.5
100	1.55 (6.5)	0.60 (2.5)	0.93 (3.9)	0.14 (0.6)	3.21 (13.4)	470	1.4
500	1.79 (7.5)	0.76 (3.2)	1.39 (5.8)	0.22 (0.9)	4.54 (19.0)	670	1.0
1,000	3.27 (13.7)	0.86 (3.6)	1.96 (8.2)	0.31 (1.3)	4.41 (26.8)	880	0.7
1–1,000	2.51 (10.5)	0.79 (3.3)	1.51 (6.3)	0.24 (1.0)	5.04 (21.1)	710	0.9

[a]Mcal (MJ) or g/kg weight gain.

[b]At various sizes or growing from 1 to 1,000 g based on assumption that the fish are reared at 12°C, growing with a thermal-unit growth coefficient (TGC) = 0.220, and fed diet with 420 g DP, 220 g lipids, and 4.54 Mcal (19 MJ) digestible energy (DE) per kg.

[c]RE (kJ/fish) = (–0.0039 (live body weight at $T_{(i+1)}$, g/fish)^2 + 5.5812 (live body weight at $T_{(i+1)}$, g/fish)) – (–0.0039 (live body weight at $T_{(i)}$, g/fish)^2 + 5.5812 (live body weight at $T_{(i)}$)).

[d]$H_eE = ((-1.04 + 3.26(T) - 0.05(T)^2) / (0.020^{0.824})) / d$.

[e]$H_iE = 0.6 \times RE$.

[f]$UE + ZE = 0.05 \times (RE + H_eE + H_iE)$.

[g]DE requirement = RE + H_eE + H_iE + (UE + ZE).

[h]Oxygen requirement = H_eE + H_iE / oxycalorific coefficient (13.6 kJ/g O_2 consumed).

[i]Expected feed efficiency (gain/feed).

TABLE 4-5 Energy and Oxygen Requirements[a] and Expected Feed Efficiency of European Sea Bass (*Dicentrarchus labrax*)[b]

Live Weight (g/fish)	RE[c]	H_eE[d]	H_iE + (UE + ZE)[e]	DE[f]	Oxygen[g] (g/kg weight gain)	Feed Efficiency[h]
	Mcal/kg (MJ/kg) Weight Gain					
1	1.15 (4.8)	0.62 (2.6)	0.57 (2.4)	2.34 (9.8)	360	2.0
5	1.43 (6.0)	0.84 (3.5)	0.72 (3.0)	2.99 (12.5)	480	1.6
10	1.58 (6.6)	1.00 (4.1)	0.81 (3.4)	3.37 (14.1)	550	1.4
50	1.98 (8.3)	1.41 (5.9)	1.08 (4.5)	4.47 (18.7)	770	1.1
100	2.15 (9.0)	1.65 (6.9)	1.22 (5.1)	5.07 (21.2)	880	0.9
250	2.41 (10.1)	2.01 (8.4)	1.41 (5.9)	5.83 (24.4)	1,050	0.8
400	2.56 (10.7)	2.22 (9.3)	1.53 (6.4)	6.26 (26.2)	1,160	0.8
1–400	2.34 (9.8)	1.89 (7.9)	1.34 (5.6)	5.59 (23.4)	1,039	0.8

[a]Mcal (MJ) or g/kg weight gain.

[b]At various sizes or growing from 1 to 400 g based on assumption that the fish are reared at 22°C, growing according to $y = 0.64 \times BW(kg)^{0.587} \times \exp^{0.07T}$ (Lupatsch et al., 2001a), and fed a diet with 500 g digestible protein, 180 g lipids, and 4.78 Mcal (20 MJ) DE per kg.

[c]RE (kJ/fish) = (5.17 (live body weight (g) at $T_{(I+1)}$)$^{0.107}$) – (5.17 kJ (live body weight (g) at $T_{(i)}$)$^{0.107}$.

[d]$H_eE = 35 \times$ (live body weight (kg) at $T_{(i)}$)$^{0.8}$ / d.

[e]H_iE + (UE + ZE) = 0.32 × (RE + H_eE).

[f]DE requirement = RE + H_eE + H_iE.

[g]Oxygen requirement = H_eE + H_iE / oxycalorific coefficient (13.6 kJ/g O_2 consumed).

[h]Expected feed efficiency (gain/feed).

TABLE 4-6 Energy and Oxygen Requirements[a] and Expected Feed Efficiency of Asian Sea Bass (*Lates calcarifer*)[b]

Live Weight (g/fish)	Growth Rate[c] (g/fish per day)	RE[d]	H_eE[e]	$H_iE + (UE + ZE)$[f]	DE[g]	Oxygen[h] (g/kg weight gain)	Feed Efficiency[i]
		Mcal/kg (MJ/kg) weight gain					
10	1.1	1.08 (4.5)	0.29 (1.2)	0.74 (3.1)	2.13 (8.9)	319	2.6
50	2.2	1.36 (5.7)	0.55 (2.3)	0.93 (3.9)	2.84 (11.9)	454	1.9
100	3.0	1.51 (6.3)	0.69 (2.9)	1.03 (4.3)	3.25 (13.6)	533	1.7
250	4.4	1.72 (7.2)	0.98 (4.1)	1.17 (4.9)	3.90 (16.3)	666	1.4
500	5.9	1.91 (8.0)	1.29 (5.4)	1.29 (5.4)	4.49 (18.8)	794	1.2
1,000	8.0	2.10 (8.8)	1.67 (7.0)	1.43 (6.0)	5.21 (21.8)	953	1.0
2,000	10.7	2.32 (9.7)	2.15 (9.0)	1.58 (6.6)	6.07 (25.4)	1,152	0.9
3,000	12.7	2.46 (10.3)	2.51 (10.5)	1.67 (7.0)	6.64 (27.8)	1,290	0.8

[a]Mcal (MJ) or g/kg weight gain.

[b]At various sizes or growing from 10 to 3,000 g based on assumption that the fish are reared at 30°C, growing according to a growth rate (g/fish per day) $= (0.54 - 0.1199T + 0.0074T^2 - 0.0001T^3) \times BW(kg)^{0.424} \times \exp^{0.07T}$ (Glencross, 2008) and fed a diet with 5.38 Mcal (22.5 MJ) DE per kg.

[c]Growth rate g/fish per day $= (0.54 - 0.1199T + 0.0074T^2 - 0.0001T^3)\ BW^{0.424}$.

[d]RE (kJ/fish) = 3.273 (live body weight gain (g/fish)) × live weight $(g/fish)^{0.143}$.

[e]H_eE (kJ/fish per day) $= (0.44624 - 0.08484T + 0.00483T^2 + 0.0008T^3) \times BW^{0.8035}$.

[f]$H_iE + (UE + ZE) = 0.684 \times (RE)$.

[g]DE requirement $= RE + H_eE + H_iE$.

[h]Oxygen requirement $= H_eE + H_iE$ / oxycalorific coefficient (13.6 kJ/g O_2 consumed).

[i]Expected feed efficiency (gain/feed).

given set of conditions (such as species, water temperature, growth rates, and diet composition) and should not be applied blindly.

The DE requirement (kcal per fish per day) is largely dependent on growth rate of the animal. In general, as temperature increases, the growth rate, DE intake, and RE increase, but efficiency of ME and DE utilization (RE/ME or RE/DE) does not change (Azevedo et al., 1998). Total energy requirement should ideally be expressed as DE because FE and, consequently, IE are highly dependent on the composition of diet fed. FE losses by animals are largely a factor of the composition of the diet and not always greatly affected by biological factors.

LIMITATIONS OF NUTRITIONAL ENERGETICS APPROACHES

Nutritional energetics models are simple and practical, but their limitations have been increasingly recognized (Birkett and de Lange, 2001; Bureau et al., 2002; Bajer et al., 2004; Dijkstra et al., 2007; Dumas et al., 2008). Energy systems simplify the partitioning of dietary components into body deposition of nutrients on the basis of their heats of combustion, disregard specific metabolic roles of different nutrients and their interaction, and ignore significant differences in contribution of protein and lipid toward live weight gain. More than a century ago, Rubner (1902) described bioenergetics as the "heat doctrine" (Dumas et al., 2008). The lumping of nutrients together solely on the basis of their energy values is largely irrational. It does not allow a complete evaluation of the effect of chemical composition of the feed and role or efficiency of use of specific nutrients. "Energy requirement" and "dietary energy utilization" are parameters and tools that help deal with the complexity of animal metabolism and nutritional requirements, not immutable concepts.

One of the practical limitations of bioenergetics models is that they assume that energy is allocated in a hierarchical fashion and that growth is the surplus of energy after all other components of the energy budget have been covered or satisfied (Elliot and Hurley, 1999). Models based on bioenergetic principles assume that growth and feed efficiency will be nil when animals are fed a maintenance ration (RE = 0). That assumption has been proven inaccurate in fish, as well as in other animals, where positive weight gain was still observed even though animals were fed on a maintenance ration of a nutritionally adequate diet (Le Dividich et al., 1980; Bureau et al., 2006). Live weight gain is mainly (but not solely) driven by protein deposition due to the close association of water with protein deposition (Shearer, 1994; Dumas et al., 2007). Lipid reserves can be mobilized to support protein deposition (Black, 1974; Campbell, 1988; Bureau et al., 2006; Figure 4-4). Azevedo et al. (2004a,b) observed dramatic changes in the feed efficiency in rainbow trout of increasing weight. Efficiency of protein retention decreased significantly in these animals but efficiency of ME utilization did not change.

Bioenergetics models rely on estimates of the cost of growth calculated empirically based on statistical interpretations of experimental data. How much of the energy cost is truly due to "biological inefficiencies" or simply due to the fact that animals are fed "imperfect" diets is not known. Furthermore, these empirical estimates cannot be legitimately extrapolated to conditions beyond which data are collected (France and Thornley, 1984; Baldwin, 1995). Although nutritional energetics frameworks are including increasingly explicit representations of metabolic use of dietary nutrients,

there is increasing evidence that current bioenergetics models are not sufficiently rational and flexible to be applied to the wide range of conditions encountered in fish culture (Bureau and Hua, 2008; Dumas et al., 2008). There is a necessity to move to an approach based on a clearer understanding of the role of specific absorbed nutrients and their metabolism in determining productive responses of the animal (Reynolds, 1999; Dumas et al., 2008). A more scientifically correct feed evaluation and requirement system should be based on characterization of nutrient fractions relevant to their actual digestion, metabolism, and deposition in the animal under varying practical conditions (Boisen and Verstegen, 1998).

Mechanistic models, based on more or less explicit representation of biochemical reactions and metabolic use of amino acids, fatty acids, and glucose, have been developed for various fish species, such as African catfish (Machiels and Henken, 1986; Conceição et al., 1998), Nile tilapia (van Dam and De Vries, 1995), rainbow trout (van Dam and De Vries, 1995; Hua and Bureau, 2010), turbot (Conceição et al., 1998), and Atlantic salmon (*Salmo salar*) (Bar et al., 2007). Construction of mechanistic models requires adequate knowledge of the system, and relies on sufficient and accurate data to quantify the perceived system (Baldwin, 1995). The process of parameterization can be a major bottleneck in the development and application of complex mechanistic models (Kyriazakis 1999; McNamara, 2004; Dumas et al., 2010). Consequently, all biochemical models have been developed with some degree of simplification of metabolic pathways, have included numerous assumptions, and have been generally driven by more or less transparent partitioning rules. However, metabolic models are often more complex than what is required to represent growth at the whole animal level (van der Honing, 1998; Birkett and de Lange, 2001). These highly detailed models can work well within the narrow range of conditions for which they are parametrized and calibrated. However, they generally fail to accurately describe nutrient utilization by fish as influenced by a wide range of conditions (including differences in feed composition, environmental conditions, husbandry practices, life stages, and genetic background of animals) encountered in fish culture (Dumas et al., 2010; Hua and Bureau, 2010). Despite these limitations, there is a need for the fish and shrimp nutrition community to increasingly embrace approaches based on explicit and integrative utilization of nutrients in order to enable the development of more rational systems aimed at better describing and predicting growth and nutrient utilization and requirements of fish and shrimp.

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