Chemical composition and pollution potential of fish and shrimp feeds

by

Sirirat Chatvijitkul

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Approved by

Claude E. Boyd, Chair, Professor of School of Fisheries, Aquaculture, and Aquatic Sciences
Donald A. Davis, Chair, Professor of School of Fisheries, Aquaculture, and Aquatic Sciences
Terrill R. Hanson, Professor of School of Fisheries, Aquaculture, and Aquatic Sciences
Julie A. Howe, Associate Professor of Crop, Soil and Environmental Sciences, college of Agriculture

Abstract

The objectives of this research were to assess the resources use for producing aquaculture feeds and to determine the waste loads of nutrients to the culture system (system loads), along with the solution to reduce nutrient loads by applying enzyme in aquatic feeds. In the first study, the literature was searched for works that allowed the estimation of land, water, nutrients, and energy embodied in the common feedstuffs used in aquaculture feeds. Results demonstrated that the embodied energy of salmon and trout feeds were greater than for other aquatic feeds. Whiteleg shrimp feed required the greatest amount of land to produce plant-based ingredients. Tilapia and pangasius feeds exhibited the highest average water requirement per unit of production.

In the second study, feed samples obtained from major aquaculture countries were analyzed for concentrations of carbon, nitrogen, and phosphorus in order to estimate system and environmental loads in natural waters. The result indicated that system waste loads for nitrogen ranged from 54.83 g/kg (or kg/tonne) for tilapia to 90.30 g/kg for channel catfish. System load of phosphorus ranged from 10.55 g/kg for salmon to 18.32 g/kg for channel catfish. System waste loads for carbon ranged from 350.74 g/kg (or kg/tonne) for salmon to 650.57 g/kg for channel catfish.

In the third study, 17 minerals elements in aquaculture feeds were analyzed by ICP-AES to quantify levels of minerals and to estimate loads from data on concentrations of elements in

feeds. Concentration of macro and micro-minerals was generally found to be higher in feed samples than in bodies of cultured species. Also, P, Mg, Cu and Zn concentrations were found to be significantly higher than animals requirement levels in the feed samples. System loads for macro-minerals and micro-minerals S, Ca, and K were found to be highest for channel catfish.

In the fourth study, this study was designed to determine the effect of carbohydrase and phytase enzymes supplemented to the diet on growth performance and nutrient retention of channel catfish. The results obtained indicate supplementing carbohydrase or phytase into diets did not result in significant ($P>0.05$) improvement in the growth performance of fish. Supplementation of diets with phytase 2000 IU/kg improved P retention and ash content in the fish, resulting in a reduction in P system load.

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Chapter1 Introduction

Presently, there is much concern about the sustainability of humankind. Providing goods and services for human population requires enormous amounts of resources and consequently causes negative environmental impacts. Based on information from environmental nongovernmental organizations (eNGOs), the ecological footprint of humanity is reported to be 1.3 to 1.7 times greater than the global capacity to supply resources and assimilate wastes (Boyd and McNevin, 2014). Therefore, it is important for the aquaculture industry to seek ways of lessening resource use and negative environmental impacts. As a general rule, these two objectives are closely linked – reducing resource use lessens negative environmental impacts.

The human population has increased exponentially since 1950, and the demand for food has increased along with it. Agricultural production increased from 3 billion ton in 1961 to 7 billion ton in 2010, with a rate of increase slightly greater than in that of population growth. This increase resulted primarily from intensification of agriculture. It has been shown that total land use by agriculture increased approximately 10% – from 4.5 billion ha in 1961 to 5 billion ha in 2010 (Boyd et al., 2013). Aquaculture is an important part of the world food system, and as the population has grown the demand for aquatic products has also increased. Aquaculture currently is the source of about half of fisheries production for human consumption, and around 8 to 9% of animal protein intake of humans (FAO, 2012).

According to FAO (2012), aquaculture has been a fast growing industry at a global level and is expected to continue this trend because of an increase in demand for aquatic products.

Aquaculture is expanding more rapidly than all other animal food industries. Over the last three decades, aquaculture industry has grown substantially on a global scale and production has increased by almost 12 times, at an average annual rate of 8.8% (FAO, 2012). For 2010, FAO (2012) also estimated the global aquaculture production to be 60 million metric tons with an estimated total value of US $119 billion.

The impact of aquaculture on the environment became apparent in late 20th. Before this culture practices relied primarily on natural productivity or low quality inputs such as fertilizers to enhance natural productivity and provide natural food organisms for the cultured species (Ackefors and Enell, 1990). Extensive aquaculture was considered to be environment friendly (Avault, 1996). Aquaculture farms then started to increase use of manufactured feeds and operate on a semi-intensive or intensive scale with the purpose of achieving increased production for each unit of land and water. Intensive aquaculture involves higher stocking rate, use of nutritionally complete feeds, and mechanical aeration to allow higher production (Marte et al., 2000; Islam, 2005). With an increase in nutrient input to enhance production, there is a corresponding increase in water pollution (Bonsdorff et al., 1996, 1997; Anderson, 1997). Thus, it is important to understand and minimize the environmental impact due to intensive aquacultural practices.

Presently, aquaculture operations are confronted with the task of complying environmental laws and regulations. A number of statutes and regulations directly affect the management of aquaculture wastes and effluents (Butz and Vans-Cappell, 1982; Handy and Poxton, 1993). For example, Europe has regulations restricting discharges of total ammonia and suspended solids discharged to 408 and 6,400 mg/ kg fish/ day, respectively. Dissolved oxygen consumption by the farm should not exceed 6,000 mg/ kg fish/ day (Highland River Purification Board, 1987). Therefore, determining the quantity of nutrient loads in the effluents from aquaculture is crucial.

There are two approaches for estimating nutrient loss to environment – direct and indirect methods (Beveridge, 1991). Nutrient loss is determined directly through sampling and analysis of the water column and of sediment particulate material. The quantity of nutrient loss is estimated indirectly using a mass balance approach which involves the composition of feed applied into the system, nutrient retention in cultured species, and feed conversion ratio of each cultured species in particular culture system (Beveridge et al., 1991; Barg, 1992; Angel et al., 1992). The mass balance approach provides insights into why and where wastes occur; therefore, the information on the quantity of nutrients in feeds is an important factor in determining nutrient loss into the environment.

Once feeds are applied into a culture system, a portion of the feed is consumed by cultured species, and the rest which is left over breaks down and is dissolved or suspended in the water. Some also becomes organic matter in sediment. Aquaculture feed contains digestible and indigestible components. Enzymes in the digestive tract help digest the feed. After the absorption process, a portion of digestible nutrients are retained in the cultured species and increase the biomass of the cultured species (growth). The rest are excreted in the form of dissolved products such as ammonia and carbon dioxide resulting from metabolic processes. Nutrients not absorbed by the intestine are excreted by animals in form of feces (solid waste) and released into the water. Fecal production depends largely on the feed composition especially the fiber content.

The portion of the feed not consumed by the aquatic animal is broken down in the water. Feeds containing a lot of small particles (fines) and those that are less stable in water easily break down and produce solid waste increasing their potential for polluting the environment. Unconsumed feeds can accumulate on the bottom of the system and give rise to anaerobic conditions at the sediment−water interface. Cage culture is an open system; solids built up in

beneath the cages. Solid accumulations ranging from 11 to 38 percent of the feed applied have been reported (McLaughlin, 1981). Solid wastes also were estimated at between 0.5 and 0.7 g/kg of fish produced (Paramatrix, 1990). Solid accumulation can cause a reduction of benthic organisms and also cause toxicity when hydrogen sulfide is into the environment during their composition (Chamberlin, 1986). Ammonia, carbondioxide, and phosphates also are released by decomposition of solids.

Unconsumed feeds also cause water quality problems in and around farms. Feeding waste can lead to low dissolved oxygen concentration, and increased levels of ammonia that stress animals resulting in diseases, phytoplankton blooms, increased level of pollutants in water recently farm effluents, and economic losses related to elevated FCR (Muller & Varadi, 1980; Bergheim *et al.,* 1982; Enell, 1987; Penczak *et al.,* 1982; Enell & Lof, 1983; Beveridge, 1985; Phillips & Beveridge 1986; Molver *et al.*, 1988). As feed input increases, more metabolic, dissolved and solid wastes enter into the water. Organic matter from both feces and unconsumed feed accumulating in the system exert an oxygen demand that can be expressed as biological oxygen demand (BOD). Wastes discharged nitrogen and phosphorus, not only increase oxygen demand but raise concentrations of phytoplankton blooms and associated eutrophication.

The composition and physical properties of waste products reflects the diet composition. The primary pollutants that are often discussed are nitrogen and phosphorus (Cho et al., 1994; Islam, 2005). But, organic carbon concentration in feed is directly related to oxygen demand, and little is known about carbon concentration in aquaculture feeds. There are many other nutrients in feed that also may negatively impact water quality. In order to minimize waste pollution from aquaculture feeds, it is necessary to know the amount of nutrients in the feed and to determine the nutrient requirements of animals. The use of feeds containing nutrient levels closely meeting, but

not exceeding the requirement of a cultured species is an effective way of minimizing waste outputs (Sugiura and Hardy, 2000; Cho and Bureau, 2001). Aquatic feeds should, however, contain all essential nutrients in amounts necessary for supporting normal growth of cultured species. Vitamin and mineral premixes are added to diets in order to provide all necessary nutrients to the culture (Avault, 1996). Information on mineral requirements for aquatic animals is still limited (Cheng et al., 2005; Wang et al., 2006). Therefore, in current feed formulations, the amount of mineral mixtures may not be optimal and can be high or low depending upon the requirement of a particular species. Also, in case of high concentration of micro-minerals, the excess can be either absorbed in the animal tissue or dispersed into water and sediments. Higher metal level in animal tissue is a source of concern as it poses a possible health risks to humans consuming the food (Agusa et al., 2007).

The environmental impacts of aquaculture from feed wastage can be reduced by implementing various practices. Culture system may be designed or refitted to allow easily control and treatment of effluent. For example, system can be design to drain water of high solid content from near the pond bottom into a settling reservoir, and this water replaced with higher quality water (Davidson and Summerfelt, 2005). Ponds can be designed to collect sludge, which can be degraded within the system naturally or removed after an extended period of time. Discharge limits maybe imposed on facilities by governmental agencies responsible for environmental protection. Several organizations have eco-label certification programs participation in which require compromise with effluent water quality limits. Good feed management and feeding practices are employed to meet effluent limits (Handy and Poxton, 1993).

In an effort to minimize nutrient loading from aquaculture effluents, many alternative technologies are being explored. In aquaponics, plants remove waste products from aquaculture

operations. Aquaponics is a well-designed nutrient recycling approach by utilizing the waste products as resources. Moreover, the development of high energy and low pollution diets can significantly improve wastewater quality. High energy or nutrient-dense diet formulated based on the protein sparing principle, typically contains high lipid level and lower level of protein (Kissil and Lupatsch, 1992; Cho et al., 1994; Anderson and De Silva, 1997). This lower protein content in feed does not affect the growth of cultured species but leads to a reduction of nitrogen loading on the environment (Cho, 1992; Johnsen et al., 1993). Enzymes have been added to animal feeds in recent years because of economic, health and environmental concerns (Lobo, 1998; Bedford, 2000; Gatlin and Li, 2008). Enzymes can improve nutrient digestibility and enhance feed efficiency, leading to a decrease in nutrient pollution in environment as well.

To evaluate the environmental impact of aquaculture effluents, it is important to know the amount of nutrients discharged from farms. Although, the types of wastes released from aquaculture farming are similar, there are differences based on the quality and quantity of nutrient input to the systems.

This research specifically aims to estimate the resources used to produce aquaculture feeds and quantify levels of nutrients in various types of aquaculture feeds in order to estimate nutrient loads in natural waters, along with the solution to reduce nutrient loads by applying enzyme in aquatic feeds. Given the interest of many consumers for environmental sustainability, it is critical to gather appropriate data to develop a solid and compelling case for the use of feed in the continued expansion of aquaculture as a sustainable industry. To achieve this goal, the following specific objectives will be carried out for feed samples obtained from different aquatic feed companies from major aquaculture countries.

1. Estimate land, water, energy, and nutrients used for producing aquaculture feed ingredients
2. Quantify the concentrations of nitrogen, phosphorus, carbon and trace elements in aquatic feed samples.
3. Quantify the concentrations of nitrogen, phosphorus, carbon and trace elements of select culture species from literature data or analyses.
4. Demonstrate how to estimate loads of pollutants from data on concentrations of elements in feed and culture species and the FCR.
5. Determine the effect of carbohydrase and phytase on growth performance and nutrient retention of channel catfish (*Ictalurus punctatus*).

References

Ackefors, H., Enell, M., 1990. Discharge of nutrients from Swedish fish farming to adjacent sea areas. Ambio. 19, 28-35.

Agusa, T., Kunito, T., Sudaryanto, A., Monirith, I., Kan-Atireklap, S., Iwata, H., Ismail, A., Sanguansin, J., Muchtar, M., Tana, T.S., Tanabe, S., 2007. In: Kan-Atireklap, S., (Eds.), Exposure assessment for trace elements from consumption of marine fish in Southeast Asia. Env. Pollut. 145, 766-777.

Anderson, T.A., De Silva, S.S., 1997. Strategies for low-pollution feeds and feeding. Aquacult Asia 2, 18-25.

Angel, D., Krost, P., Zuber, D., Mozes, N., Neori, A., 1992. The turnover of organic matter in hypertrophic sediments below a floating fish farm in oligotrophic Gulf of Eilat (Aqaba). Bamidgeh. 44, 143-144.

Avault Jr, J.W., 1996. Fundamentals of Aquaculture: A step by step guide to commercial aquaculture, AVA Publishing Company Inc. Baton Rouge, Louisiana, USA.

Barg, U., 1992. Guidelines for the promotion of environmental management of coastal aquaculture development. FAO Fisheries Technical Paper 328. FAO, Rome.

Bedford, M., 2000. Exogenous enzymes in monogastric nutrition-their current value and future benefits. Anim. Feed Sci. Tech. 86, 1-13.

Bergheim, A., Hustveit, H., Kittelsen, A., Selmer-Olsen, A., 1982. Estimated pollution loadings from Norwegian fish farms. I. Investigation 1978-79. Aquaculture 28, 346-361.

Beveridge, M.C.M., Phillips, M.J., Clarke, R.M., 1991. A quantitative and qualitative assessment of wastes from aquatic animal production. In: Brune, D.E., Tomasso, J.R. (Eds.), Aquaculture and Water Quality. World Aquacult. Soc. Baton Rouge, LA, pp. 506–533.

Beveridge, M.C.M., 1985. Cage and pen fish farming. Carrying capacity models and environmental impact. FAO Fish. Tech. Pap. 255, 131.

Bonsdorff, E., Blomqvist, E.M., Mattila, J., Norkko, A., 1996. Long-term changes and coastal eutrophication; examples from Aland Islands and Archipelago Sea, northern Baltic Sea, Oceanol. Acta 20, 319-329.

Bonsdorff, E., Blomqvist, E.M., Mattila, J., Norkko, A., 1997. Coastal eutrophication: causes consequences and perspectives in the archipelago areas of the northern Baltic Sea. Estuarine, Coastal Shelf Sci. 44, 63-72.

Boyd, C., Tucker, C.S., 1998. Pond aquaculture water quality management: waste management, Kluwer Academic Publishers. 541-573.

Boyd, C.E., Queiroz, J., McNevin, A., 2013. Perspectives on the responsible aquaculture movement. World Aquacult. 44, 14-21.

Boyd, C.E., McNevin, A.A., 2014. Aquaculture, resource use, and the environment. Wiley-Blackwell, New York, NY, USA.

Butz, I., Vans-Cappell, B., 1982. Organic load from the metabolic products of rainbow trout fed with dry food. In report of the EIFAC workshop on fish farm effluents. EIFAC Tech. Pap. 41, 73-82.

Casado-Martinez, M.C., Smith, B.D., Luoma, S.N., Rainbow, P.S., 2010. Bioaccumulation of arsenic from water and sediment by a deposit-feeding polychaete (*Arenicola marina*): a biodynamic modelling approach. Aquat. Toxicol. 98, 34–43.

Chamberlain, G.W., 1986. Grow-out research. Coastal Aquacult. 3, 7-8.

Chen, C.Y., Stemberger, R.S., Klaue, B., Blum, J.D., Pickhardt, P.C., Folt, C.L., 2000. Accumulation of heavy metals in food web components across a gradient of lakes. Limnol. Oceanogr. 45, 1525-1536.

Cheng, Z., Man, Y.B., Nie, X.P., Wong, M.H., 2013. Trophic relationships and health risk assessments of trace metals in the aquaculture pond ecosystem of Pearl River Delta, China. Chemosphere 90, 2142-2148.

Cheng, K.M., Hu, C.Q., Liu, Y.N., Zheng, S.X., Qi, X.J., 2005. Dietary magnesium requirement and physiological responses of marine shrimp *Litopenaeus vannamei* reared in low salinity water. Aquacult. Nutr. 11, 385-393.

Cho, C.Y., 1992. Feeding systems for rainbow trout and other salmonides with reference to current estimates of energy and protein requirements. Aquaculture 100, 107-123.

Cho, C.Y., Bureau, D.P., 2001. A review of diet formulation strategies and feeding systems to reduce excretory and feed wastes in aquaculture. Aquac. Res. 32, 349-360.

Cho, C.Y., Hynes, J.D., Wood, K.R., Yoshida, H.K., 1994. Development of high-nutrient-dense, low pollution diets and prediction of aquaculture wastes using biological approaches. Aquaculture 124, 293-305.

Davidson, J., Summerfelt, S.T., 2005. Solids removal from a cold water recirculating system-comparison of a swirl separator and a radial-flow settler. Aquacult. Eng. 33, 47-61.

Enell, M., 1987. Environmental impact of cage fish farming with special reference to phosphorus and nitrogen loadings. Common Meeting of the International Council for Exploration of Sea, CMICES/F, pp. 44.

Enell, M., Lof, J., 1983. Environmental impact of aquaculture-sedimentation and nutrient loadings from fish cage culture. Vatten. 39, 364-375.

Food and Agriculture Organization of the United Nations (FAO), 2012. The state of world fisheries and aquaculture. FAO Fisheries and Aquaculture Department. Food and agriculture organization of the United Nations. Rome.

Fernandes, C., Fontainhas-Fernandes, A., Peixoto, F., Salgado, M.A., 2007. Bioaccumulation of heavy metals in Liza saliens from the Esmoriz–Paramos coastal lagoon, Portu- gal, Ecotoxicol. Environ. Saf. 66, 426–431.

Gatlin, D.M., III, Li, P., 2008. Use of the diet additives to improve nutritional value of alternative protein sources. In: Lim, C., Webster, C.D., Lee, C.S. (Eds.), Alternative Protein Sources in Aquaculture Diets in Haworth Press. New York, pp. 501-522

Hakanson, L., Ervik, A., Makinen, T., Moller, B. 1988. Basic concepts concerning assessments of environmental effects of marine fish farms. Nordic Council of Ministers, Copenhagen, pp. 103.

Handy, R.D., Poxton, M.G., 1993. Nitrogen pollution in mariculture: toxicity and excretion of nitrogenous compounds by marine fish. Reviews in Fish Biology and Fisheries 3, 205–241.

Highland River Purification Board, 1987. Policy for controlling pollution from fish farms. Dingwall: HRPB, Tech. Rep. No. TR4/87. pp.10.

Islam, M.S., 2005. Nitrogen and phosphorus budget in coastal and marine cage aquaculture and impacts of effluent loading on ecosystem: review and analysis towards model development. Mar. Pollut. B. 50, 48–61.

Johnsen, F., Hillestad, M., Austreng, E., 1993. High energy diets for Atlantic salmon. Effects on pollution. In: Kaushik, S.J., Luquet, P., (Eds.), Fish Nutrition in Practice, Proceedings of the IVth International Symposium on Fish Nutrition and Feeding, Biarritz in INRA Editions. Paris, France. pp. 391–401.

Kirkpatrick, D.C., Coffin, D.E., 1974. The trace metal content of representative Canadian diets in 1970 and 1971. Can. Inst. Food Sci. Tech. J. 7, 56–58.

Kissil, G.W., Lupatsch, I., 1992. New approaches to fish feed in Israeli mariculture as a result of environmental constraint. Isr. J. Aquacult. Bamidgeh. 44, 125.

Lacerda, L.D., Santos, J.A., Madrid, R.M., 2006. Copper emission factors from intensive shrimp aquaculture. Mar. Pollut. Bull. 52, 1823–1826.

Lobo, J.P., 1998. How can enzymes benefit me. Feed Manag. 49, 12-16.

Marte, C.L., Cruz, P., Flores, E.E.C., 2000. Recent development in freshwater and marine cage aquaculture in the Philippines. In: Liao, I.C., Lin, C.K. (Eds.), Cage Aquaculture in Asia: Proceedings of the First International Symposium on Cage Aquaculture in Asia. Asian Fisheries Society, Manila, and World Aquaculture Society—Southeast Asian Chapter, Bangkok, pp. 21–37.

McLaughlin, T.W., 1981. Hatchery effluent treatment -U.S. Fish and Wildlife Service. In: Allen, L.J., Kinney, E.C., (Eds.), Proceeding of the Bio-Engineering Symposium for fish culture. Fish Culture Section, American Fishery Society, Bethesda, Md.

Molver, J., Stigebrandt, A., Bjerkenes, V., 1988. On the excretion of nitrogen and phosphorous from salmon. Proc. Aquaculture lnt. Congress, Aquaculture International Congress, Vancouver, BC. pp. 80.

Muller, F., Varadi, L., 1980. The results of cage fish culture in Hungary. Aquacult. Hung. 2, 154-167.

Paramatrix, Inc., 1990. Final programmatic environmental impact statement: Fish culture in floating net pens. Prepared for the Washington Department of Fisheries, Olympia, Wash.

Phillips, M., Beveridge, M., 1986. Cage and the effect on water condition. Fish Farmer 9, 17-19.

Penczak, T., Galicka, W., Molinski, M., Kusto, E., Zalewski, M., 1982. The enrichment of a mesotrophic lake by carbon, phosphorus and nitrogen from the cage aquaculture of rainbow trout, *Salmo gairdneri*. J. Appl. Ecol. 19, 371–393.

Sugiura, S., Hardy, R.W., 2000. Environmental friendly feeds. In: Stickney, R.R. (Ed.), Encyclopedia of Aquaculture. Wiley, New York, pp. 299–310.

Wang, W.N., Wang, A.L., Zhang, Y.J., 2006. Effect of dietary higher level of selenium and nitrite concentration on the cellular defense response of *Penaeus vannamei*. Aquaculture 256, 558-563.

Wu, R.S.S., Lam, K.S., MacKay, D.W., Lau, T.C., Yam, V., 1994. Impact of marine fish farming on water quality and bottom sediment: a case study of the sub-tropical environment. Mar. Environ. Res. 38, 115-145.

Zhou, H.Y., Wong, M.H., 2000. Mercury accumulation in freshwater fish with emphasis on the dietary influence. Water Res. 34, 4234–4242.

Chapter 2 Reviews of literature

1. Aquaculture feeds

Feeds currently used in aquaculture are fortified with nutrients and energy by including proteins, lipids, vitamins, and minerals for growth and various physiological functions. Formulation of manufactured feeds depends on knowing the amounts of energy and nutrients required by the animals (Li and Robinson, 2015). Because there are about 40 nutrients required for each species it is difficult to gather the information to build a sufficient database for feed composition. Nutritional databases for aquatic animals are not complete, but dietary nutritional requirements have been determined for most of the main aquaculture species (NRC, 2011).

Energy is the most important component of feed because it regulates the quantity of feed needed by animals. In theory, when dietary energy is high, feed intake usually declines. Aquatic animals can use dietary lipid and protein as energy sources more efficiently than carbohydrates. The ability to use carbohydrates as an energy source varies considerably among species (NRC, 2011). Aquatic animals do not have a specific requirement for dietary carbohydrates. However they are essential to the feed manufacturing purposes, the level of carbohydrates in feed usually depends on nutrient density of the feed. High levels of carbohydrates can be included in feeds for common carp, tilapia, catfish, but not in rainbow trout and some marine fish (Li and Robinson, 2015). Feeds for species that cannot utilize carbohydrates well often contain high concentrations of protein and lipid as primary energy sources. Lipids are highly digested by most species so they are an excellent source of energy. The amount of dietary lipid required is also affected by the

concentrations of dietary protein and carbohydrates in feeds. Although an optimum level of dietary lipid cannot be defined for a species, a number of studies have been conducted to provide a range of dietary lipid concentration in feeds.

Aquatic animals require minerals for normal life processes, and they may derive these elements from their diet and from the surrounding water (Phillips et al., 1960; Hunn and Fromm, 1966). The amount of trace elements should be maintained within narrow ranges in cells and tissues for normal metabolic activities. Minerals have a wide range of functions such as: in the formation of skeleton, maintenance of colloidal systems, regulation of acid-base equilibrium as well as for biologically important compounds such as hormones and enzymes. Mineral deficiencies have effects on biological, functional, and structural pathologies depending on several factors such as the duration and degree of mineral deprivation or excess (Hilton, 1989; Davis and Gatlin, 1996; Watanabe, 1997).

The physiological functions of minerals are well recorded for humans and some other animals (Davis and Gatlin, 1996). However, information of trace mineral requirements in aquatic animals is limited, especially because many are required only in small quantities, and excesses or deficits of these minerals can have a negative impact. Although trace minerals are needed in very small amounts, they are certainly required for normal growth. Toxicity may develop in animals, if excess amounts of these elements are assimilated (Kromhout et al., 1985). Because the mineral levels need to be maintained in balance within the body of animals, it is important to consider factors such as uptake, storage, and excretion while formulating feeds (Watanabe et al., 1997).

Trace elements are naturally present entering the aquatic environments through various geochemical processes (Ismail et al., 1999). Anthropogenic sources of metals including pollution from mining process, metal smelting, oil burning, coal and petroleum combustion and other

industrial emissions (Yang and Rose 2005) also contribute to environmental concentrations of metals. This can reduce water and sediment quality and may affect fish health and other biological attributes because of their toxicity, persistence and bioaccumulation in the food chain (Kirkpatrick & Coffin, 1974; Fernandes et al., 2007). Most aquatic animals are able to accumulate minerals from their environment directly; however, the distribution of minerals in different tissues relies on the mode of exposure such as dietary or aqueous exposure (Alam et al., 2002; Canbek et al., 2007). Moreover, trace elements are dissolved in water and absorbed into particulates in the water and in the sediment. Aquatic organisms may obtain these elements from the water directly or by ingestion of particulates from the water column or sediment (Chen et al., 2000; Casado-Martinez et al., 2010). Cheng et al. (2013) reported that pelleted feeds contained the higher concentrations of trace minerals than zooplankton, mud carp, and trash fish. Trash fish, a kind of small fish collected from capture fisheries, contained the lowest concentrations of all elements. Therefore, fish feeds can be an important source of trace minerals to aquatic species (Zhou and Wong, 2000; Lacerda et al., 2006).

There are a number of problems related to the quantification of mineral requirements, including identification of potential contribution of minerals from the water and natural food particles, leaching of minerals from diets, availability of proper concentrations for targeted species, and limited data on mineral bioavailability. The concentrations of minerals are extremely variable due to a number of factors such as the differences in the basic raw ingredients used in diet formulation and the addition of specific macro and trace mineral premixes and contaminants presented in feed ingredients.

2. Estimated waste production from aquatic feeds

It is crucial to have an accurate estimate of the quantities of wastes produced by a farm and discharged into the surrounding waters. Hakanson et al. (1988) stated that production of the major polluting agents, including organic matter, nitrogen and phosphorus, was equal to the difference between the amount of nutrients added by the feed and the amount of nutrients utilized for animal production. The waste loading entering the culture system unit can be calculated based on data on compositions of feed, feed consumption, nutrient retention and production of the cultured species. Boyd and Queiroz (2001) referred to the difference in the input an element in feed (or fertilizer) and the amount of the element is harvest biomass or the system load of the element.

Hakanson et al. (1988) calculated a mass balance for rainbow trout grown on a diet containing 50% protein, 20% fat, 12% carbohydrate and 3.8 Mcal metabolizable energy; the feed conversion ratio was 1.5; the digestibility of protein and energy were 85% and 81%, respectively. The waste load based on the production of 1 kg of fish and the quantity of organic matter is expressed as energy and BOD. The results showed that 35% of the energy is utilized for fish growth, 47% for respiration and 18% is excreted as feces. Fish utilize about 25% of dietary nitrogen, whereas 15% is lost in the feces and 60% is excreted as ammonia from the gills (Table 2.1). They also summarized the mass balance based on a daily growth rate of 0.007 kg/day. It was shown that 1 kg of trout with growth rate of 0.007 kg/day fed on 55 kcal/day would consume 6.9 g O_2/day. Assuming no removal of water from the culture unit, discharge to the receiving water would be 4.0 g BOD/day, 0.63 g N/day and 0.07 g P/day (Table 2.2).

Boyd and Tucker (1998) calculated inputs in feed and outputs in fish for dry matter, carbon, nitrogen and phosphorus in catfish pond for production of 1,000 kg live fish. By using feed conversion ratio as 2, waste loads to the pond, which were calculated from inputs in feed minus outputs in fish, represented 87.5% of dry matter, 84.9% of carbon, 81.7% of nitrogen and 79.6%

of phosphorus presented in the feed (Table 2.3). Boyd and Teichert-Coddington (1995) also estimated system waste loads for white shrimp (*Penaeus vannamei*), which were fed a low protein content diet (21.7 % crude protein) in semi-intensive farm. Waste loads to the pond consisted of 86.1% of dry matter, 88.5% of carbon, 54.7% of nitrogen and 78.7% of phosphorus contained in feed (Table 2.4). In case of intensive shrimp farms where it was common to use feed with higher protein contain (30-40% crude protein), nitrogen waste loading would be greater (Boyd and Teichert-Coddington, 1995).

Waste loads from feed can be assimilated by processes in ponds. Uneaten feed, feces, as well as dead phytoplankton and cultured animals can be decomposed by microorganisms in the water column or in the sediment. The organic matter will be converted to mineral components, including water, carbon dioxide, ammonia and phosphates. Some parts of the organic matter will be degraded to dissolved organic matter. Detritus and dissolved organic matter also can be lost from ponds during overflowing water or draining of ponds (Boyd and Tucker, 1998).

It is difficult to determine the amount of feed waste in fish culture because uneaten food cannot be easily separated from other solid wastes (Merican and Phillips, 1985). The types of feed used along with the feeding strategies applied are major factors in determining waste loading. Wastage can ranged from 1 to 38%, depending on the feed type, feed practice, culture method and animal species (Warren-Hansen, 1982a; Ove Arup *et al.,* 1989; Thorpe *et al.,* 1990; Beveridge *et al.,* 1991). Warrer-Hansen (1982a, b) reported that values of wasted feeds were 1-5% in dry diets and 10-30% in moist diets. The amount of feed waste generated by aquaculture farm is related to various factors such as stocking density, feeding regime, and feeding rate as these three factors together control the total amount of feeds to be used (Wu, 1995). The amount of fecal wastes

produced is determined by two important factors; the actual amount of supplied feed, which is consumed by fish, and its digestibility.

3. Environmental impacts related to aquatic feeds

The addition of nutrients to natural waters is one of the most significant impacts arising from aquacultural wastes as it causes an increase in plankton and microbial populations ultimately having an adverse effect on our environment (Enell, 1987; Cohen and Neori, 1991; Boyd and McNevin, 2015). When the fish cannot consume all feed provided, the waste settles and accumulates at the bottom of the water body (Wu et al., 1994). Various nutrients leach from feed pellets polluting the waters; however, the organic components added in the pellet are eventually decomposed by microorganisms (Moriarty, 1997; Burford and Glibert, 1999). Of the feed that is eaten, a proportion of it is indigestible and excreted as solid fecal waste that adds to the nutrient loading into the surrounding environment. Some nutrients absorbed by the animal's body are used in metabolic processes and the rest for tissue growth. This process also produces wastes that enter the water in dissolved form (Cho, 1994).

3.1 Effluent discharges

Considerable attention has been given to the effects of effluent discharge from certain types of aquaculture sites, especially in marine or freshwater cage culture (FAO-NACA, 1995). Nutrients and particulate matter are brought in the culture systems with the water supply but this input typically is smaller than the input of nutrients and organic matter from feed (Phillip, 1997). Waste feed is mineralized along with accumulated fecal matter, adding to the nutrient content of the water for natural organic production by photosynthesis (Butz and Vens-Cappell, 1982). At a well-managed farm, nutrients from feed wastage can be minimized (Warren-Hansen, 1982; Ove Arup et al., 1989; Thorpe et al., 1990; Beveridge et al., 1991). The initial waste released during

aquaculture consists of matter (feces and uneaten feed) and metabolic wastes (Beveridge, 1996; Alongi et al., 2003). The solid waste, containing mainly of organic carbon and nitrogen compounds, may remain suspended or settle and accumulate in the sediments. But some of the solid waste decomposes into particulate or soluble components or soluble compounds leach from them (Lawrence and Lee, 1997). Thus, the discharge from aquaculture system contains soluble and particulate organic matter, and in ponds much of the particulate matter consists of microscopic organisms.

The biological oxygen demand (BOD) of waste, which is a measure of oxygen required by microorganisms to decompose organic matter, is an important variable for assessing the waste pollution strength of an effluent. An increase in BOD results in a decline in dissolved oxygen concentration and often occurs near the effluent outfall of aquaculture farms (Muller and Varadi, 1980; Bergheim et al., 1982; Beveridge, 1985; Molver et al., 1988). Dissolved oxygen concentrations are often returned to normal within 30 m downstream (Gowen and Bradbury, 1987).

3.2 Hypernutrification and Eutrophication

Hypernutrification and eutrophication can result in natural waters receiving aquaculture effluents. A substantial increase in the concentration of dissolved nutrients is called hypernutrification, and its consequence is to increase phytoplankton growth and productivity, which is called eutrophication (Pillay, 2004). The main effect of eutrophication is a decrease in dissolved oxygen concentration (Muller and Varadi, 1980; Bergheim et al., 1982; Gowen and Bradbury, 1987). Organic matter also accumulates in the sediment near aquaculture effluent outfalls resulting in increased sediment oxygen demand that can result in anoxic sediment (Wu, 1990a; Wu *et al.,* 1994). This leads to important changes in the biological and chemical processes in the sediment and on the ecology of benthic organisms.

The main sources of organic material in sediments at cage culture sites are feed and feces (Brown et al., 1987; Vethe et al., 1990; Frogh, 1991). Thus, water under marine cage farms – especially where water turbulence is low – can become anoxic for long periods (Gowen and Bradbury, 1987). In anoxic environments, reduced substances produced by microbial metabolism are released into the water. Some of these substances, in particular ammonia, nitrite, and hydrogen sulfide, can be toxic to aquatic animals. Enell and Lof (1983) estimated the rate of ammonia release under freshwater cage farms as 2.6 to 3.3 times greater than from undisturbed oxic sediments.

Bubbles often are released from sediments beneath fish cages. Schaaning (1991) mentioned that bubbles released from sediments contained 60-80% methane. These bubbles have been reported to contain large amounts of carbon dioxide, methane, and hydrogen sulfide (Hall et al., 1990). Schaaning (1991) also found that the bubbles consisted of about 10-20% carbon dioxide and 1-5% hydrogen sulfide. It is recognized that hydrogen sulfide gas from the sediments can damage the gills of the fish (Braaten et al., 1983).

Boyd (1995) stated that most phosphorus added to ponds is soon bound to the sediment. Soluble P is taken up by plants for growth, or bind to Fe^{3+}, Al^{3+}, Ca^{2+} or colloids. A number of studies showed that the most important pools of P in the system were fish and pond sediments (Boyd, 1995). Since fish were harvested, the P contained in their biomass was removed from a pond system. However, over half of the P input, mainly from aquatic feed, becomes bound to the sediments in an insoluble form. The ability of sediment to absorb P is considerable; however, it is possible to saturate soil with P and diminish its absorption capacity. Phosphorus binds strongly with the mineral particles under oxygenated conditions. This situation changes steadily as the water over the soil becomes anoxic.

3.3 Algal bloom

The negative effect of algal blooms is the possible release of toxins, which may toxic to cultured animals, or in the case of molluse, accumulate in their flesh (Pillay, 2004). The major concern caused by algal blooms is dinoflagellates or red tides, which affects both the farming site and also the surrounding environment (Pillay, 2004). For instance, the catastrophic blooms of *Chrysochromulina polylepis* wiped out entire salmon farms in Norway (Pillay, 2004). The red tide from dinoflaggellate *Pyrodinium bahamense* caused paralytic shellfish poisoning (PSP) in many countries around the world (Khoo, 1985). Similarly, blooms of *Gyrodinium aureolum* resulted in the mortality of marine organisms in northern Europe, but there has not been a significant relationship with the discharges from aquaculture industries (Tangen, 1977; Doyle et al., 1984).

4. Case Studies

4.1 Pollution problems related to marine cage culture practice

Being an essentially open-culture system, cage culture has more potential to cause negative impacts in both onshore and offshore environments (Aldridge, 1988; Islam, 2005). Cage aquaculture produces wastes rich in organic matter and nutrients that are directly released into the environment (Soley et al., 1994; Wu et al., 1994; Wu, 1995). The adverse effects of cage culture include eutrophication, cross-transmission of parasites and pathogens, and contamination by xenobiotics (Wu, 1995). However, such environmental impacts can be minimized through good feeding practices and environmental monitoring to maintain the suitable conditions for farms stocks in the cages and to preserve the surrounding environment.

Most marine cage culture takes place in open coastal waters. Unlike pond aquaculture systems, cage culture does not use organic and inorganic fertilizers (Ackefors and Enell, 1990). The major problems resulting from marine cage culture are those to the local environment around the farms, predominantly on the benthos (Gowen and Bradbury, 1987). Wu (1995) estimated that

85% of phosphorus, 80-88% of carbon, and 52-95% of nitrogen input into a marine fish culture might be lost into the environment. In a separate study, loadings of nitrogen and phosphorus from a Northern Ireland fish farm were reported as 124.2 and 25.6 kg/ton/yr respectively (Foy and Rosell, 1991). Oxygen demand of bottom sediments in fish cage farming can be 2-5 times more than in control areas (Wu, 1990a; Wu *et al.,* 1994). Sediment metabolism has been observed to be 10 times higher under cage culture than in control areas (Holmer & Kristensen, 1992).

Changes in macrobenthic community structure in cage farm areas also have been reported (Brown *et al.*, 1987; Weston, 1990). The impacts of nutrient loadings may persist for many years after the culture activity has ceased. Guo and Li (2003) carried out research in a static lake in China that contain 20,000 m^2 of cage area and produced 16 metric tons of fish. They reported the impact of cage culture was higher near the farm site and extended 20 m outside the cage area. This conclusion was supported by Leung et al (1999) who found high levels of nutrient loading e.g., nitrate, nitrite, ammonia, and reactive phosphate near fish culture sites in many parts of the world. Problems arising from high organic and nutrient loading also may cause conflicts with other resource users in coastal zones (Hammond, 1987; Waldichuck, 1987a, b; Morton, 1989; Miki et al., 1992).

4.2 Pond effluents from shrimp farm

Effluents from shrimp farms have received considerable attention because of their highly polluting nature (Naylor et al., 1998). The use of fertilizers and artificial feeds results in the build-up of organic matter, leading to hypernutrification and eutrofication (Penczak et al., 1982; Tacon and Akiyama, 1997). The large amount of organic matter in effluents will increase the suspended solids and nutrient levels, decrease the dissolved oxygen level, as well as increase the BOD in coastal waters (Muller & Varadi, 1980; Bergheim *et al.,* 1982). This eventually may cause the

bottom sediments to become anoxic leading to changes in the benthic community (Phillips and Beveridge 1986; Molver *et al.,* 1988).

Hypernutrification may cause a change in the composition of plankton species, whereas eutrophication may lead to phytoplankton blooms (Enell, 1987). Occurrences of harmful algal bloom in aquaculture areas are reported to be increasing. In Malaysia in 1983, red tide occurred in the coastal areas, which resulted in mass mortality of shrimp in shrimp farms located in the vicinity (Khoo, 1985). Over the last two decades, red tide incidences have also increased in China, and this has been linked with the expansion of mariculture (Qi et al., 1992).

Although effluents from shrimp farms have been related to high organic load, a number of studies have shown that the level of pollution from shrimp farms is less than the effluents from some other agricultural activities (Ho et al., 1984; Chew and Yeoh, 1987; Tookwinas, 1998; Choo and Tanaka, 2000). For instance, intensive shrimp farming in Thailand contributed only 0.1% phosphorus and 0.5% nitrogen to the total nitrogen loading from major rivers of the central region (Phillips, 1997). Studies in China indicated that the shrimp farms contributed only 1.2-5.1% of total COD load in two coastal provinces (FAO-NACA, 1995, cited from Phillips, 1997). Because of the large volume of effluents from shrimp ponds, recent advances in shrimp farming have developed and incorporated semi-closed or closed systems in the grow-out phase with minimal water exchange. In Thailand, semi-closed or closed systems are normally used in areas that are prone to spread disease and related problems (Kongkeo, 1995). Water from the sea is pumped into reservoirs and treated before being added into ponds.

5. Practical feed management to minimize waste loading

As aquaculture operations continue to expand, feeds become a more important part of intensive farming (Chamberlain, 1993; Lawrence and Lee, 1997). Presently, most farms rely on

commercial feed, and it is important to use good management proctices (Avault, 1996; Marte et al., 2000, Islam, 2005). It is necessary for cultured species to be fed to reach their optimal growth requirements, but efforts should be made to minimize waste to the environment. Unconsumed feed also results in an economic loss and contributes to degradation of the rearing environment (Ballestrazzi et al., 1994). In order to estimate proper amount of feed input, monitoring of growth, feed delivery and waste outputs need to be well managed (Hensey, 1992; Bureau et al., 2003; Hua and Bureau, 2006).

5.1 Feed management

The purpose of feeding is to provide required nutrients that maintain good health and optimal growth of cultured species while at the same time minimizeing wastes and optimizing profits. Feed wastage is one of the most important factors leading to organic and nutrient loadings (Ackefors & Enell, 1990; Seymour & Bergheim, 1991). One of the obvious differences between feeding aquatic animals and feeding terrestrial animals is that once aquatic animals have been fed, the excess feeds cannot be retrieved from the water. Therefore, in order to reduce nutrient loading into the environment, it is important to feed cultured species with the correct amounts.

Feed conversion ratio (FCR) is a measure of an animal's efficiency in converting feed into harvestable biomass (Avault Jr., 1996) i.e. the amount of feed required to produce a unit of fish. The lower the FCR, the more efficient the cultured species is in converting feed into flesh, leading to less feed wastage released into the environment. Feed conversion ratio is affected by species, culture systems and water quality. Beveridge (1984) demonstrated that FCR of trout reared in cage was approximately 20% higher than in ponds because of the greater proportion of feed wasted in ponds. Moreover, FCR can provide important information on the management at a farm. It is an

indicator of how well feed is being used and as indirect indicator of animal health and waste production.

Because of improvement in nutrition and feed manufacturing technology, the feed conversion ratio can be reduced to 1.0-1.4 with several species both in freshwater and seawater. In many Northern European countries, the FCR has been decreased from 2.3 to less than 1.3 because of improved knowledge. The nitrogen and phosphorus content of commercial feeds also have decreased because of better science and technology. As a result, the discharge of nitrogen and phosphorus are less than 53 kg and 10 kg per tonne of fish produced, respectively (Ackefors and Enell, 1994).

Feed wastage can be reduced by enhancing the stability and decreasing the sinking rate of feed, as well as providing an optimal size of feed at different stages of development (Wu, 1995). Feed is the most expensive aquaculture input and a cause of water pollution (Lawrence and Lee, 1997). Therefore, it is necessary to feed optimally to assure good growth of animals with a minimum of feed wastage (Naylor et al., 1998). Optimal feeding requires detailed knowledge in many aspects such as feeding behavior of the fish, information on environmental conditions, and the density and size of fish in culture system.

5.2 Change in diet composition

Feed ingredients play a role in waste production. Fishmeal is one of the most common protein sources utilized in commercial feed because of its high protein content, balanced amino acid profile, high digestibility and palatability (Abdelghany, 2003). There is considerable concern over the sustainability and level adequacy of fishmeal supplies for the future Moreover, high level of phosphorus (16.7-42.1 mg/g) in fishmeal leads to large amounts of phosphorus release (Uyan et al., 2006). Hence, one strategy to reduce phosphorus output from aquaculture operations is to

reduce fishmeal levels in aquatic feeds (Jauncey and Ross, 1982; Fontainhas- Fernandes et al., 1999; Coyle et al., 2004; Brinker and Reiter, 2011).

The use of renewable plant-based proteins has become the focus of protein substitution studies in aquatic feeds, because they are less expensive, have acceptable protein level and amino acid ratios, are of consistent quality, and cause less pollution (Watanabe, 2002). Vegetable protein sources are an efficient approach to reduce nutrient pollution from aquaculture farming. Soybean meal contains lower levels of phosphorus (6.8 mg/g) compared to fishmeal (16.7-42.1 mg/g) (Davis and Kurmaly, 1993). However, the dietary phosphorus in soybean is present in the form of phytate that is poorly available to aquatic animals. Supplementation of enzyme into diet can hydrolyze phytate to digestible phosphorus. Sookying and Davis (2011) indicated that use of high levels of soybean meal as a main protein source in formulated diets for *L. vannamei* is a viable way to provide essential nutrients in diet that are properly balanced to meet shrimp nutritional requirements.

Finding the minimal phosphorus requirement for aquatic animals is complicated because the availability of phosphorus in feed ingredients to cultured species varies considerably (NRC, 1993). Phosphorus can interact with other elements, such as calcium, and be influenced by pH of the gastrointestinal tract (Sugiura, 1998). Phytate is the organic form of phosphorus, which accounts for 67% of phosphorus in plant-based ingredients. Phytate is not available to aquatic animals because they lack phytase, the enzyme required to liberate phosphorus from phytase. Adding phytase enzyme into feed formulation has been shown to increase phosphorus availability to several aquatic species (Eya and Lovell, 1997). Storebakken (1998) studied the availability of phosphorus in fishmeal and soybean meal supplemented with phytase in Atlantic salmon (*Salmo*

salar) and found a difference in fecal and metabolic excretion between fish fed fishmeal-based diet (10.5 g/kg gain) and those fed soybean meal with phytase diet (7.2 g/kg gain).

Nitrogen excretion by animals is a function of protein intake, and protein content in feeds should not be higher than necessary. Thus, high energy and low pollution diets can improve wastewater quality and also reduce production costs. High energy or nutrient-dense diet are formulated on the protein sparing principle. This diet typically contains higher lipid and carbohydrate levels and lower concentration of protein than do traditional feeds (Kissil & Lupatsch, 1992; Cho et al., 1994; Anderson and De Silva, 1997).

A properly formulated low protein content in feed does not negatively affect fish growth as long as feed intake is not limited, and it also leads to less nitrogen excretion (Robinson and Li, 1997). It has been reported that the level of protein retention in *Sparus aurata* can be increased from 24.3 to 31.3% by increasing the dietary lipid by 37% (Kissil and Lupatsch, 1992). The protein content of the whole fish seems to be fixed for each cultured species at a given size (Shearer 1994). Helland and Grisdale-Helland (1998) indicated that increasing levels of dietary protein are used less efficiently for growth in juvenile Atlantic salmon. They also reported that diets with more protein and less fat led to fish lower carcass and visceral fat. Shyong et al. (1998) observed that muscle protein was increased by feeding higher protein level diets in juvenile *Zacco babata* but fish weight also increased; therefore, the higher protein levels appear to be result from fish size alone.

On the contrary, Webster et al. (1997) reported that high levels of dietary protein resulted in elevated body protein. An increase in dietary protein can enhance growth up to the point where protein intake exceeds the ability of animals to synthesize additional protein (Kestin and Warriss, 2001). At this point, the addition of protein is deaminated and stored as fat. Kaushik et al. (1995)

indicated that high levels of protein might suppress growth. The source of protein seems to have insignificant effect on body protein content (Kestin and Warriss, 2001). Smith et al. (1988) fed rainbow trout with isonitrogenous and isoenergetic diets containing plant or animals protein, and found no difference in carcass composition corresponding to the diets.

5.3 Feed processing technology

Advanced processing methods for commercial feeds are of importance in minimizing feed wastages (Tacon and Akiyama, 1997). Pellet water stability is an important factor contributing to the quality of feeds. High water stability of pellets favors longer retention and the physical integrity of pellets, leading to less nutrient leaching into the water (Lawrence and Lee, 1997). Poor stability and high solubility of feed pellets results in greater nutrient loss to surrounding environment. During the last decade, there has been increased use of extruded feeds in aquaculture. The extruded pellet has a great floating property, slow sinking rate and high water stability; hence, it becomes available to cultured species more effectively than compressed pellets.

5.4 Feed storage and handling

The feed efficiency can be affected by poor feed handling and storage. Rough handling causes physical damage to pellets, leading to an increase in the amount of fines in feeds. Fine particles of feed are wasted, as fish cannot consume them. The fine is suspended easily in the water but not directly available to culture animals. If feed is stored for a long time or under poor conditions, such as high humidity or excessive heat, before use, its nutritional quality can be impaired. This results in a greater proportion of the feed being voided as feces. High humidity enhances fungal growth and loss in quality of feed. Food palatability also has an effect on feed efficiency by reducing feed intake and increasing the proportion of wasted feed. Pelleted feeds should be used within their recommended shelf lives.

6. Sustainability

Aquaculture currently produces about 50% of global fish production for human consumption. Aquaculture, in common with other food production, is facing challenge for sustainable development in which production should be environmentally and socially responsible. Awareness of potential environmental problems from aquaculture operations has increased significantly. Efforts are under way to improve resource use and environmental management in aquaculture.

Aquaculture has been reported to discharge large amount of wastes containing organic matter and nutrients into the environment (Gowen and Bradbury, 1987; Wu et al., 1994; Wu, 1995; Leung et al., 1999). Aquaculture produces much less pollution than many other aspects of the world food system and various other major human endeavors (Boyd and McNevin, 2015). Nevertheless, Boyd and McNevin (2015) emphasized that aquaculture can be responsible for considerable water pollution at the local level resulting in negative impacts on aquatic biodiversity. Aquaculture, as all industries, should be held accountable for water pollution and other negative environmental impacts by being expected to use better management practices, comply with governmental environmental standards, and monitor operations to determine whether or not they are causing negative impacts.

Table 2.1 Mass balance for energy, organic matter (BOD), nitrogen and phosphorus from the production of 1 kg of rainbow trout (Hakanson et al., 1988).

	Energy (kcal kg^{-1})	BOD (g kg^{-1})	Nitrogen (g N kg^{-1})	Phosphorus (g P kg^{-1})
Feed used	7829	2416	120.0	15.0
Growth	2746	848	29.6	4.5
Faeces	1439	444	18.0	10.5
Excretion, ammonia	430	133	72.4	-
Respiration	3213	992	-	-
Waste load:				
Feces and excretion	1869	577	90.4	10.5

Table 2.2 Mass balance for energy, organic matter (BOD), nitrogen and phosphorus from the production of 1 kg of rainbow trout based on a daily growth rate of 0.007 kg (Hakanson et al., 1988).

	Energy (kcal/kg/day)	BOD (g/kg/day)	Nitrogen (g N/kg/day)	Phosphorus (g P/kg/day)
Feed used	54.8	16.9	0.84	0.11
Growth	19.2	5.9	0.21	0.03
Faeces	10.1	3.1	0.13	0.07
Excretion, ammonia	3.0	0.9	0.51	-
Respiration	22.5	6.9	0.51	-
Waste load:				
Feces and excretion	13.1	4.0	0.63	0.07

Table 2.3 Inputs, outputs and loadings of carbon, nitrogen and phosphorus for the production of 1,000 kg channel catfish calculated by using feed conversion ratio of 2:1 (Boyd and Tucker, 1998).

	Amount			Amount		
Input	%	kg	Output	%	kg	Loading (kg)
Feed		2000	Live fish		1000	
Dry matter	91.7	1834		23.0	230	1640
Carbon	45.8	840		55.0	126.5	713.5
Nitrogen	5.58	102.3		8.15	18.7	83.6
Phosphorus	0.87	15.96		1.42	3.26	12.7

Table 2.4 Inputs, outputs and loadings of carbon, nitrogen and phosphorus for the production of 1,000 kg *Penaeus vannamei* calculated by using feed conversion ratio of 2:1 (Boyd and Teichert-Coddington, 1995).

	Amount			Amount		
Input	%	kg	Output	%	kg	Loading (kg)
Feed		2000	Live fish		1000	
Dry matter	92.0	1840		25.5	255	1585
Carbon	52.1	959		43	110	849
Nitrogen	3.47	64		11.2	29	35
Phosphorus	0.82	15		1.25	3.2	11.8

References

Abdelghany, A.E., 2003. Partial and complete replacement of fishmeal with gambusia meal in diets for red tilapia *Oreochromis niloticus* × *O. mossambicus*. Aquacult. Nutr. 9, 145–154.

Ackefor, H., Enell, M., 1994. The release of nutrients and organic matter from aquaculture systems in Nordic countries. J. Appl. Ichthyol. 10, 225-41.

Ackefors, H., Enell, M., 1990. Discharge of nutrients from Swedish fish farming to adjacent sea areas. Ambio. 19, 28-35.

Alam, M.G.M., Tanaka, A., Allinson, G., Laurenson, L.J.B., Stagnitti, F., Snow, E.T., 2002. A comparison of trace element concentrations in cultured and wild carp (*Cyprinus carpio*) of Lake Kasumigaura, Japan. Ecotoxicol. Environ. Saf. 53, 348–354.

Aldridge, C. H., 1988. Atlantic salmon pen strategies in Scotland. Proc. Aquaculture International Congress, Aquaculture International Congress, Vancouver, BC, 28 pp.

Alongi, D.M., Chong, V.C., Dixon, P., Sasekumar, A., Tirendi, F., 2003. The influence of fish cage aquaculture on pelagic carbon flow and water chemistry in tidally dominated mangrove estuaries of peninsular Malaysia. Mar. Environ. Res. 55, 313–333.

Avault Jr, J.W., 1996. Fundamentals of Aquaculture: A step by step guide to commercial aquaculture, AVA Publishing Company Inc. Baton Rouge, Louisiana, USA.

Ballestrazzi, R., Lanari, D., Agaro, E.D., Mion, A., 1994. The effect of dietary protein level and source on growth, body composition, total ammonia and reactive phosphate excretion of growing sea bass (*Dicentrachus labrax*). Aquaculture 127, 197-206.

BC Ministry of Environment, 1990. Environmental Management of Marine Fish Farms. British Columbia Ministry of Environment, Victoria (Canada), 28 pp.

Bergheim, A., Hustveit, H., Kittelsen, A., Selmer-Olsen, A., 1982. Estimated pollution loadings from Norwegian fish farms. I. Investigation 1978-79. Aquaculture 28, 346-361.

Beveridge, M. C. M., 1985. Cage and pen fish farming. Carrying capacity models and environmental impact. FAO Fish. Tech. Pap. 255, 131.

Beveridge, M.C.M., Phillips, M.J., Clarke, R.M., 1991. A quantitative and qualitative assessment of wastes from aquatic animal production. In: Brune, D.E., Tomasso, J.R. (Eds.),

Aquaculture and Water Quality. The World Aquaculture Society. Baton Rouge, LA, pp. 506–533.

Beveridge, M.C.M., 1996. Cage Aquaculture. Blackwell Science, Oxford. 345 pp.

Beveridge, M.C.M., 1984. Cage and pen fish farming. Carrying capacity models and environmental impact. FAO Doc. Tech. Peches. No. 255. FAO, Rome, pp. 126.

Boyd, C.E., 1995. Bottom soils, sediment and pond aquaculture. Chapman & Hall. New York, pp. 341.

Boyd, C.E., 1990. Water quality in ponds for aquaculture. Auburn University, Alabama Agricultural Experiment Station, pp. 482.

Boyd, C.E., 1985. Chemical budget for channel catfish ponds. Trans. Amer. Fish. Soc. 114, 291-8.

Boyd, C.E., Queiroz, J., 2001. Nitrogen and phosphorus loads by system, USEPA should consider system variables in setting new effluent rules. Global Aquaculture Advocate 4, 84-86.

Boyd, C.E., Teichert-Coddington, D., 1995. Dry matter, ash and elemental composition of pond-cultured *Penaeus vannamei* and *P. stylirostis*. J. World Aquacult. Soc. 26, 88-92.

Boyd, C., Tucker, C.S., 1998. Pond aquaculture water quality management: waste management, Kluwer Academic Publishers. 541-573.

Braaten, B., Aure, J., Ervik, A., Boge, E., 1983. Pollution problems in Norwegian fish farming. ICES CM 1983/F. 26, 11.

Brinker, A., Reiter, R., 2011. Fish meal replacement by plant protein substitution and guar gum addition in trout feed, Part I: Effects on feed utilization and fish quality. Aquaculture 310, 350-360.

Brown, J. R., Gowen, R. J., McLusky, D. S., 1987. The effect of salmon farming on the benthos of a Scottish sea loch. J. *Exp. Mar. Biol. Ecol.* 109, 39-51.

Bureau, D.P., Gunther, S.J., Cho, C.Y., 2003. Chemical composition and preliminary theoretical estimates of waste outputs of rainbow trout reared in commercial cage culture operations in Ontario. N. Am. J. Aquacult. 65, 33–38.

Burford, M.A., Glibert, P.M., 1999. Short-term N uptake and regeneration in early and late growth phase shrimp ponds. Aquacult. Res. 30, 215–227.

Butz, I., Vans-Cappell, B., 1982. Organic load from the metabolic products of rainbow trout fed with dry food. In: Alabaster, J.S. (Ed.), report of the EIFAC workshop on fish farm effluents. EIFAC Tech. Pap. 41, 73-82.

Casado-Martinez, M.C., Smith, B.D., Luoma, S.N., Rainbow, P.S., 2010. Bioaccumulation of arsenic from water and sediment by a deposit-feeding polychaete (*Arenicola marina*): a biodynamic modelling approach. Aquat. Toxicol. 98, 34–43.

Canbek, M., Demir, T.A., Uyanoglu, M., Bayramoglu, G., Emiroglu, O., Arslan, N., Koyuncu, O., 2007. Preliminary assessment of heavy metals in water and some Cyprinidae species from the Porsuk River, Turkey, J. Appl. Biol. Sci. 1, 91–95.

Chamberlain, G.W., 1993. Aquaculture trends and feed projections. World Aquacult. 24, 19–29.

Chen, C.Y., Stemberger, R.S., Klaue, B., Blum, J.D., Pickhardt, P.C., Folt, C.L., 2000. Accumulation of heavy metals in food web components across a gradient of lakes. Limnol. Oceanogr. 45, 1525–1536.

Cheng, Z., Man, Y.B., Nie, X.P., Wong, M.H., 2013. Trophic relationships and health risk assessments of trace metals in the aquaculture pond ecosystem of Pearl River Delta, China. Chemosphere 90, 2142-2148.

Chew, T.Y., Yeoh, B.G., 1987. Palm oil mill effluent as a source of bioenergy. Processings of the National Symposium on Oil Palm By-products for agro-based Industry, pp. 155-168.

Cho, C.Y., 1992. Feeding systems for rainbow trout and other salmonids with reference to current estimates of energy and protein requirements. Aquaculture 100, 107-123.

Cho, C.Y., Bureau, D.P., 2001. A review of diet formulation strategies and feeding systems to reduce excretory and feed wastes in aquaculture. Aquacult. Res. 32, 349–360.

Cho, C.Y., Hynes, J.D., Wood, K.R., and Yoshida. 1994. Development of high-nutrient-dense, low pollution diets and prediction of aquaculture wastes using biological approaches. Aquaculture 124, 293-305.

Cholik, F., Poernomo, A., 1986. Development of aquaculture in mangrove areas and its relationship to its mangrove ecosystem. In: Mepham, R.H., Petr, T. (Eds.). The paper contributed to the workshop on strategies for management of fisheries and aquaculture in mangrove ecosystems. Bangkok, Thailand, pp. 93-104.

Choo, P.S., Tanaka, K., 2000. Nutrient levels in ponds during the grow-out and harvest phase of *Penaeus monodon* under semi-intensive or intensive culture. Japanese International Research Center for Agricultural Sciences (JIRCAS) 8, 13-20.

Cohen, I., Neori, A., 1991. *Ulva lactuca* biofilter for marine fishpond effluents. I. Ammonia uptake kinetics and nitrogen content. Bot. Mar. 34, 475-482.

Coyle, S.D., Mengel, G.J., Tidwell, J.H., Webster, C.D., 2004. Evaluation of growth, feed utilization, and economics of hybrid tilapia, *Oreochromis niloticus* × *Oreochromis aureus*, fed diets containing different protein sources in combination with distillers dried grains with solubles. Aquacult. Res. 35, 365– 370.

Davis, D.A., Gatlin, III, D.M., 1996. Dietary mineral requirements of fish and marine crustaceans. Fisheries Sci. 4, 75-99

Davis, D.A., Kurmaly, K., 1993. Advances in mineral nutrition for aquatic species. Conference Contribution at VICTAM ASIA'93. 25 October, Bangkok, Thailand.

Doyle J. et al. 1984. The impact of blooms on mariculture in Ireland. ICES Special Meeting on the Causes, Dynamics and effects of exceptional marine blooms and related events. Copenhagen.

Eliason, E.J., Higgs, D.A., Farrell, A.P., 2007. Effect of isoenergetic diets with different protein and lipid content on the growth performance and heat increment of rainbow trout. Aquaculture 272, 723-736.

Enell, M., 1995. Environmental impact of nutrients from Nordic fish farming. Water Sci. Tech. 31, 61-71.

Enell, M., 1987. Environmental impact of cage fish farming with special reference to phosphorus and nitrogen loadings. Common Meeting of the International Council for Exploration of Sea, CMICES/ F, 44.

Enell, M., Ackefors, H. 1991. Belastning av fosfor och kveve fran fiskodlingar i Norden, pa omgivande havsomraden. In: Hoffman E., Persson, R., Gaard, E., Jonsson, G.S., (Eds). Nordisk Ministerrad, Kobenhavn. Havbrug og Miljo. pp. 83-101.

Enell, M., Lof, J., 1983. Miljoeffekter av vattenbruk—sedimentation och narsaltbelastning from fishkasseodingar. Vatten 39, 364-375.

Eya, J.C., Lovell, R.T., 1997. Net absorption of dietary phosphorus from various inorganic sources and effect of fungal phytase on net absorption of plant phosphorus by channel catfish *Ictarulus punctatus*. J. World Aqucult. Soc. 28, 386-391.

FAO-NACA., 1995. Regional study and workshop on the environmental assessment and management of aquaculture development. Food and Agricultural Organization of the United Nations and Network of Aquaculture Centers in Asia-Pacific, Bangkok, Thailand.

Fontainhas-Fernandes, A., Gomes, E., Reis-Henriques, M.A., Coimbra, J., 1999. Replacement of fishmeal by plant proteins in the diet of Nile tilapia: digestibility and growth performance. Aquacult. Int. 7, 57–67.

Foy, R.H., Rosell, R., 1991. Loadings of nitrogen and phosphorus from a Northern Ireland fish farm. Aquaculture 96, 17-30.

Fernandes, C., Fontainhas-Fernandes, A., Peixoto, F., Salgado, M.A., 2007. Bioaccumulation of heavy metals in Liza saliens from the Esmoriz–Paramos coastal lagoon, Portu- gal, Ecotoxicol. Environ. Saf. 66, 426–431.

Frogh, M., 1991. Nedbrytning av organisk material I bunnsedimenter pavirket av havbruksvirksomhet. In: Hoffman E., Persson, R. Gaard, E., Jonsson, G.S. (Eds). Nordisk Ministerrad, Kobenhavn. Havbrug og Miljo. Nord. pp. 10.

Gowen, R.J., Bradbury, N.B., 1987. The ecological impact of salmonid farming in coastal waters: a review. Oceanography and Marine Biology Annual Review 25, 563–575.

Guo, L., Li, Z., 2003. Effects of nitrogen and phosphorus from fish cage-culture on the communities of a shallow lake in middle Yangtze River basin of China. Aquaculture 226, 201–212.

Hakanson, L., Ervik, A., Makinen, T., Moller, B. 1988. Basic concepts concerning assessments of environmental effects of marine fish farms. Nordic Council of Ministers, Copenhagen, pp. 103.

Hall, P.O.J., Anderson, L.G., Holby, O., Kouberg, S., Samuelson, M.O., 1990. Chemical flux and mass balances in a marine fish cage farm. I. Carbon. Mar. Ecol. Prog. Ser. 61, 61-73.

Hammond, L. S., 1987. Mariculture pollution. Mar. Pollut. Bull. 18, 148.

Handy, R.D., Poxton, M.G., 1993. Nitrogen pollution in mariculture: toxicity and excretion of nitrogenous compounds by marine fish. Reviews in Fish Biol. Fisheries 3, 205–241.

Helland, S.J., Grisdale-Helland, B., 1998. The influence of replacing fish meal in the diet with fish oil on growth, feed utilization and body composition of Atlantic salmon (*Salmo salar*) during the smoltification period. Aquaculture 162, 1-10.

Hensey, M.P., 1992. Environmental monitoring for fish farms in Ireland. In: Depauw, N., Joyce, J. (Eds.). *Aquaculture and the Environment.* Special Publ. Eur. Aquacult. Soc. 16, 145-154.

Hilton, J.W., 1989. The interaction of vitamins, minerals and diet composition in the diet of fish. Aquaculture 79, 1-4.

Ho, Y.C., Chee, Y.S., Chong, C. N., Choy, C.K., Yeoh, B.G., Yew, K.K., 1984. Environmental, social and economic considerations in the management of swine wastes in Malaysia. Proceedings of the Regional Seminar on the Technical Utilization and Management of Agricultural Wastes.

Holmer, M., Kristensen, E., 1992. Impact of marine fish cage farming on metabolism and sulfate reduction of underlying sediments. Mar. Ecol. Prog. Ser. 80, 191-201.

Hua, K., Bureau, D.P., 2006. Modelling digestible phosphorus content of salmonid fish feeds. Aquaculture 254, 455-465.

Hunn, J.B., Fromm, P.O., 1966. In vivo uptake of radioiodide by rainbow trout. J. Water Pollut. Contr. Fed. 38, 1981-1985.

Ibrekk, H.O., Molvar, J., Faafeng, 1990. Nutrient loading to Norwegian coastal waters, and its contribution to the pollution of the North Sea. In: Processings from international conference

on North Sea pollution “Technical strategies for improvement”. Amsterdam. Aquatech 90. pp. 680.

Islam, M.S., 2005. Nitrogen and phosphorus budget in coastal and marine cage aquaculture and impacts of effluent loading on ecosystem: review and analysis towards model development. Mar. Pollut. B. 50, 48–61.

Ismail, A., Yap, C.K., Tanabe, S., 1999. Distribution of Heavy Metals in the Tissues of Green-Lipped Mussel *Perna viridis* (Linnaeus). In: Saadon, M.N., Abdullah, S.A., Sheriff, S.M., Ariffin, N.A., (Eds.), Proceedings of the tenth JSPS/VCC joint seminar on marine and fishery sciences—sustainable utilization of marine resources. Malaysia: College University Terengganu.

Jauncey, K., Ross, B., 1982. A guide to Tilapia feeds and feeding. Institute of Aquaculture, University of Stirling, Stirling, Great Britain.

Juell, J.E., 1991. Hydroacoustic detection of food waste--a method to estimate maximum food intake of fish populations in sea cages. Aquacult. Eng. 10, 207-217.

Khoo, E.W., 1985. Occurrences of red tide along Johore Straits, Malaysia, resulted in heavy mortality of shrimp. World Mariculture Society Newsletter 16, 4.

Kestin, S.C., Warriss, P.D., 2001. Farmed fish quality: The effect of diet composition and feeding regime on the proximate composition of farmed fishes. Blackwell Science, Berlin, Germany.

Kirkpatrick, D. C., Coffin, D. E., 1974. The trace metal content of representative Canadian diets in 1970 and 1971. Can. Inst Food Sci. Tech. J. 7, 56–58.

Kissil, G. W., Lupatsch, I., 1992. New approaches to fish feed in Israeli mariculture as a result of environmental constraint. Isr. J. Aquacult. Bamidgeh. 44, 125.

Kongkeo, H., 1995. How Thailand make it to the top. INFOFISH International. 1/95, 25-31.

Krom, M.D., Neori, A., 1989. A total nutrient budget for an experimental intensive fish pond with circularly moving seawater. Aquaculture 83, 345-358.

Kromhout, D., Bosschieter, F.B., Lezenne, C.C., 1985. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N Engl J Med. 312, 1205–1209.

Lacerda, L.D., Santos, J.A., Madrid, R.M., 2006. Copper emission factors from intensive shrimp aquaculture. Mar. Pollut. Bull. 52, 1823–1826.

Lawrence, A.L., Lee, P.G., 1997. Research in the Americas. In: D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds), Crustacean Nutrition, Advances in World Aquaculture vol. 6. World Aquacult. Soc. Baton Rouge, pp. 566–587.

Leung, K.M.Y., Chu, J.C.W., Wu, R.S.S., 1999. Nitrogen budget for the areolated grouper *Epinephelus areolatus* cultured under laboratory conditions and in open-sea cages. Mar. Ecol. Prog. Ser. 186, 271–281.

Li, M.H., Robinson, E.H., 2015. Nutritional requirements of cultured fish: formulating nutritionally adequate feeds. In: Davis, D.A., (Ed.), Feed and feeding practices in aquaculture. Woodhead publishing, Cambridge, UK, pp. 53-100.

Marte, C.L., Cruz, P., Flores, E.E.C., 2000. Recent development in freshwater and marine cage aquaculture in the Philippines. In: Liao, I.C., Lin, C.K. (Eds.), Cage Aquaculture in Asia: Proceedings of the First International Symposium on Cage Aquaculture in Asia. Asian Fish. Soc., Manila, and World Aquacult. Soc.—Southeast Asian Chapter, Bangkok, pp. 21–37.

Merican, Z.O., Phillips, M.J., 1985. Solid waste production from rainbow trout, *Salmo gairdneri Richardson*, cage culture. Aquacult. Fish. Manage. 1, 55–69.

Midlen, A., Redding, T.A., 1998. Environmental management for aquaculture: Environmental impact of aquaculture. Chapman and Hall Aquaculture series 2, 21-56.

Miki, K., Sano, M., Bailly, D., 1992. The role and problems of coastal fish culture in Japan. Oceanis-Doc. Oceanogr. 18, 385-395.

Molver, J., Stigebrandt, A., Bjerkenes, V., 1988. On the excretion of nitrogen and phosphorous from salmon. Proc. Aquaculture lnt. Congress. Aquaculture International Congress, Vancouver, BC. pp. 80.

Moriarty, D.J.W., 1997. The role of microorganisms in aquaculture ponds. Aquaculture 151, 333–349.

Muller, F. & Varadi, L. (1980). The results of cage fish culture in Hungary. Aquacult. Hung. 2, 154-167.

National Research Council (NRC), 2011. Nutrition requirements of fish. The National Academic Press, Washington.

National Research Council (NRC), 1993. Nutrient Requirements of Fish in National Academies Press. Washington, DC.

Naylor, R.L., Goldburg, R.J., Mooney, H., Beveridge, M., Clay, J., Folke, C., Kautsky, N., Lubchenco, J., Primavera, J., Williams, M., 1998. Nature's subsidies to shrimp and salmon farming. Science 282, 883–884.

Ove Arup, F., 1989, Hydraulic Research (Asia) & WRc (Asia). Assessment of the Environmental Impact of Marine Fish Culture in Hong Kong. Final Report submitted to the Environmental Protection Department, Hong Kong Government.

Phillips, M.J., Macintosh, D.J., 1997. Aquaculture and the environment: challenges and opportunities. In: Nambiar, K.P.P., Singh, T., (Eds.). Sustainable Aquaculture. Processings of INFOFISH AQUATECH 1996 International Conference on Aquaculture, Kuala Lumpur, Malaysia.

Phillips, M.J., Lin, C.K., Beveridge, M.C.M., 1993. Shrimp culture and the environment: lessons from the world's most rapidly expanding warmwater aquaculture sector. In: Pullin, R.S.V., Rosenthal, H., Maclean, J.L., (Eds.). Environment and aquaculture in developing countries), ICLARM Conference Proceedings 36, International center for living aquatic resource management, Manila; Deutsche Gessellschaft fur Technische Zusammenarbeit (GTZ) GmbH, Eschborn, pp. 171-196.

Phillips, M., Beveridge, M., 1986. Cage and the effect on water condition. *Fish Farmer* 9, 17-19.

Phillips, A.M., Podiliak, H.A., Livingston, D.L., Dumas, R.F., Hammer, G.L., 1960. Metabolism of trace levels of dietary cobalt by brook trout. Fish. Res. Bull. N.Y. 23, 36-46.

Penczak, T., Galicka, W., Molinski, M., Kusto, E., Zalewski, M., 1982. The enrichment of a mesotrophic lake by carbon, phosphorus and nitrogen from the cage aquaculture of rainbow trout, *Salmo gairdneri*. J. Appl. Ecol. 19, 371–393.

Pillay, T.V.R., 2004. Aquaculture and the environment: Nature of environmental impacts. Blackwell Publishing, 7-19.

Qi, Y., Hong, Y., Qian, H., Lu, S., 1992. Problems caused by harmful algal blooms in China. BMTC-IOC-POLARMAR International workshop on Training Requirements in the field of eutrophication in semi-enclosed seas and harmful algal blooms, Bremerhaven, 29 September- 3 October 1992. Intergovermental Oceanographic Commission Workshop Report No. 94, NESCO.

Robinson, E.H., Li, M.H., 1997. Low protein diets for channel catfish *Ictalurus punctatus* raised in earthen ponds at high density. J. World Aquacult. Soc. 28, 224-229.

Schaaning, M., 1991. Cu, Zn and organic matter in sediments at a fish farm location on the northwest coast of Norway. In: Hoffman E., Persson, R., Gaard, E., Jonsson, G.S. (Eds). Nordisk Ministerrad, Kobenhavn. Havbrug og Miljo. Nord. 10, pp. 55-56.

Seymour, E.A., Bergheim, A., 1991. Towards a reduction of pollution from intensive aquaculture with reference to the farming of salmonids in Norway. Aquacult. Eng. 10, 73-88.

Shearer, K.D., 1994. Factors affecting proximate composition of cultured fishes with the emphasis on salmonids. Aquaculture 119, 63-88.

Shyong, W.J., Huang, C.H., Chen, H.C., 1998. Effect of dietary concentration on growth and muscle composition of juvenile *Zacco barbata*. Aquaculture 167, 35-42.

Smith, R.R., Kincaid, H.L., Regenstein, J.M., Rumsey, G.L., 1988. Growth, carcass composition, and taste of rainbow trout of different strains fed diets containing primarily plant or animal protein. Aquaculture 70, 309-21.

Sookying, D., Davis, D.A., 2011. Pond production of Pacific white shrimp (*Litopenaeus vannamei*) fed high levels of soybean meal in various combinations. Aquaculture 319, 141-149.

Storebakken, T., 2002. Atlantic salmon, *Salmo salar*. In: Webster, C.D., Lim, C., (Eds.), Nutritional requirements and feeding of finfish for aquaculture. CABI, New York, pp. 79-102.

Storebakken, T., Shearer, K.D., Roem, A.J., 1998. Availability of protein, phosphorus and other elements in fish meal, soy-protein concentrate and phytase-treated soy-protein-concentrate-based diets to Atlantic salmon, *Salmo salar*. Aquaculture 161, 365-379.

Sugiura, S.H., Dong, F.M., Hardy, R.W., 1998. Effects of dietary supplements on the availability of minerals in fish meal: preliminary observations. Aquaculture 160, 283-303.

Tacon, A.G.J., Akiyama, D.M., 1997. Feed ingredients. In: D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds.) Crustacean Nutrition, Advances in World Aquaculture vol. 6. World Aquaculture Society, Baton Rouge, pp. 411–472.

Tangen, G., 1977. Blooms of *Gyrodinium aureolum* (Dynophyceae) in North European waters, accompanied by mortality in marine organisms. Sarsia. 63, 123-33.

Thorpe, J. E., Talbot, C., Miles, M.S., Rawlings, C., Keay, D.S., 1990. Food consumption in 24 hours by Atlantic salmon *Salmon salar* L, in a sea cage. *Aquaculture 90,* 41-47.

Tookwinas, S., 1998. The environmental impact of intensive marine shrimp farming effluents and carrying capacity estimation at Kung Krabaen Bay, Eastern Thailand. Asian Fisheries Sci. 11. 303-316.

Uyan, O., Koshio, S., Teshima, S., Ishikawa, M., Thu, M., Alam, M.S., Michael, F.R., 2006. Growth and phosphorus loading by partially replacing fishmeal with tuna muscle by-product powder in the diet of juvenile Japanese flounder, *Paralichthys olivaceus,* Aquaculture 257, 437–445.

Vethe, O., Schaaning, M., Linjordet, R., 1990. Avfall og forurensning fra fiskeoppdrett. Arsmelding, Jordforsk, 27-29.

Waldichuk, M., 1987a. Fish farming problems. Mar. Pollut. Bull. 18, 2-3.

Waldichuk, M., 1987b. TBT in aquaculture salmon a concern. Mar. Pollut. Bull. 18, 265-266.

Warren-Hansen, I., 1982a. Evaluation of matter discharged from trout farming in Denmark. In: Albaster, J.S. (Ed.). Report of the EIFAC Workshop on Fish-Farm Effluents EIFAC Tec. Pap. 41, pp. 166.

Warrer-Hansen, I., 1982b. Methods of treatment of wastewater from trout farming. In: Alabaster, J.S. (Ed.), Report of the FIFAC Workshop on Fish Farm Effluents, Denmark, May 1981. FIFAC Technical Paper 41, pp. 113–121.

Watanabe, T., 2002. Strategies for further development of aquatic feeds. Fisheries Sci. 68, 242-252.

Watanabe, T., Kiron, V., Satoh, S., 1997. Trace minerals in fish nutrition. Aquaculture 151, 185-207.

Webster, C.D., Tiu, L.G., Tidwell, J.H., 1997. Growth and body composition of juvenile hybrid bluegill *Lepomis cyanellus* × *L. macrochirus* fed practical diets containing various percentage of protein. J. World Aquacult. Soc. 28, 230-40.

Webster, C.D., Tiu, L.G., Tidwell, J.H., Wyk, P.V., Howerton, R.D., 1995. Effects of dietary protein and lipid levels on growth and body composition of sunshine bass (*Morone chrysops* × *M. Saxatilis*) reared in cages. Aquaculture 131, 291-301.

Weston, D., 1990. Quantitative examination of macrobenthic community changes along an organic enrichment gradient. Mar. Ecol. Progr. Ser. 61,233-244.

Wu, R.S.S., 1995. The environmental impact of marine fish culture: towards sustainable future. Mar. Pollut. B. 31, 159–166.

Wu, R.S.S., Lam, K.S., MacKay, D.W., Lau, T.C., Yam, V., 1994. Impact of marine fish farming on water quality and bottom sediment: a case study of the sub-tropical environment. Mar. Environ. Res. 38, 115-145.

Wu, R.S.S., 1990a. A respirometer for continuous, in-situ measurements of sediment oxygen demand. *Wat. Res.* 24, 391-394.

Yang, H.D., Rose, N., 2005. Trace element pollution records in some UK lake sediments, their history, influence factors and regional differences. Env. Int. 31, 63–75.

Zhou, H.Y., Wong, M.H., 2000. Mercury accumulation in freshwater fish with emphasis on the dietary influence. Water Res. 34, 4234–4242.

Chapter 3 Embodied Resources Use for Production of Aquaculture Feeds

3.1 Abstract

Aquaculture production currently depends heavily upon the use of manufactured feeds. Prepared feed is typically the most expensive aquacultural input. Production of feed ingredients (feedstuffs) and feed manufacturing consume a large amount of the energy input to aquaculture. Therefore, estimates of the quantities of resources used in aquacultural feed production are needed. The purpose of this study was to obtain a better understanding of the rangeof resources to include embodied energy, embodied nutrients, land, and water that are required to produce aquacultural feeds. Typical feed formulations for major cultured species including channel catfish, tilapia, pangasius catfish, salmon, trout and whiteleg shrimp were used for the calculations. Data on the use of land, water, nutrients and energy necessary to produce a unit quantity of each of the feedstuffs needed for the formulations were estimated from literature data on the production of the feedstuffs. These values were used to calculate the average embodied energy, land, water, and nutrients associated with the production of 1 tonne of each feed. The embodied energy of salmon and trout feeds were greater than for other feeds. Whiteleg shrimp feed required the greatest amount of land to produce plant-based ingredients. Tilapia and pangasius feeds exhibited the highest average water requirement per unit of production. Whiteleg shrimp feed required the highest average embodied nitrogen input, and tilapia feed required the highest average embodied phosphorus input.

3.2 Introduction

Use of manufactured feeds is increasing in the aquaculture industry because a greater level of production can be achieved by using high quality feeds. According to FAO (2012), approximately two-thirds of aquaculture production systems received some feed input in 2012 as compared to about one-half of the systems in 1980. In 2014, 41 million tonne (Mt) of aquaculture feeds were produced (Allteach, 2015). Although this quantity is rather small as compared to production of poultry feed (439 Mt), ruminant feed (196 Mt) and swine feed (256 Mt), aquaculture feed production is a large industry with significant promise for future growth. However, resources are used and negative environmental impact result from use of aquaculture feed (New and Wijkstrom, 2002).

Feeds are composed of a variety of feedstuffs mainly from agriculture and fisheries such as fishmeal, squid meal, feather meal, bone meal, plant meals, corn, wheat flour, rice flour, fish oil, vegetable oil, vitamin premixes and mineral supplements. The choice of ingredients to be used in a feed depends largely on the species for which the feed is intended, and the availability, nutritional quality, price and sanitation of feedstuffs. Manufactured feed causes a large proportion of the environmental impacts associated with aquaculture (Henriksson et al., 2012). The amount of resources used, i.e., energy, land, water and feedstuffs, in producing aquaculture feeds also has led to resource use and environmental concerns (Boyd and McNevin, 2015).

The purpose of this study was to gain a better understanding of the amounts of direct and embodied resources required for feed production for common aquacultural species.

3.3 Material and Methods

The literature was searched for works that allowed the estimation of land, water, nutrients, and energy embodied in the common feedstuffs used in aquaculture feeds. Representative feed ingredient formulae for common aquacultural species are provided in Tables 3.1 to 3.6.

3.3.1 Embodied Energy

The amount of embodied energy needed to produce feedstuffs (FS) – mainly plant and animal meals used in feeds for production of 1 tonne (t) of feed can be determined using Equation 3.1;

$$\textit{Embodied energy (GJ/t)} = (\%\ FS \times 100) \times \textit{embodied energy (MJ/kg)} \times 10^3 \quad (3.1)$$

3.3.2 Land requirement

The amount of agricultural land needed to produce plant based meals used in feeds for production of 1 t of feed can be determined using Equation 3.2;

$$\textit{Land requirement (ha/t)} = (\%\ FS \times 100) \times \textit{Land to produce FS } (m^2/kg) / 10 \quad (3.2)$$

3.3.3 Water use

Water used to produce plant-based meals used in feeds for production of 1 ton of feed can be determined using Equation 3.3;

$$\textit{Water use } (m^3/t) = (\%\ FS \times 100) \times \textit{water to produce the FS } (m^3/kg) \times 10^3 \quad (3.3)$$

3.3.4 Embodied Nitrogen and Phosphorus from crop fertilizer use

Values of embodied N and P from fertilizer use for major crops used in manufacturaing aquacultural feeds are given in Table 3.8. The values are the median quantity of fertilizer N and P reported in the literature to be used for the various crops from which the feedstuffs were derived. The average global yields of the feedstuffs per hectare were multiplied by the N and P contents of

the feedstuffs to estimate the recovery of N and P in the feedstuff. The amount of N and P recovered per hectare in the feedstuffs were subtracted from N and P added in fertilizer to obtain the embodied N and P values. Embodied nutrient use in aquaculture feed ingredients can be evaluated using Equation 3.4;

$$\textit{Embodied nutrient (kg/t)} = \textit{Land used to produce FS} \times \textit{Embodied N or P (kg/ha)} \quad (3.4)$$

3.3.5 Statistical Analysis

All data were statistically analyzed using one-way analysis of variance to determine significant differences ($p < 0.05$) among treatments, which was followed by the Fisher's Least Significant Difference test to determine significant differences among treatment means. All statistical analyses were performed using SAS (V9.2 SAS Institute, Cary, NC, USA).

3.4 Results

The embodied energy, land use, and water use for feedstuffs were presented in Table 3.7. The average embodied nitrogen and phosphorus were presented in Table 3.8.

3.4.1 Embodied Energy

The estimated values for embodied energy used for producing channel catfish, tilapia, pangasius catfish, salmon, trout and whiteleg shrimp feeds were given in Table 3.9 and 3.10. The highest mean embodied energy requirement for manufactured feeds was for salmon species (11.27 GJ/t). The values ranged from 10.81 to 11.83 GJ/ton. Whereas, the lowest average embodied energy requirement was found to be for catfish species (4.13 GJ/t), which ranged from 3.38 to 4.94 GJ/t. Average energy input required for production of trout fish species was particularly high – 6.64 GJ/t (Figure 3.1).

3.4.2 Land requirement

The estimated values for land area required to produce channel catfish, tilapia, pangasius catfish, salmon, trout and whiteleg shrimp feeds, are given in Table 3.9 and 3.10. Tilapia feeds showed highest average land use (0.287 ha/t of production). The values ranged from 0.113 to 0.373 ha/t. Salmon feeds had the lowest average amount of embodied land (0.137 ha/t) (Figure 3.2).

3.4.3 Water Use

The estimated values of water requirement to produce channel catfish, tilapia, pangasius catfish, salmon, trout and whiteleg shrimp feeds are given in Table 3.9 and 3.10. Pangasius catfish feed exhibited the highest average water requirement for per unit of producion at 1,661 m^3/t. Catfish feed production was found to have lowest water requirement (average=1,227 m^3/t of produce) (Figure 3.3).

3.4.4 Embodied Nutrients

The estimated values of embodied nitrogen and phosphorus to produce channel catfish, tilapia, pangasius catfish, salmon, trout and whiteleg shrimp feeds are given in Table 3.9 and 3.10. Catfish feeds exhibited the highest average embodied nitrogen per unit of production at 3.42 kg/t. While Pangasius feeds exhibited the lowest average embodied nitrogen per unit of production at 1.52 kg/t (Figure 3.4). The average embodied phosphorus was highest in tilapia feed production (4.47 kg/t) and lowest in salmon feed production (2.08 kg/t) (Figure 3.5).

3.5 Discussion

The basic ingredients in aquaculture feeds are the same ingredients which are used in other animal feeds. Most of the ingredients are products of capture fisheries or agriculture, which have been delivered to a processing facility, refined and transported to a feed mill for use in manufacturing aquaculture feed. Some of the ingredients are by-products of agriculture and fisheries. The energy used for producing aquaculture feeds includes the energy used in agriculture and the capture fisheries to acquire raw materials for making feed ingredients, processing of raw materials into feed ingredients and milling of feed ingredients. Moreover, transportation of ingredients and feeds also use energy.

Avadi et al. (2015) determined the amount of energy used to produce one tonne of commercial feed were 1.2 and 0.68 GJ/t for trout and tilapia, respectively. According to FAO (2011), estimated energy use for aquaculture is energy used for producing and delivering feeds which was estimated to be 0.45 exajoules (EJ) connect to GJ/t of feed or 450,000,000 GJ. This value made up for about 28.8% of total energy use for aquaculture calculated from the LCA studies mentioned above (Boyd and McNevin, 2015).

Various life cycle analyses (LCAs) of aquaculture species reported estimates of energy use (Gronroos et al., 2006; Pelletier et al., 2009; Winther et al., 2009; Iribarren et al., 2010; Pelletier and Tyedmer, 2010; Mungkung et al., 2012). However, it is not possible to estimate the amount of energy used specifically for feed production from these studies. In the present study it was not possible to obtain energy use for transportation of ingredients to feed mill, milling, and delivery to farmers. However, Boyd et al. (2008) reported these energy costs for channel catfish feed and found that 1.57 GJ/t were required. The energy required for these purposes for channel catfish feed is possibly similar for other feeds. However, it does not agree well with two other estimates WHO.

They reported the average energy use for all species to be roughly 26 GJ/t of production of live fish.

Fishmeal is made from small, pelagic, ocean fish, i.e., anchovies, herring, menhaden and sardines. The production of fishmeal involves pulverization of the small fish, followed by oil and water extraction. The remaining solids are then cooked and pulverized into a meal. Water is separated from the remaining liquid to obtain fish oil. Production of fishmeal itself is a very energy intensive process. Fishmeal has been used in animal feeds as it contains high levels of protein, calcium, phosphorus and other minerals. Fishmeal is particularly useful in aquacultural feeds because of its high protein content and excellent balance of amino acids for aquatic animals (Abdelghany, 2003). This has caused the fishmeal and fish oil resources to be vulnerable to over-exploitation for aquaculture feeds. About 60% of the world's fishmeal and 80% of the fish oil supply is devoted to aquaculture feeds (Boyd and McNevin, 2015).

Currently, many efforts around the globe are working to reduce the level of fishmeal and oil in aquaculture feeds (Welch et al., 2010). Because fishmeal production is an energy intensive process, reduction of fishmeal usage in feed manufacturing also would lessen energy use for feed production. A number of studies involving use of feeds containing only plant protein for cultured species have shown success. Fish meal-free feed normally contains a rendered meat product which provides a good source of protein. Feeds for some cultured species can be formulated without any fishmeal, e.g., feeds for channel catfish often contain no fishmeal (Boyd et al., 2007). In the present study, the representative feed formulae are considered to be typical of commercial feed, and contain fishmeal and plant meals in different ratios which varies greatly among species. It has found that the greatest percentage of fishmeal use was in salmon and trout feeds; percentage were

intermediate for shrimp feed; lowest for tilapia feed. Channel catfish feed, however, contained no fishmeal.

Lipids are included in aquaculture feeds in order to provide energy and essential fatty acids to the animals. Vegetable oil, fish or other marine oil, or combination of both are some common lipid sources. Feeds for cold-water species, such as salmon and trout, contain a high level of oil, especially fish oil, as compared to warm-water species such as channel catfish, tilapia and marine shrimp. Use of fish oil in the fish diet helps in build up essential fatty acids in the animals. Although vegetable oil has been used in aquaculture feeds, cultured animals receiving diets containing mainly vegetable oil have been shown to exhibit a lower ratio of omega-3: omega-6 fatty acids than found in wild caught fish (Bell et al., 2001; Alasalvar et al., 2002; Lenas and Nathanailides, 2011).

Approximately two-thirds of the total fish oil used in aquacultural feeds goes to salmonid feed manufacturing, and most of the rest is used in marine fish and crustacean feeds (www.iffo.net). The inclusion of fishmeal in aquaculture feed has decreased from 25.5% to 14.0%, and fish oil inclusion fell from 7.5% to 4.4%. However, the total amount of fish meal and fish oil used in aquaculture feeds was still increased during this period because of the huge increase in feed-based aquaculture. In the present study, the representative feeds also contained fish oil just as they contained fishmeal. Salmon and trout feeds had the highest amount of fish oil followed by shrimp feeds which had intermediate fish oil levels, whereas the content was lowest for tilapia feeds. Catfish feeds did not have any fish oil in them. Trout C feed had lower energy content than rest of the feeds as soybean oil was used to replace fish oil. This practice reduces energy use for manufacturing oil and conserves fish oil. Similarly, catfish A and D feeds did not have distiller dried grain which requires a higher amount of energy to produce, as a result, embodied energies

of these two channel catfish feeds were less than other feeds. Tilapia D feed contained more rice bran compared to other feeds. And because rice bran requires less energy to produce than many other plant meals, the feed contains less embodied energy.

Boyd et al. (2007) estimated the total land area used for an aquacultural facility to be 1.25 to 1.50 times greater than the water surface area of production units. This additional land is used for structures such as canals, storage or sedimentation reservoirs, embankments, buildings, parking lots and staging areas. Intensive culture systems such as cages and raceways do not make use of such large surface areas. For instance, it takes approximately 200 m^2 of surface area for a cage system to produce 40 ton of tilapia; however, approximately 16 ha of agricultural land would be used to produce plant feedstuffs. Similarly, intensification of pond aquaculture lessens the amount of land to be converted to water surface area and support area at the facility; however, the amount of agricultural land per unit area needed to support such systems for more intensive production increases.

Land is required to produce meals and oils for manufactured feeds. Soybean meal, corn meal, peanut meal, cottonseed meal, wheat middling, rice flour, and vegetable oils are common plant feedstuffs for aquafeeds. Cottonseed meal and wheat middlings are by-products of cotton fiber and wheat flour production, respectively. Vegetable oils are extracted from soybeans, corn, peanuts, and other seeds. Thus, their use in manufactured feeds usually does not require land dedicated specifically to aquacultural production.

A typical feed for swine contains 74.4% corn and 23.4% soybean meal, whereas broiler chicken feed usually contains 67% corn and 23.7% soybean meal. Also, typical FCRs for swine and broilers are 2.80 and 1.88, respectively (Boyd and McNevin, 2014). Therefore, the land requirements for plant ingredients in feed for 1 t net production were reported to be 0.52 ha and

0.33 ha for swine and broilers, respectively. Cattle fed grain usually have an FCR of 6 (Schnepf, 2011) and the amount of corn needed to produce 1 t of cattle would require approximately 0.64 ha of agricultural land. This study estimated that the average land area required to produce plant meal animal diet ingredients for 1 t production of aquatic animals is about 0.20 ha which is lower than land required for feed producyion of other species. The main plant ingredients used in aquaculture feeds requiring more land area are canola seed, soybean meal, wheat and corn. Tilapia species, being herbivorous, feed mainly on plant-based ingredients in a diet requiring more land area to produce their diet for unit weight production. Whiteleg shrimp feed had high embodied nitrogen as the main ingredients in their diets, which were derived from wheat and canola crops requiring large amounts of nitrogen. Tilapia and pangasius catfish feeds had high embodied phosphorus due to soybean and corn contents which require greaterer phosphorus to grow.

The aquacultural production area is much smaller than the area needed other type of agricultural production. Total agricultural area in 2011 was about 6,325 million ha − 1,396 million ha for arable land, 1,570 million ha for permanent crops and 3,359 million ha for permanent meadows and pastures. This area represents approximately 38% of the earth's land surface (Boyd and McNevin, 2014). The total aquacultural area is about 0.45% of the total agricultural area, and it is estimated to be around 0.17% of the earth's land surface.

Most of the water used in animal feed production is for producing plant based feed ingredients. The amount of water used to produce 1 t of plant feedstuff varies depending on plant species and climate. However, Chapagain and Hoekstra (2004) suggested using a value of 2,000 m^3/t in general water use calculations. The plant ingredient content of aquaculture feeds ranged from 40% to nearly 90% (Boyd et al., 2007; Verdegem and Bosma, 2009) with the average of these two studies at 71.3%. Therefore, water use for plant ingredients in 1 t of aquaculture feed

averages about 14,000 m^3/t. Based on this relationship in 2012 approximately 48 billion m^3 water was required to produce feed ingredients for 34.4 Mt of aquaculture feed. World's annual water usage is about 9,695 billion m^3 and aquaculture feeds are responsible for 0.5% of world water use. Chapagain and Hoekstra (2004) reported that most of the water used in agricultural production is from rainwater which falls on the fields. In this study, tilapia and pangasius catfish feeds consisted mainly of plant feedstuff, and thus required more water for their production than feeds for other aquacultural species.

Even though aquaculture feeds require water for production, their use enhances the level of animal production in a grow-out unit. Intensification of production by applying more feed results in a decrease in water use in spite of the high water demand associated with feed production. For example, water use in channel catfish ponds in Alabama was found to be 12,700 m^3/ha (Boyd and McNevin, 2014). In unaerated ponds with feeding, when production is about 2,000 kg/ha and FCR of 2.0, water use for producing feed ingredients would be about 5,600 m^3 – total water use of 18,300 m^3/ha or 9,150 m^3/t. At a production rate of 6,000 kg/ha, water use in an aerated pond would be around 4,917 m^3/t. Total water use in aquaculture varies considerably depending upon types of culture methods. Cage and net pen culture uses the least amount of water, while raceway culture uses the most. Water use in ponds varies depending on the intensity of production, frequency of draining, and amount of water exchange (Boyd and Gross, 2000).

Research on feed and feeding practices has proven to be useful in conservation and efficient use of natural resources. Between 1995 and 2007 the average FCR for major aquatic species decreased from 1.95 to 1.75 (Naylor et al., 2009). The FCR is an important variable associated with the efficiency of feed ingredient use and the amount of energy and resources used to produce feed. For instance, a facility which produces fish at an FCR of 1.5 is much more efficient than one

where the FCR is 2.0. Thus, the amount of energy, land, water, and nutrients used to produce feeds per unit of animal production decreases as the FCR declines.

3.6 Conclusion

Production of feed ingredients (feedstuffs) and feed manufacturing consume a large amount of the resource input to aquaculture. In the present study, the amount of energy used to produce one tonne of commercial feed ranged from 4.13 to 11.27 GJ/Mt. Land is required to produce plant meals and oils for manufactured feeds. The result from this study demonstrated that aquaculture feed required land 0.137 to 0.287 ha to produce 1 Mt feed. Most of the water used in animal feed production is for producing plant-based feed ingredients. The amount of water used to produce 1 t of plant feedstuff ranged from 823.6 to 1,661.0 m^3.

Table 3.1 Channel catfish feed ingredient formulas.

Ingredient	Inclusion rate in feed (%)				
	A[1]	B[2]	C[3]	D[4]	E[5]
Fish meal	0.0	0.0	0.0	0.0	0.0
Rendered products	6.0	5.0	0.0	0.0	0.0
Soybean meal	34.5	17.4	21.4	33.2	21.3
Cottonseed meal	12.0	20.0	20.0	25.0	20.0
Corn grain	20.4	15.0	15.0	18.2	15.0
Corn meal	0.0	20.0	20.0	20.0	0.0
Wheat meal	0.0	0.0	0.0	0.0	0.0
Wheat middlings	20.0	9.8	5.4	0.0	24.1
Distiller's dried solubles	0.0	10.7	16.1	0.0	15.0
Fat/oil	2.0	1.5	1.5	2.0	2.0
Mineral pack	0.5	0.5	0.5	0.5	0.5
Dicalcium phosphate	1.0	1.0	1.0	1.0	1.0
Vitamin pack	0.5	0.5	0.5	0.5	0.5
Lysine HCl	0.0	0.4	0.4	0.3	0.4

[1]Boyd and Polioudakis (2006).

[2,3]Robinson and Li (2012).

[4,5]Li and Robinson (2013).

Table 3.2 Tilapia feed ingredient formulas.

Ingredient	Inclusion rate in feed (%)				
	A[1]	B[2]	C[3]	D[4]	E[5]
Fish meal	0.0	5.0	0.0	10.0	6.0
Bone meal	0.0	0.0	16.0	0.0	0.0
Soybean meal	50.0	40.0	28.0	21.1	38.3
Canola meal	5.0	5.0	0.0	0.0	0.0
Corn grain	0.0	0.0	20.0	3.1	48.8
Corn meal	10.0	9.0	0.0	0.0	0.0
Cassava	0.0	0.0	0.0	10.0	0.0
Peanuts	0.0	0.0	16.0	0.0	0.0
Cottonseed meal	5.0	5.0	0.0	0.0	0.0
Sorghum	0.0	0.0	20.0	0.0	0.0
Wheat flour	11.3	10.5	0.0	0.0	4.0
Wheat bran	8.0	20.0	0.0	10.0	0.0
Rice bran	0.0	0.0	0.0	35.0	0.0
Fish oil	0.0	0.0	0.0	3.0	1.5
Vegetable oil	2.2	2.2	0.0	0.0	0.0
Mineral pack	1.0	1.0	0.0	0.1	0.7
Limestone	0.0	0.0	0.0	0.8	0.0
Monocalcium phosphate	1.5	1.5	0.0	1.0	0.0
Vitamin pack	1.0	1.0	0.0	0.1	0.7

[1,2]Swick (2001).

[3,4]Workagegn (2014).

[5]Boyd and Polioudakis (2006).

Table 3.3 Pangasius catfish feed ingredient formulas.

Ingredient	Inclusion rate in feed (%)				
	A[1]	B[2]	C[3]	D[4]	E[5]
Fish meal	15.0	8.5	12.8	15.0	0.0
Soybeans	10.0	0.0	0.0	0.0	0.0
Soybean meal	0.0	35.0	20.7	15.0	52.8
Corn meal	25.0	0.0	0.0	0.0	6.0
Wheat	0.0	0.0	0.0	0.0	23.6
Wheat middlings	0.0	0.0	0.0	0.0	10.0
Cassava	0.0	12.5	18.0	0.0	0.0
Canola meal	0.0	8.5	0.0	0.0	0.0
Rice	0.0	7.0	10.7	15.0	0.0
Rice bran	50.0	7.5	21.9	30.0	0.0
Wheat bran	0.0	15.0	13.2	0.0	0.0
Peanuts	0.0	0.0	0.0	24.0	0.0
Fish oil	0.0	0.0	1.9	0.0	3.5
Mineral pack	0.0	0.3	0.3	0.5	0.0
Vitamin pack	0.0	0.3	0.3	0.5	0.1
Calcium phosphate	0.0	0.0	0.0	0.0	2.7
Soy lecithin	0.0	0.0	0.0	0.0	1.0

[1]Annonymous (2012).

[2,3]Bosma et al. (2009).

[4]Paripatananont (2002).

[5](www.soyaqua.org/sites/default/files/reports/02pangasiustrhainan.pdf).

Table 3.4 Salmon feed ingredient formulas.

Ingredient	Inclusion rate in feed (%)		
	A[1]	B[2]	C[3]
Fish meal	28.3	32.4	40.0
Fish oil	9.0	19.4	15.9
Poultry by-product meal	11.0	13.0	0.0
Wheat	16.0	0.0	0.0
Corn gluten meal	11.0	10.0	0.0
Canola seed	11.0	0.0	0.0
Canola oil	7.0	0.0	0.0
Soybean mean	4.5	12.0	27.3
Premixes	2.0	2.0	0.7
Blood meal	0.0	3.0	0.0
Wheat flour	0.0	8.2	11.5
Wheat middlings	0.0	0.0	0.0
DL methionine	0.0	0.0	0.5
Wheat starch	0.0	0.0	2.3
Cellulose	0.0	0.0	1.7

[1]Pelletier and Tyedmers (2007).

[2](www.fao.org//fileadmin/user_upload/affris/docs/tabl3.pdf).

[3]Carter and Hauler (2000).

Table 3.5 Rainbow trout feed ingredient formulas.

Ingredient	Inclusion rate in feed (%)				
	A[1]	B[2]	C[3]	D[4]	E[5]
Fish meal	30.0	34.0	0.0	25.0	0.0
Poultry by-product meal	6.0	0.0	0.0	12.0	35.0
Feather meal	6.0	4.0	0.0	5.0	27.4
Meat and bone meal	0.0	12.0	0.0	0.0	0.0
Blood meal	4.0	8.0	0.0	5.0	0.0
Crab meal	0.0	0.0	0.0	0.0	0.0
Canola meal	0.0	6.0	0.0	0.0	0.0
Corn gluten meal	4.0	8.7	6.0	12.0	18.2
Wheat	22.0	0.0	0.0	12.0	0.0
Wheat flour	0.0	0.0	0.0	0.0	0.0
Wheat middlings	0.0	0.0	8.0	0.0	0.0
Soybean meal	12.0	10.0	70.0	8.0	0.0
Brewer's grain	0.0	10.0	0.0	0.0	0.0
Fish oil	9.0	6.2	0.0	17.0	16.7
Soybean oil	5.0	0.0	10.0	0.0	0.0
Mineral pack	0.0	1.0	0.2	1.0	0.0
Salt	0.0	0.0	0.0	0.0	0.1
Calcium phosphate	0.0	0.0	0.0	0.0	0.0
Vitamin pack	1.5	0.1	0.4	1.0	1.5
Vitamin C	0.0	0.0	0.0	0.0	0.3
Chlorine chloride	0.0	0.0	0.3	0.0	0.5
DL-methionine	0.0	0.0	0.0	0.5	0.0
Lysine HCl	0.0	0.0	0.0	0.5	0.3
Binder	0.0	0.0	0.0	0.0	0.0
Brewer's yeast	0.0	0.0	5.0	0.0	0.0
Ascorbic acid	0.0	0.0	0.1	0.0	0.0

[1](www.fao.org/fisheries/affris/species-profiles/trout/feedformulation/en/).

[2]Tacon (1990).

[3]Hughes et al. (2008).

[4]Bureau (2006).

[5]Hardy and Cheng (Undated).

Table 3.6 Whiteleg shrimp feed ingredient formulas.

Ingredient	Inclusion rate in feed (%)			
	A[1]	B[2]	C[3]	D[4]
Fish meal	19.0	30.0	0.0	6.0
Animal protein mix	0.0	0.0	50.0	0.0
Soybean meal	16.0	17.7	0.0	52.7
Soy lecithin	0.0	0.5	1.0	1.0
Corn meal	0.0	0.0	15.8	0.0
Corn gluten meal	0.0	0.0	0.0	8.0
Wheat	0.0	0.0	0.0	13.0
Wheat starch	0.0	35.9	0.0	0.0
Wheat flour	37.5	0.0	17.6	0.0
Wheat bran	10.0	0.0	0.0	0.0
Vegetable oil	2.1	0.0	0.0	0.3
Mineral premixes	3.2	0.5	4.2	0.5
Calcium phosphate	0.0	0.2	0.0	2.5
Vitamin premixes	0.0	2.1	2.0	2.1
Meat and bone meal	10.0	0.0	0.0	0.0
Fish oil	2.1	4.1	4.2	0.0
Cellulose	0.0	5.0	0.7	0.0
Wheat gluten	0.0	4.0	4.0	0.0
Cholesterol	0.0	0.0	0.5	0.1
Corn starch	0.0	0.0	0.0	8.0

[1]Yaemsooksawat et al. (2009).

[2]Davis and Arnold (2000).

[3]Lim (2008).

[4]Fang et al. (2016).

Table 3.7 Average crop production and yields of feedstuffs from crops of selected plant-based feedstuffs and embodied energy, land use, and water use in plant and animals-based feedstuffs.

Feed ingredient	Embodied Energy (MJ/kg)	Land use (m^2/kg)	Water use (m^3/kg)
Alfalfa	8.07[1]	---	0.25[15]
Alfalfa meal	18.63[2]	1.6	0.25[15]
Barley	2.70[2]	---	1.42[15]
Brewer's dried grain	11.62[2]	---	0.10[9]
Canola	4.94[3]	---	2.27[15]
Canola meal	8.31[3]	9.47	1.12[15]
Canola oil	12.24[3]	---	4.30[15]
Cassava	0.86[4]	---	0.56[15]
Cassava flour	4.78[5]	5.19	1.88[15]
Cassava starch	4.78[5]	3.61	2.25[15]
Corn	4.23[2]	1.95	1.22[15]
Corn gluten feed	12.46[6]	2.14	0.66[9]
Corn gluten meal	12.46[6]	2.14	0.1[9]
Corn oil	12.46[6]	2.14	2.57[15]
Corn starch	12.46[6]	2.14	1.08[15]
Corn meal (flour)	5.01[7]	2.53	1.67[15]
Cottonseed meal	1.29[2]	---	0.86[15]
Distiller's dried grain solubles	11.62[2]	---	0.10[9]
Dried whey	53.22[2]	---	0.10[9]
Fat and oils:	---	---	---
Animal fat and oil	9.94[2]	---	0.10[9]
Fish oil	9.94[2]	---	0.10[16]
Vegetable oil (average)	14.24[2]	---	---
Fish and squid meal	18.60[2]	---	0.10[9]
Meat and bone meal	8.60[4]	---	0.10[9]
Oats	2.62[2]	---	1.79[15]
Peanuts	3.93[8]	---	2.78[15]
Peanut meal	5.32[9]	15.49	1.48[15]
Poultry by-product meal	9.71[2]	---	0.01[15]
Premixes and additives:	---	---	---
Amino acids	0.38[6]		0.10[9]
Calcium carbonate	0.12[10]	---	0.10[9]
Calcium phosphate	17.5[11]	---	0.10[9]
Mineral mixes	0.38[6]	---	0.10[9]

Vitamin mixes	0.38[6]	---	0.10[9]
Rice	3.49	2.35	1.67[15]
Rice bran	0.32[2]	---	2.23[15]
Rice flour	7.14[13]	3.14	2.63[15]
Shrimp head meal	8.60[6]	---	0.10[16]
Sorghum	5.80[2]	7.41	3.05[15]
Soybeans	1.84[3]	---	2.15[15]
Soybean meal	3.88[3]	5.05	2.52[15]
Soybean oil	9.37[3]	---	4.19[15]
Sugarcane	0.43[14]	---	0.21[15]
Sugarcane molasses	5.81[2]	4.70	0.53[15]
Wheat	3.96[2]	3.28	1.83[15]
Wheat bran	0.32[2]	---	---
Wheat flour	7.71[9]	4.25	1.85[15]
Wheat middlings	0.32[2]	---	---

[1]Mobtaker et al. (2011).
[2]Davulis and Frick (1977).
[3]Smith et al. (2007).
[4]Bamgboye and Koseman (2015).
[5]Thylmann et al. (2013).
[6](http://www.fao.org/wairdocs/lead/x6100e/x6100e03.htm).
[7]Brekke (1970).
[8]Firouzi and Aminpanah (2012).
[9]Estimated.
[10](www.oregon.gov/energy/renew/biomass/docs/forum/fossilenergyuse.pdf).
[11]Helsel (1992).
[12]Chauhan et al. (2006).
[13]Nishita and Bean (1982).
[14]Mendoza and Samson (2002).
[15]Mekonnen and Hoekstra (2011).
[16](www.fao.org/docrep/003/X6899e/X6899E08.htm)
[17]Jones and Kerns (1974).

Table 3.8 Embodied nutrients from fertilizer use in major crops that are sources of animal feedstuffs.

Crop	Embodied nutrient (kg/ha)[1]	
	Nitrogen	Phosphorus
Alfalfa	0.0	38.9
Barley	45.0	9.7
Canola	41.0	17.1
Cassava	95.0	11.8
Corn	54.0	17.6
Oats	37.1	11.2
Peanuts	0.0	8.5
Rice	90.0	15.6
Sorghum	55.0	15.2
Soybeans	0.0	21.2
Sugarcane	57.0	24.8
Wheat	64.0	8.0

[1]Calculated as difference between typical fertilizer nutrient application rate per hectare and estimated concentration of nutrients in part of plant used to make feedstuffs.

Table 3.9 Embodied energy, embodied nitrogen, embodied phosphorus, land use, and water use for each particular feed formulation. Values represent mean and standard error with minimum and maximum.

Feed Type	Embodied Energy (GJ/t)	Embodied Nitrogen (kg/t)	Embodied Phosphorus (kg/t)	Land use (ha/t)	Water use (m^3/t)
Catfish A	3.38	2.15	4.39	0.214	1226
Catfish B	4.66	4.34	3.28	0.168	1142
Catfish C	4.94	4.34	3.71	0.189	1248
Catfish D	3.76	4.68	5.09	0.255	1613
Catfish E	3.91	1.58	2.80	0.137	910
Tilapia A	4.40	6.40	7.00	0.373	1740
Tilapia B	4.87	6.04	5.85	0.317	1461
Tilapia C	5.10	0.00	3.00	0.329	2022
Tilapia D	3.52	0.00	2.26	0.113	1423
Tilapia E	5.13	1.09	4.24	0.306	1646
Pangasius A	4.39	3.46	1.13	0.064	1762
Pangasius B	4.07	3.30	5.12	0.274	1342
Pangasius C	4.02	0.00	2.22	0.130	1307
Pangasius D	4.94	0.00	1.61	0.111	1982
Pangasius E	4.23	0.83	5.92	0.359	1912
Salmon A	10.81	1.27	0.90	0.099	1006
Salmon B	11.83	3.39	1.94	0.117	521
Salmon C	11.17	3.75	3.39	0.197	1016
Trout A	10.23	0.46	1.44	0.141	969
Trout B	12.14	3.33	2.37	0.126	401
Trout C	4.45	0.69	7.72	0.366	2196
Trout D	10.65	1.39	1.31	0.105	487
Trout E	9.69	2.10	0.69	0.039	65
Shrimp A	8.38	10.20	2.99	0.240	1131
Shrimp B	9.91	10.85	3.25	0.259	1285
Shrimp C	7.91	8.06	1.45	0.132	728
Shrimp D	6.79	1.85	6.24	0.343	1960

Table 3.10 Average embodied energy, embodied nitrogen, embodied phosphorus, land use, and water use to produce feed ingredients for different cultured species. Values represent mean and standard error with minimum and maximum.

Feed type	Embody energy (GJ/t)	Land (ha/t)	Water (m^3/t)	Embody nitrogen (kg/t)	Embody phosphorus (kg/t)
Channel	**4.13±0.29[b]**	**0.193±0.020[a]**	**1227.8±113.4[a]**	**3.41±0.64[a]**	**3.85±0.40[a]**
	(3.38-4.94)	(0.137-0.255)	(910.1-1613.0)	(1.58-4.68)	(2.79-5.09)
Tilapia	**4.60±0.30[b]**	**0.287±0.045[a]**	**1658.2±107.9[a]**	**2.70±1.45[ab]**	**4.47±0.88[a]**
	(3.52-5.13)	(0.113-0.373)	(1423.0-2021.6)	(0.00-6.04)	(2.26-7.00)
Pangasius	**4.33±0.17[b]**	**0.188±0.055[a]**	**1661.0±142.0[a]**	**1.52±0.77[b]**	**3.19±0.97[a]**
	(4.02-4.94)	(0.064-0.359)	(1306.6-1982.1)	(0.00-3.46)	(1.13-5.92)
Salmon	**11.27±0.30[a]**	**0.137±0.052[a]**	**847.5±163.4[a]**	**2.80±0.77[ab]**	**2.08±0.72[a]**
	(10.81-11.83)	(0.099-0.197)	(520.7-1015.7)	(1.27-3.75)	(0.90-3.39)
Trout	**9.43±1.31[a]**	**0.155±0.055[a]**	**823.6±372.2[a]**	**1.60±0.52[b]**	**2.70±1.28[a]**
	(4.45-12.14)	(0.039-0.366)	(65.5-2195.9)	(0.46-3.33)	(0.68-7.72)
Whiteleg shrimp	**8.25±0.65[a]**	**0.243±0.087[a]**	**1276.0±256.5[a]**	**2.01±0.20[ab]**	**3.48±1.00[a]**
	(6.79-9.91)	(0.132-0.343)	(728.0-1960.0)	(1.85-10.85)	(1.45-6.24)
P-value	<0.001	0.253	0.044	0.013	0.618
PSE	1.447	0.097	468.7	2.409	2.021

Values with different letters in a column are different from each other (α=0.05)

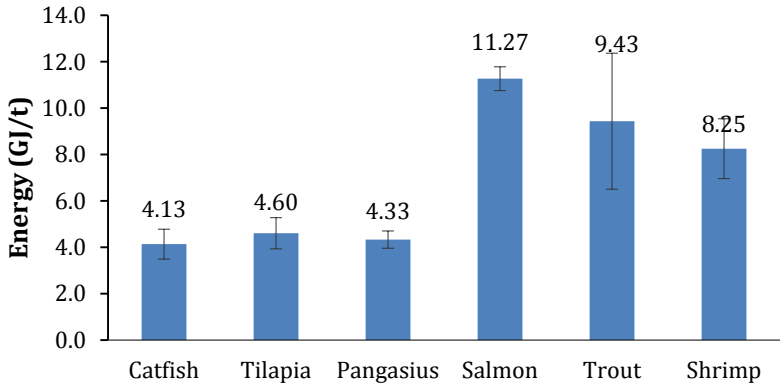

Figure 3.1 Embodied energy used to produce 1 ton of feed for main aquacultural species.

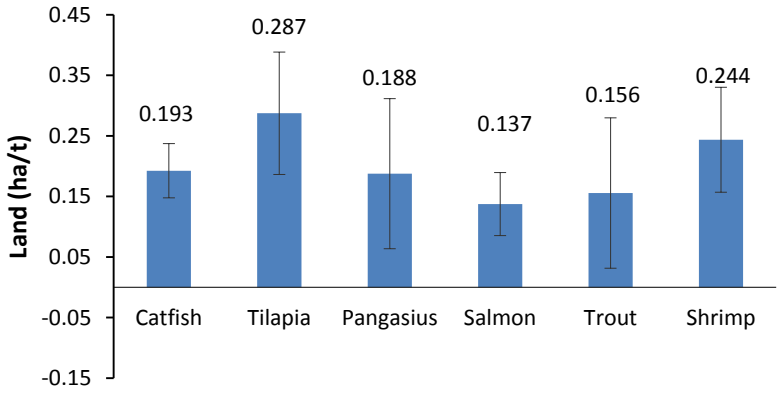

Figure 3.2 Land used to produce 1 ton of feed for main aquacultural species.

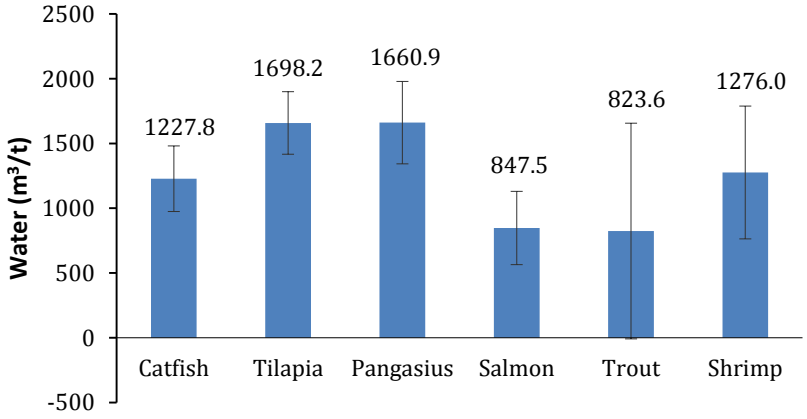

Figure 3.3 Water used to produce 1 ton of feed for main aquacultural species.

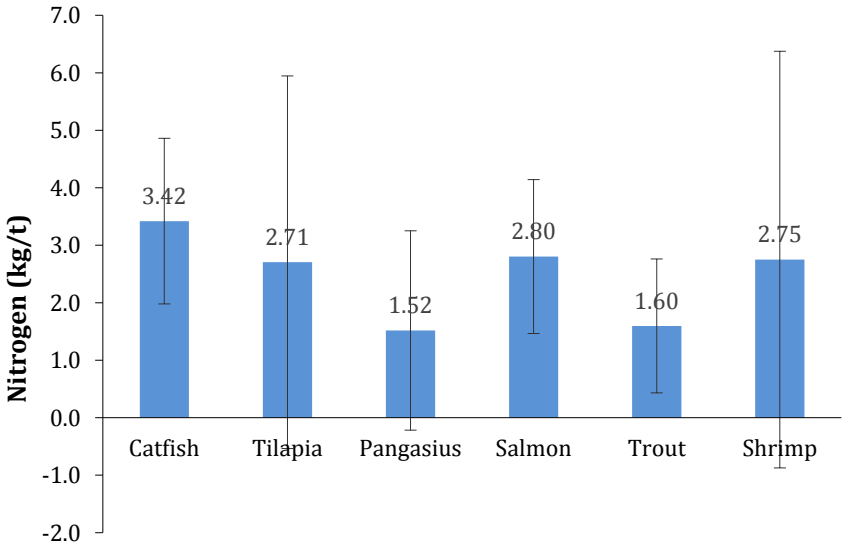

Figure 3.4 Embodied nitrogen used to produce 1 ton of feed for main aquacultural species.

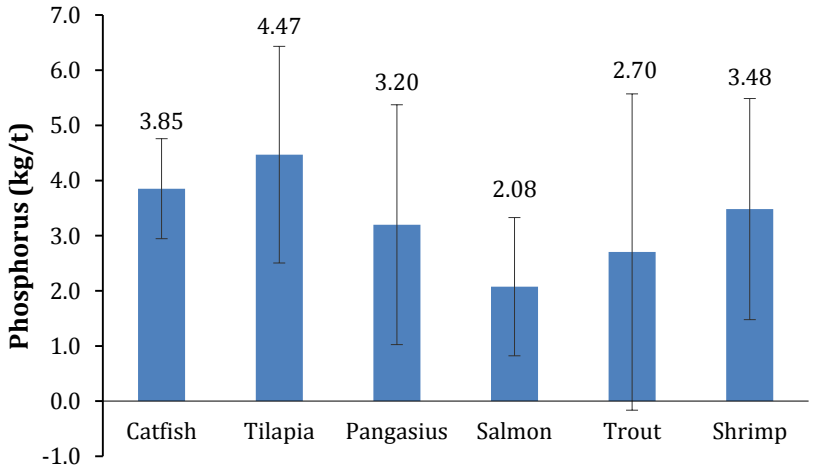

Figure 3.5 Embodied phosphorus used to produce 1 ton of feed for main aquacultural species.

References

Abdelghany, A.E., 2003. Partial and complete replacement of fishmeal with gambusia meal in diets for red tilapia *Oreochromis niloticus* × *O. mossambicus*. Aquacult. Nutr. **9**, 145–154.

Alasalvar, C., Taylor, K.D.A., Zubcov, E., Shahidi, F., Alexis, M., 2002. Differentation of cultured and wild sea bass (*Dicentrarchus labrax*): total lipid content, fatty acid and trace mineral composition. Food Chem. 79, 145-150.

Angel, A., Pelletier, N., Aubin, J., Ralite, S., Nunes, J., Freon, P., 2015. Comparative environmental performance of artisanal and commercial feed use in Peruvian freshwater aquaculture. Aquaculture 435, 52-66.

Alltech, 2014. Alltech Global Feed Survey. Alltech, Nicholasville.

Bamgboye, A.I., Koseman, B.S., 2015. Energy input in the production of cassava. Energy and Env. Res. 5, 42-48.

Bell, J.G., McEvoy, J., Tocher, D.R., McGhee, F., Campbell, P.J., Sargent, J.R., 2001. Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism. J. Nutr. 131, 1535-1543.

Boyd, C.E., McNevin, A.A., 2014. Aquaculture, resource use, and the environment. Wiley-Blackwell, New York, NY, USA.

Boyd, C.E., Polioudakis, M., Whitis, G.N., 2008. Direct energy use in channel catfish production. Global Aquaculture Advocate 11, 48-50.

Boyd, C.E., Polioudakis, M., 2006. Land use for aquaculture production. Global Aquaculture Advocate 9, 64-65.

Brekke, O.L., 1970. Dry-milling artificially dried corn; roller-milling of degerminator stock at various moistures. Cereal Science Today 15, 37-42.

Bureau, D.P., 2006. Rendered products in fish aquaculture feeds, In: Meeker, D.L. (Ed), Essential Rendering: All about the Animal By-products Industry. National Rendering Association, Alexandria, Virginia, USA, pp.179-194.

Chapagain, A.K., Hoekstra, A.Y., 2004. Water Footprints of Nations. In: Value of Water Research Report Series No. 16, vol. 1. UNESCO-IHE Delft, The Netherlands.

Chauhan, N.S., Mohapatna, P.M.J., Pandey, K.P. 2006. Improving energy productivity in paddy production through benchmarking – An application of data development analysis. Energ. Conserv. Manage. 47, 1063-1085.

Chiu, A., Li, L., Guo, S., Bai, J., Fedor, C., Naylor, R.L., 2013. Feed and fishmeal use in the production of carp and tilapia in China. Aquaculture 414–415, 127–134.

Davulis, J.P., Frick, G.E., 1977. Potential for energy conservation in feeding livestock and poultry in the United States. Bulletin 560, Agricultural Experiment Station, University of New Hampshire, Durham, New Hampshire, USA.

Davis, D.A., Arnold, C.R., 2000. Replacement of fish meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture 185, 291-298.

Fang, X., Yu, D., Buentello, A., Zeng, P., Davis, D.A., 2016. Evaluation of new non-genetically modified soybean varieties as ingredients in practical diets for Litopenaeus vannamei. Aquaculture 451, 178-185.

FAO (Food and Agriculture Organization), 2012. The state of world fisheries and aquaculture. FAO Fisheries and Aquaculture Department, Food and agriculture organization of the United Nations, Rome.

FAO (Food and Agriculture Organization), 2011. "Energy-Smart" Food to People and Climate. FAO, Rome.

Firouzi, S., Aminpanah, H., 2012. Energy use efficiency for groundnut (*Arachis hypogaea L.*) production in a semi-mechanized production system. Ann. Biol. Res. 3, 3994-3997.

Gronroos, J., Seppala, J., Silvenius, F., Makinen, T., 2006. Life cycle assessment of Finnish cultivated rainbow trout. Boreal Environ. Res. 11, 401-414.

Hardy, R.W., Cheng, Z.J. Undated. Effect of poultry by-product meal supplement with L-lysine, DL-methionine, and L-histidine as a replacement for fish meal on the performance of rainbow trout (*Oncorhynchus mykiss*). Publication 317, Director's Digest. Fats and Proteins Research Foundation, Inc., Bloomington, Illinois, USA.

Helsel, Z.R., 1992. Energy and alternatives for fertilizer and pesticide use. In: Fluck, R.C. (Ed), Energy in Farm Production, Volume 6. Elsevier, Amsterdam, The Netherlands. pp. 177-201.

Henriksson, P.J.G., Guinée, J.B., Kleijn, R., Snoo, G.R., 2012. Life cycle assessment of aquaculture systems — a review of methodologies. Int. J. Life Cycle Assess. 17, 304–313.

Hughes, S.G., Montgomery, R., Nelson, J.J., Piper, M.P., 2008. Creation of organic feed formulations for rural Pennsylvania aquafarms. The Center for Rural Pennsylvania, Harrisburg, Pennsylvania, USA.

Iribarren, D., Hospido, A., Moreira, M.T., Feijoo, G., 2010. Carbon footprint of canned mussels from a business-to consumer approach. A starting point for mussel processors and policy makers. Environ. Sci. Policy 13, 509-521.

Jones, H.B.Jr., Kerns, W.R., 1974. Wastewater treatment costs in poultry rendering plants in Georgia. Southern Journal Agricultural Economics Dec, 65-71.

Lenas, D.S., Nathanailides, C., 2011. High content of n-6 fatty acids in the flesh of farmed fish sea bass *Dicentrarchus labrax L*. and gilthead sea bream *Sparus aurata L*.: implications for the human health. Int. Aquat. Res. 3, 181-187.

Li, M.H., Robinson, E.H., 2013. Feed ingredients for feeds for channel catfish. Publication 1806, Southern Regional Aquaculture Center, Stoneville, Mississippi, USA.

Lim, C., 2008. Replacement of marine animal protein with peanut meal in diets for juvenile white shrimo, *Penaeus vannamei*. J. App. Aquacult. 7, 67-78.

MeKonnen, M.M., Hoekstra, A.Y., 2011. The green, blue, and gray water footprint of crops and derived crop products. Hydrol. Earth Syst. Sc. 15, 1577-1600.

Mendoza, T.C., Samson, R., 2002. Energy cost of sugar production in the Philippine context. Philipp. J. Crop Sci. 27, 17-26.

Mobtaker, H.G., Akram, A., Keyhani, A., Mohammadi, A., 2011. Energy consumption in alfalfa production: A comparison between two irrigation systems in Iran. Afr. J. Plant Sci. 5, 47-51.

Mungkung, R., Gheewala, S.H., Tomnantong, A., 2012. Carbon footprint of IGF peeled tail-on breaded shrimp *Litopenaeus vannamei*. How big is it compared to other aquatic products? Environ. Nat. Resour. J. 10, 31-36.

Naylor, R.L., Hardy, R.W., Bureau, D.P., Chiu, A., Elliott, M., Farrell, A.P., Forster, I., Gatlin, D.M., Goldburg, R.J., Hua, K., Nichols, P.D., 2009. Feeding aquaculture in an era of finite resources. Proc. Natl. Acad. Sci. 106, 15103-15110.

New, M.B., Wijkström, U.N., 2002. Use of fishmeal and fish oil in aquafeeds: further thoughts on the fishmeal trap: FAO Fisheries Circular No. 975. pp.61.

Nishita, K.D. Bean, M.M., 1982. Grinding methods: Their impact on rice flour properties. Cereal Chem. 59, 46-49.

Pelletier, N., Lammers, P., Stender, D., Pirog, R., 2010. Life cycle assessment of high- and low-profitability commodity and deep-bedded niche swine production systems in the upper Midwestern United States. Agric. Syst. 103, 599-608.

Pelletier, N., Tyedmer, P., Sonesson, U., Scholz, A., Ziegler, A., Flysjo, F., Kruse, A., Cancino, B., Silverman, H., 2009. Not all salmon are created equal: life cycle assessment (LCA) of global salmon farming systems. Environ. Sci. Technol. 43, 8730-8736.

Robinson, E.H., Li, M.H., 2012. Composition and formulation of channel catfish feeds. Bulletin 1200, Mississippi Agricultural and Forestry Experiment Station, Mississippi State University, Mississippi State, Mississippi, USA.

Schnepf, R., 2011. U.S. livestock and poultry feed use and availability: Background and emerging Issue. Congressional Research Service, Washington.

Smith, E.G., Janzen, H.H., Newlands, N.K., 2007. Energy balances for biodiesel production from soybeans and canola. Can. J. Plant Sci. 87,793-801.

SOFIA, 2012. The state of world fisheries and aquaculture 2012. FAO Fisheries and Aquaculture Department, Rome.

Swick, R.A., 2001. Feed based tilapia culture. Technical Bulletin AQ49-2001, American Soybean Association, Singapore.

Tacon, A.G.J., 1990. Fish feed formulation and production, People's Republic of China. FI-CRR/88/077, field Document 8 (www.fao.org/3/a-u4173e).

Thylmann, D., Druzhinia, E., Deimling, S. (editors). 2013. The Ecological Footprint of Cassava and Maize Post-harvest-losses in Nigeria. Duetsche Gesellschaft fur Internationale Zusammenarbeit Gmbh, Bonn, Germany.

Verdegem, M.C.J., Bosma, R.H., 2009. Water withdrawal for brackish and inland aquaculture, and options to produce more fish in ponds with present water use. Water Policy 11, 52-68.

Welch, A., Hoenig, R., Stieglitz, J., Benetti, D., Tacon, A., Sims, N., O'Hanlon, B., 2010. From fishing to the sustainable farming of carnivorous marine finfish. Rev. Fish. Sci. 18, 235–247.

Winther, U., Ziegler, F., Hognes, E.S., Emanuelsson, A., Sund, V., Ellingsen, H., 2009. Carbon footprint and energy use of Norwegian seafood products. SINTFF Fisheries and Aquaculture Report SFH80A096068, Trondheim, Norway.

Workagegn, K.B., 2014. Growth performance of the Nile tilapia (*Oreochromis niloticus*) fed different types of diets. Journal of Aquaculture Research and Development 5, 1000235.

Yaemsooksawat, N., Jintasataporn, O., Areechon, N., Puntamaopas, S., Thongtuak, C., 2009. Effect of dietary protein level on growth and immunity of *Litopenaeus vannamei*, Boone 1931. Songklanakarin J. Sci. Technol. 31, 15-20.

Chapter 4 Carbon, Nitrogen, and Phosphorus Compositions of Aquaculture Feeds; Relationship to Waste Loads

4.1 Abstract

Feed composition is important with respect to the dietary requirements aquaculture animals and environmental sustainability. It is critical that the nutrient compositions in feeds meet the requirements of cultured animals but not exceed them in order to assure efficient production and lessen pollution of the environment. Hence, a study was recently initiated to obtain data on the chemical composition of feeds for a variety of aquaculture species from several major aquacultural countries. A total of 203 manufactured feeds for a number of cultured species were collected from various countries. All feed samples were analyzed for the concentrations of nitrogen, carbon, phosphorus and ash content. The results indicated that nitrogen and phosphorus concentrations in feeds were highest in starter feeds, intermediate in fingerling feeds, and lowest in grower feeds. Carbon concentrations in feeds were similar for all stages. For grower feeds, nitrogen concentrations varied from 4.1% to 8.5%. Phosphorus concentrations in the feeds were similar for all species and varied from 1.1% to 1.8%. Carbon concentration in the feeds varied from 31.2% to 46.7%. The feed BOD was calculated for feeds of each of the five main cultured species; the values ranged from 1.03 to 1.16 kg O_2/kg feed. System waste loads for nitrogen ranged from 57.2 g/kg for salmon to 94.0 g/kg for channel catfish. System waste loads of phosphorus ranged from 11.5 g/kg for salmon to 22.1 g/kg for channel catfish.

4.2 Introduction

Proper nutrition of aquaculture animals is crucial for efficient, profitable production. Manufactured feed is a major expense of semi-intensive and intensive aquaculture, because it often represents 60-70% of the production cost (Tan and Dominy, 1997). It is important to use a high quality feed and good feeding practices that maximize production per unit of feed. Advantages in applying feeds for aquaculture include providing the right amount of nutrients for maximum growth, uniformity in product quality, and high production (FAO, 2012). Manufactured feeds are specially formulated to contain all essential nutrients to support animal growth. Nutritional requirements of different species vary considerably and feeds are designed specificially for each species.

Nutrients are chemical substances necessary for maintenance, growth and health of cultured species. Primary organic nutrients in feeds are proteins, carbohydrates, and lipids. Carbohydrates and lipids contain primary carbon (C), oxygen (O), and hydrogen (H), while proteins also have a large amount of nitrogen and some sulfur (S). Protein sources for feeds usually contain more phosphorus (P) than found in other feed ingredients. The water content of different feeds is variable, and the concentrations of nutrients in feed are commonly expressed on a dry matter basis. The dry matter of a feed can be separated into organic matter (mainly C, H, O, and N) and mineral matter. The organic matter in a feed can be combusted by subjecting it to a high temperature, and the residual mineral matter remaining after ignition is known as ash.

Nitrogen is an important constituent of protein, and on an average, proteins contain about 16% N. The percentage of N in feeds can be estimated from the crude protein concentration (% crude protein ÷ 6.25 = % N) and vice versa (Mariotti et al., 2008). Proteins are formed by linkages of individual amino acids (Brody, 1999). Aquatic animals cannot synthesize all amino acids;

therefore, they must acquire these essential amino acids from their diets (Cowey and Walton, 1989). Protein requirements of cultured animals usually are lower for herbivorous and omnivorous species than those for carnivorous species (Rosamond et al., 2000). Because protein is the most expensive component of aquatic feed, it is important to precisely determine the protein requirements of a species and provide a feed containing the proper kinds and amounts of amino acids and protein.

Intensive aquaculture relies on manufactured feeds to promote animal growth. Through addition of supplementary feed, production can be increased many times above that possible with fertilization alone (Ackefors and Enell, 1990). However, an increase in feed input without appropriate management increases the potential risk of water pollution in aquaculture systems and in water bodies receiving aquaculture effluents that contain uneaten feed, feces, dissolved inorganic nitrogen and organic matter, and plankton (Bonsdorff et al., 1996, 1997; Anderson and De Silva, 1997). In commercial feed-based production, only a portion of the applied feed – usually between 70 and 95% – is directly consumed by the cultured species. The unconsumed feed, along with feces and metabolic wastes enter the water body and can adversely impact water quality. Moreover, manufactured feeds can lose nutrients through leaching almost immediately after immersion into water; hence, some nutrient loses occur even before the feed is consumed. It has been estimated that around 60-80% of nitrogen and phosphorus in the feeds pass into the water column unused by the cultured species (Hall et al., 1990; Holby and Hall, 1991; Hall et al., 1992).

The composition and physical nature of feeds reflect the characteristics of the wastes resulting from its use (Pillay, 2004). Nutrients from feeding waste can lead to a number of problems including dense phytoplankton blooms, low dissolved oxygen concentration, and increased concentrations of ammonia and other nitrogenous substances within culture systems.

Impaired water quality stresses fish and shrimp, lessening their appetite and growth rate as well as predisposing them to parasites and diseases (Muller and Varadi, 1980; Bergheim et al., 1982; Penczak et al., 1982; Beveridge, 1985). The organic C and N in feeds that are not converted to biomass of the culture species exert an oxygen demand. Part of the oxygen demand results from oxidation of organic C by the culture species and of organic wastes (feces and uneaten feed) by bacteria. The remainder of the oxygen demand is caused by biological nitrification. In nitrification ammonia excreted by metabolism of the culture species and released during decomposition of organic matter by bacteria and other organisms of decay is oxidized to nitrate by nitrifying bacteria (Boyd and Tucker, 1998). The summary equation for nitrification is:

$$NH_4^+ + 2O_2 \longrightarrow NO_3^- + 2H^+ + H_2O$$

The process consumes oxygen and releases acidity (H^+) that neutralizes alkalinity.

In order to perform a mass balance analysis for feed nutrients in aquaculture, a knowledge of the elemental composition of inputs (mostly from feeds) and outputs for a particular culture system is necessary. One of the greatest needs related to evaluation of environmental pollution caused by aquacultural activities is a comprehensive data based on concentrations of various elemental components in formulated feeds especially organic C, N, and P. The present study aims to determine the composition of major elements in feeds and in the corresponding whole bodies of cultured animals. Data on the concentration of these elements in feed will be useful for estimating system loads of elements that is defined as feed input of an element minus output of the element in harvested biomass (Boyd and Queiroz, 2001). Knowledge of the system loads for different species and feeds could be benefit in formulating feeds that produce less waste and cause less pollution within culture units. Moreover, information on system loads would be useful in designing

better management practices to lessen environmental loads − the portions of system loads of elements discharged into receiving water bodies.

4.3 Materials and Methods

4.3.1 Sample Collection and Preparation

A total of 203 feed samples were collected for different species as follows: channel catfish, *Ictalurus punctatus*, clarias catfish, *Clarias microcephalus* × *C. gariepinus*; pangasius catfish, *Pangasius hypophthamus*; tilapia, *Oreochromis niloticus and O. aureus*; snakehead, *Channa micropeltes*; Asian sea bass, *Lates calcarifer*; common carp, *Cyprinus carpio*; salmon, *Salmo salar*; trout, *Oncorhynchus mykiss*; whiteleg shrimp, *Litopenaeus vannamei*; black tiger prawn, *Penaeus monodon*. The samples were obtained the United States, Canada, Guatemala, Ecuador, Brazil, China, Vietnam, Thailand, and Ghana. Feeds were categorized into three groups, 'starter', 'fingerling' and 'grower' feeds based on the growth stage of the cultured species for which they were intended. Catfish, tilapia, salmon, trout and whiteleg shrimp samples (whole body) were procured from fish markets in Alabama and Georgia, USA. Three individual animals of each species were obtained for analysis. Information from literature for whole body elemental composition of cultured species from literatures were included in this study as follows: tilapia; (Boyd and Green, 1998), whiteleg shrimp; (Boyd and Teichert-Coddington, 1995), black tiger prawn; (Gomes and Boyd, 2003).

Feed samples were pulverized with a mortar and pestle. Fish and shrimp samples were chopped into small pieces and then were ground using an IKA Economical Analytical Mill (Cole-Parmer, 27 Vernon Hills, IL, USA). Samples were dried in a convection oven at 105 ^{o}C. Moisture contents of whole bodies and feed samples were calculated using Equation 4.1 by recording their original weight (wet) and dried weight (dry). For moisture content, samples in ceramic crucibles

were placed in a convection oven at 105°C for 8 hr. Crucibles and contents were then transferred to desiccators until they were at room temperature before weighing. The crucibles and dry samples were incinerated in a furnace (62700 Barnstead Thermolyne, Dubuque, Iowa, USA) at 500°C for 8 hr and ash concentrations were determined using Equation 4.2. The loss of volatile matter from the dry samples during ignition allowed an estimate of total organic matter in samples by using Equation 4.3.

$$Moisture\ content\ (\%) = \frac{Wd - Wc}{Ww} \times 100 \quad (4.1)$$

$$Ash\ content\ (\%) = \frac{Wa - Wc}{Wd - Wc} \quad (4.2)$$

$$Organic\ matter\ (\%) = 100 - \%\ Ash \quad (4.3)$$

where:

Ww = wet weight of the sample (g)

Wd = weight of sample and crucible after drying (g)

Wa = weight of sample and crucible after ashing (g)

Wc = weight of crucible (g)

4.3.2 Phosphorus Analysis

Ash in crucibles was dissolved in an acidic solution (1 N HNO_3 plus 1 N HCl in 1:1 ratio). Five mililiter acidic solution was added into each crucible containing the ash. Crucibles were held on a hot plate until nearly dry. The contents of crucibles were quantitatively transferred into a 100-ml volumetric flask that was then brought to volume with distilled water. The resulting solution was filtered through Whatman Number 42 acid-washed filter paper. The phosphorus concentration in cultured species and feed samples was determined using the vanadomolybdophosphoric yellow color method (Jackson, 1958). Exactly 1.00 mL digestate was placed into a 50 mL volumetric flask, and 10 mL of vanadomolybdate reagent was added. The solutions were diluted to a volume 50 mL with distilled water. Solutions were allowed to stand for 10 min so that the color could fully

develop. A blank also was carried through the procedure along with the samples. A stock P solution (1 mL = 50 µg P) was prepared by dissolving 0.2195 g KH_2PO_4 in 1,000 mL of distilled water. A working standard for calibrating the spectrophotometer containing 5 mg/L PO_4-P was made by diluting 50 mL stock solution with 500 mL distilled water. The absorbances of the standard, blank, and digestates were read at a wavelength of 490 µm.

4.3.3 Carbon and Nitrogen Analysis

The whole bodies and feed samples were further pulverized with a porcelain mortar and pestle. Aliquots of ground samples of know weights (between 10.00 and 15.00 mg) were used for determination of C and N concentrations using a CN analyzer (LECO® Truspec CN Analyzer, Michigan, USA).

4.3.4 Feed Oxygen Demand

Feed oxygen demand (BOD_f) was calculated as described by Boyd (2009) using Equation 4.4:

$$BOD_f = [C_f – (FCE \times C_c)] \times 2.67 + [N_f – (FCE \times N_c)] \times 4.57 \quad (4.4)$$

Where, BOD_f = biological oxygen demand of feed (kg O_2/ kg feed)

C_f, C_c, N_f, N_c = decimal fractions of carbon and nitrogen in feed and live weight of the cultured species respectively

FCE = feed conversion efficiency

4.3.5 Acidification Potential of Feed

The acidification potential of feed - given as the calcium carbonate ($CaCO_3$) equivalent of the potential acidity that could result from nitrification of ammonia nitrogen resulting from the feed was estimated using Equation 4.5 developed by Boyd and Tucker (2014):

$$AP_f = [N_f – (FCE \times N_c)] \times 7.14 \quad (4.5)$$

where, AP_f = acidification potential of feed (kg $CaCO_3$/kg feed)

N_f = % nitrogen in feed / 100

N_c = % nitrogen in culture species (live weight) / 100

FCE = feed conversion efficiency

7.14 = ratio $CaCO_3$: N

4.3.6 System Waste Load

The quantities of nitrogen and phosphorus entering culture systems can be estimated by subtracting amounts of each element removed in harvest of 1 kg of the cultured species from the quantity of each element applied in feed necessary to produce 1 kg of biomass using Equation 4.6.

$$E_w = [(E_f \times FCR) - E_c] \times 10^3 \quad (4.6)$$

Where, E_w = elements (C, N, or P) waste in g/kg culture species,

FCR = feed conversion ratio

E_f and E_c = decimal fractions of elements (C, N, or P) in feed and culture species respectively.

4.3.7 Statistical Analysis

All data were statistically analyzed using one-way analysis of variance to determine significant differences ($p < 0.05$) among treatments, which was followed by the Fisher's Least Significant Difference test to determine significant differences among treatment means. All statistical analyses were carried out using SAS (V9.2 SAS Institute, Cary, NC, USA).

4.4 Results

4.4.1 Compositional Analysis of Feeds

4.4.1.1 Starter Feeds

The average concentrations of ash, organic matter, organic N, C and P of starter feeds for different cultured species are presented in Table 4.1. Moisture, ash and organic matter concentrations ranged from 5.1% (salmon feed) to 8.9% (white shrimp feed), 9.9% (tilapia feed) to 14.7% (black tiger prawn feed), and 85.3% (black tiger prawn feed) to 90.1% (tilapia feed), respectively. Highest C concentration was found in feeds for trout (47.0%). Marine and salmon feeds had the highest N concentration (9.7%). Lowest C and N concentrations were found in black tiger prawn feed (40.4% C, 7.1% N). Marine feed had greater P concentration (2.0%) ($P<0.05$) than found in salmon feed (1.5%) and clarias catfish feed (1.3%).

4.4.1.2 Fingerling Feeds

Average concentrations of ash, organic matter, organic N, C and P of fingerling feeds for different cultured species are presented in Table 4.2. Moisture concentrations varied from 7.1% (channel catfish, snakehead, and trout feeds) to 10% (pangasius catfish feed). Concentration of ash varied from 6.4% (carp feed) to 18.6% (pangasius catfish feed), while organic matter component ranged from 81.4% (pangasius catfish feed) to 93.6% (carp feed). Mean C content in salmon feed (49%) was higher ($P<0.05$) than all other feeds except trout feeds. Mean N concentration varied from 4.4% (pangasius catfish feed) to 8.2% (salmon feed). Mean P concentration varied from 0.6% (carp feed) to 2.3% (black tiger prawn feed).

4.4.1.3 Grower Feeds

Average concentrations of ash, organic matter, organic N, C and P of grower feeds for different cultured species are presented in Table 4.3. Mean moisture concentration varied from

4.1% (salmon feed) to 9.5% (snakehead and whiteleg shrimp feeds). Mean ash concentration varied from 8.4% (trout feed) to 15.8% (Asian sea bass feed). Mean organic matter concentration varied from 84.2% (Asian sea bass feed) to 91.6% (trout feed). Mean C concentration varied from 31.2% (pangasius catfish feed) to 48.6% (salmon feed). Carbon concentration in salmon feed was higher ($P<0.05$) than in all other feeds except marine fish (47.2%) and trout (46.7%) feeds. Mean N concentration varied from 4.1% (pangasius catfish feed) to 8.5% (marine fish feed). Mean P concentration varied from 1.1% (clarius catfish feed) to 1.8% (snakehead and Asian sea bass feeds).

4.4.1.4 Components in feed for each stage of cultured species

Concentrations of C in feeds were similar for feed in all stages and did not have a specific trend (Figure 4.1a). Protein concentrations in feed for all species were highest in starter feeds, intermediate in fingerling feeds, and lowest in grower feeds (Figure 4.1b). Phosphorus concentrations of all feeds were highest in starter feeds. However, the values for fingerling feeds were intermediate, and those for grower feeds were lowest except for channel catfish and salmon feeds (Figure 4.1c).

4.4.1.5 Protein concentration in feeds

Overall concentrations of protein in grower feeds for cultured species were above their nutritional requirements. Only protein concentration in feed for tilapia and carp were slightly below the requirement (Figure 4.2). Results from this study (Figure 4.3) indicated that the quotients, crude protein concentrations in feeds reported by the manufacturers divided by 6.25, were generally good indicators of measured N concentrations (r^2 = 0.93). Thus, the feed N concentration can usually be estimated with a reasonable degree of certainty from the crude protein concentration given on the feedbag. The differences of protein concentration between the levels

provided by manufacturer and the levels estimated from measured concentrations of Asian feed were more variable from estimated values more than were those of African and North American feeds (Figure 4.4).

4.4.1.6 Relationship between different feed constituents

Simple linear regression analyses revealed that organic C concentration increased as nitrogen concentration increased, but the correlation was poor (r^2=0.38) (Figure 4.5a). An even lower correlation was observed between P and N concentrations in feeds (r^2=0.28) (Figure 4.5b). Carbon and P concentrations were not correlated (Figure 4.5c).

4.4.1.7 Compositional analysis of feeds from different continents

Two feed samples from Africa contained protein at a level higher than culture species requirement. In Asia, most of the protein concentrations for tilapia feeds were lower than their requirements. Amoung Asian feeds, only clarias catfish feeds had protein concentration above the protein requirement. Most of the feeds in North America including catfish catfish, tilapia, salmon, trout feeds had protein concentrations higher than their requirement. Feed samples from South America had protein concentrations slightly higher than the requirements (Figure 4.6a). Feed samples from every continent had phosphorus concentrations higher than cultured species requirements (Figure 4.6b).

The N concentrations in tilapia feeds from North America (6.1%) were higher ($P<0.05$) than those from Asia (3.9%), but not different from feeds from Africa (6.0%) and South America (4.6%) (Figure 4.7a). The C concentrations in tilapia feeds from Africa (44.2%) and North America (44.1%) were higher ($P<0.05$) than those from South America (41.7%) and Asia (41.3%) (Figure 4.7b). The P concentrations in tilapia feeds from South America (1.8%) were higher ($P<0.05$) than

those from Asia (1.1%), but not different from North America (1.3%) and Africa (1.3%) (Figure 4.7c).

For whiteleg shrimp feeds, no differences ($P<0.05$) were found in N concentration in feeds from different continents (Figure 4.8a). Mean N concentration in these feeds varied from 6.2% (Asia) to 5.3% (South America). South American feeds exhibited the highest mean C concentration (44.8%) and had higher ($P<0.05$) C concentration than Asian feeds (41.9%), but not different from North American feed (43.4%) (Figure 4.8b). Asian samples exhibited the highest mean P concentration (1.6%) and were higher in P concentration than South American feeds (0.9%), but not different from North American feeds (1.2%) (Figure 4.8c).

4.4.2 Compositional Analysis of Whole Bodies

Concentrations of ash, organic matter, organic C, N, and P in whole bodies of five important culture animals are provided (Table 4.4). Mean moisture concentrations varied from 65.9% (channel catfish) to 76.5% (trout) whereas mean ash concentrations varied from 9.3% (salmon) to 19.7% (black tiger prawn). Mean organic matter concentrations varied from 80.3% (black tiger shrimp) to 90.7% (salmon). Mean C concentrations of salmon (52.1%) were higher ($P<0.05$) than those of whiteleg shrimp (41.5%), tilapia (45.1%), and black tiger prawn (45.9%). Although whole bodies of black tiger prawn had the highest apparent mean N concentration (11.4%), they did not differ ($P>0.05$) from trout where the mean N concentration was 9.7%. Tilapia had the highest apparent mean P concentration (1.8%), but only different ($P<0.05$) from black tiger shrimp and salmon for this element.

4.4.3 Biological Oxygen Demand (BOD_f) and Acidification Potential (AP_f) of feed

The BOD_f was calculated for feeds of each of the five species (Table 4.5). The mean values ranged from 1.03 kg O_2/kg feed for whiteleg shrimp to 1.16 kg O_2/kg feed for trout. The total

oxygen demand per kilogram of production was estimated by multiplying BOD_f values by the FCR. The mean total oxygen demand from feed for production of the five species ranged from 1.19 kg O_2/kg for salmon to 2.10 kg O_2/kg for channel catfish (Table 4.6). The total oxygen demand for channel catfish feed was higher ($P<0.05$) than that for the other species. Trout feeds exhibited the highest acidity potential (38.7 kg $CaCO_3$/kg feed), while tilapia feeds had the lowest acidity potential (27.2 kg $CaCO_3$/kg feed) (Table 4.5).

4.4.4 Nutrient loads

Nitrogen and P retentions of tilapia (31.5%N, 25.1%P) were higher than those of other cultured species whereas channel catfish had the lowest N retention (26.6%) and whiteleg shrimp had the lowest P retention (21.7%). Mean system loads for N ranged from 57.2 g/kg (or kg/t) for salmon to 94.0 g/kg for channel catfish. Nitrogen load from channel catfish production was higher ($P<0.05$) than the loads for other species ($P<0.05$) (Figure 4.9a). For P, system loads ranged from 11.5 g/kg for salmon to 22.1 g/kg for channel catfish. Phosphorus load from channel catfish production was also higher ($P<0.05$) than the loads from other species, aside from tilapia production (Figure 4.9b).

4.5 Discussion

Nitrogenous substances in feeds not only consist of protein and amino acids, but they also contain numerous other compounds such as nucleic acids, amines, urea, ammonia, nitrates and nitrites (Mariotti et al., 2008). The sum of individual amino acids in feeds accounts for about 80 to 90% of the protein contents (Helland et al., 2010). Thus, besides amino acids, other nitrogen-containing compounds may account for 10 to 20% of the crude protein content in feeds. The protein requirements of aquatic animals appear to be higher than those of terrestrial animals; however, the absolute protein intake per kilogram of body weight gain is highly comparable. This

is because the energy required for maintaining homeostasis in aquatic animals is lower than that of warm-blooded terrestrial animals. According to NRC (1993), recommended dietary protein levels for various cultured species in grow-out stage were reported to be 280 to 320 g/kg (28-32%) diet for channel catfish, 260 to 300 g/kg diet for Nile tilapia, 280 to 320 g/kg diet for common carp, 340 to 400 g/kg diet for Atlantic salmon, 360 to 380 g/kg diet for rainbow trout, 400 g/kg diet for black tiger prawn, and 350 to 400 g/kg diet for whiteleg shrimp. Results from the comparison of feed for different species in each continent revealed that tilapia feeds from Asia and whiteleg shrimp feeds from South America contained lower level of protein at 243.7 g/kg and 331.2 g/kg, respectively, compared to other continents.

The metabolism of poikilothemic aquatic animals is affected by various environmental factors but especially by temperature (Bureau et al., 2002). Therefore, it often is assumed that temperature might have a significant impact on the nutritional requirement of a cultured species. Nonetheless, there was no convincing evidence that the protein requirement of fish and shrimp is affected by water temperature, dissolved oxygen or salinity (Lupatsch et al., 2001; Lupatsch and Kissil, 2005). However, Bermudes et al. (2010) found an increase in protein requirement in Asian sea bass (Barramundi) cultured at temperatures that are above the thermal optima. This may be attributed to a lower feed intake of the animals than optimal temperature.

Nutrient wastage is considered to be a major problem in aquaculture farming. The load of nutrients released to the environment in aquaculture, particularly N and P loads, generally arise from feeds and animal excretions (Gowen and Bradbury, 1987; Handy and Poxton, 1993; Burford and Williams, 2001). The amounts of N and P released vary considerably depending on fish species as a result of differences in their feeding behavior, feeding efficiency, and nutrient retention efficiency (Gavine et al., 1995; Anderson and De Silva, 1997; Cho and Bureau, 1997). These

factors bring about variation in the proportions of the feed nutrients that are transformed to uneaten, excreted, or converted to biomass of the cultured species. Islam (2005) estimated the N loading from fish cages varied from 47.3 to 320.6 kg/t of production for different species and diets. Results from the present study estimated that system loadings for N varied from 57.2 to 90.4 kg/t of production. This suggests that many of the fish cage operations considered by Islam (2005) had much high FCRs than assumed in calculation done here. Islam also revealed that N waste varied for the same species. For example, N waste from cultured rainbow trout varied from 47.3 to 124.2 kg/t. The average value of 65.1 kg N/t of trout in the present study is within this range. Differences in nutrient wastes for different species resulted from different feeding behaviors and interactions among nutrients. This leads to variation in the proportion of applied feed that is transformed into flesh compared to excreted and lost into the culture system or directly to environment in the case of cage culture (Islam, 2005). The ingredient compositions of feeds affect nutrient loading. For example, according to Islam (2005), fish fed with commercial diets caused less N loading (47.3 to 130 kg/t) as compared to those fed with trash fish (320.6 kg/t). It follows that aquaculture in tropical and subtropical regions in Asia (Thailand, China, Taiwan, Japan, Philippines etc.), where trash fish is the main diet used in cage culture, produces higher waste loads of nutrients as compared to temperate regions where higher quality pelleted feeds are used more commonly.

Handy and Poxton (1993) studied the relationship between feed loss and nutrient retention in the fish body. They reported that when only 1.45% of feed went uneaten, N retention in the fish body was higher, and only 52.2% of feed N entered the culture system. Whereas when feed wastage 40%, N retention was lower and the amount of N loss could be as great as 95%. Handy and Poxton (1993) stressed the need to consider the physiology, feeding behavior and nutrient requirement of fish, when formulating feeds to reduce nutrient loss. Langaker (1988) reported

results of an experiment in which 32% of feed N was used for growth and 68% was released into the environment. However, in another study, only 8.6% of feed N input was retained in areolated grouper, *Epinephelus areolatus* (Leung et al., 1999). The low N retention by this species was attributed to uneaten feed containing 30 to 46% of the N input leading to N loss of 88% of input. Low retention of N and other nutrients by cultured species can result from several factors including overfeeding, suboptimal feed formulation, imbalance of macronutrients in feeds, quality of ingredients and poor water stability of feeds (Grisdale-Helland and Helland, 1997; Burford and Williams, 2001).

Edwards (1978) determined the N concentration in fish feeds as 7.7% and, Bromley and Smart (1981) gave a value of 2.7% N in fish. Islam (2005) used data on feed and fish N concentration from Edwards (1978) and Bromley and Smart (1981) to determine the quantity of N lost. Assuming a FCR of 2.0 and feed wastage of 20%, it was estimated that 123 kg of N were consumed but only 27 kg (22%) were retained. The remaining 78% was entered the culture system as waste. These estimates of N waste were similar to those in trout cage culture but only almost 20 to 30% of N input was harvested in fish and about 70 to 80% was lost to the environment (Penczak et al., 1982; Gowen et al., 1985; Phillips et al., 1985; Enell, 1987; Hall et al., 1992). However, Penczak et al. (1982) concluded that 36.1% of consumed N was retained in rainbow trout resulting in 32 kg of ammonia production per ton of feed fed.

Christensen and Horstedt (1991) found that 27-36% of feed P was used for growth by rainbow trout, 26-30% released in dissolved form, 38-45% as particulate P. Persson (1987) estimated the critical load of P to be 7.3 g/kg for rainbow trout, and the actual P content in the fish to be 3.8-4.5 g/kg (Wiesmann et al., 1988). They also explained that although a large variation was

found in solubility between various types of feed, approximately 60% of P in feeds would be released and lost to the water.

Islam (2005) used a FCR-based regression model for predicting nutrient loading from marine cage aquaculture for a given diet and calculated the release of N and P per ton of production. The conceptual model showed that 132.5 kg N and 25.0 kg P are released into the environment for each tonne of fish produced. Hence, the annual N and P loading from 10,000 t fish were 1,325 t N and 250 t P at the usual feed conversion rate. Hall et al. (1992) estimated the total environmental loss of N from marine fish cage farm was between 95 to 102 kg N/t production. Moreover, cage culture is an open system releases all waste directly into the environment (Ackefors and Enell, 1990).

Feed BOD increases as feed conversion ratio increases because less organic nutrients are retained by the cultured animals and more enters into the water. Therefore, better feed conversion can improve water quality by reducing the quantity of oxygen consumed in various biological processes. Boyd (2009) stated that feed oxygen demand comprises of three components: animal respiration, bacterial decomposition of feces and uneaten feed and nitrogenous oxygen demand expressed by nitrifying bacteria. The present study reported feed biological demand to be 1.03 to 1.16 kg O_2 per kg feed. It was slightly lower than the observation of Boyd (2009) that estimated the average oxygen demand of aquaculture feed to be 1.2 to 1.3 kg O_2 per kg feed. This might be because the BOD_f in this study was calculated only from grower feeds which contained less N than fingerling and starter feeds. Boyd (2009) explained that the proportion of oxygen demand of feed was animal respiration = 0.4 to 0.6 kg O_2 per kg feed; bacterial decomposition = 0.4 to 0.6 kg O_2 per kg feed; and nitrogenous oxygen demand = 0.15 to 0.25 kg O_2 per kg feed. It has been shown

that more than half of the feed oxygen demand is expressed by processes other than respiration by cultured animals. This has important implications for aquaculture management and practices.

Comparatively few data on the amount of BOD generated per tonne of production of aquaculture species are available, but it is well known that aquaculture effluents typically are relatively low in BOD compared to municipal wastewater but higher in BOD than water bodies into which they are discharged (Boyd, 1978; Boyd and Gross, 1999; Tucker and Hargreaves, 2008). It also is commonly known that ammonia N inputs into natural water tend to be a source of acidity through nitrification and that nitrification can cause acidification in aquaculture systems – especially intensive systems with large inputs of feed (Boyd and Tucker, 2014). The discussion above reveals that system loads of N in aquaculture vary greatly depending upon the species, composition and quality of feed, and especially with factors affecting FCR. No doubt the system BOD load and acidification potential also vary with these same factors. For a particular species, feeds do not appear to vary tremendously in composition, and the major factor affecting system loads of N, P, and BOD and the system acidification potential are related most closely to FCR. Thus, average system loads of N, P, and BOD and acidification potential (Table 4.5 and 4.6) may be used as general indication of water pollution generated in aquaculture systems by feed. These general estimates of N and P loads tend to be within the range of N and P loads estimated in studies of individual production systems where manufactured feeds were used, but lower than loads for production systems in which trash fish were used as feed. There are, however, no good estimates of BOD generation or potential acidification for specific feed-based production systems. The estimates of N, P, and BOD loads and acidification potential provided in Table 4.5 and Table 4.6 are for typical FCRs. But, if there estimates are calculated for the average feed composition and

an FCR of 1.0, (Table 4.7), the values can be adjusted for any other FCR simply by multiplying values for Table 4.7 by the other FCR.

4.6 Conclusion

The rapid growth of the aquaculture industry has led to growing concerns over its environmental impacts. These environmental effects depend largely on species, culture method, stocking density, feed type, hydrography of the site and husbandry practices. In general, excessive nutrient loadings from feeding of the cultured species can be, in general, caused by either one or a combination of the following three feed-related factors: (1) feed wastage from improper feed management practices; (2) poor feed quality such as feed pellets with poor stability and high solubility; and (3) limited digestion, absorption and retention of nutrients in species. Moreover, poor water quality management leading to stressful condition in ponds and other culture system can influence the appetite, growth, and health of cultured animals. This also can lead to a higher than normal FCR. The integration of research on nutrient cycling in cultured systems, optimization of feed formulations can potentially improve nutrient retention, reduce feed wastage, and minimize water pollution.

The prospects for sustainable aquaculture are greater if pollutants from farming practices are managed well and do not exceed the assimilation capacity of the surrounding water bodies. The discharge of water entering the natural environment should be treated to reduce nutrient and suspended solid loads. Impacts can be significantly reduced by careful site selection, control of stocking density, high quality of formulated feed, improved feed formulation and integrated culture. Environmental impact assessment and monitoring should also be employed to ensure environmental sustainability.

The present study demonstrated that the total oxygen demand of production of the five species ranged from 1.19 kg O_2/kg for salmon to 2.10 kg O_2/kg for channel catfish. System loads for N and P ranged from 57.4 kg/t (salmon) to 94.0 kg/t (channel catfish) and 10.55 g/kg (salmon) to 18.32 g/kg (channel catfish), respectively. Effluent standards for land-based aquaculture farming such as those presented by Alabaster (1982) and should be employed. Nevertheless, it would be difficult to set effluent standards in open-water cage farming systems. Regular monitoring of water and sediment quality at aquatic culture sites would be required and may become mandatory in some countries.

Table 4.1 Moisture and dry matter-based concentrations of ash, organic matter, carbon, nitrogen and phosphorus of starter feeds. Values represent mean and standard error with minimum and maximum concentration.

Feed type	N	Moisture	Dry Weight (%)				
		(%)	Ash	OM	Carbon	Nitrogen	Phosphorus
Channel	**4**	**7.1±0.75^{bc}**	**10.8±0.82^{c}**	**89.2±0.82^{a}**	**46.7±0.36^{a}**	**9.4±0.12^{a}**	**1.8±0.15^{ab}**
		(6.2-8.4)	(9.5-11.5)	(88.5-90.5)	(45.9-47.6)	(9.2-9.7)	(1.5-2.1)
Clarias	**1**	**6.5±1.50^{abc}**	**12.9±1.63^{abc}**	**87.1±1.63^{abc}**	**43.1±0.00^{b}**	**7.9±0.00^{bc}**	**1.3±0.29^{b}**
		(6.5-6.5)	(12.9-12.9)	(87.1-87.1)	(43.1-43.1)	(7.9-7.9)	(1.3-1.3)
Tilapia	**2**	**8.5±1.06^{ab}**	**9.9±1.15^{c}**	**90.1±1.15^{a}**	**43.8±0.16ab**	**8.2±0.11^{abc}**	**1.9±0.21^{ab}**
		(6.7-10.2)	(9.4-10.3)	(89.7-90.6)	(43.6-43.9)	(8.1-8.3)	(1.7-2.1)
Salmon	**6**	**5.1±0.61^{c}**	**11.6±0.67^{bc}**	**88.4±0.67^{ab}**	**46.7±0.31^{a}**	**9.7±0.22^{a}**	**1.5±0.12^{b}**
		(4.0-7.4)	(10.5-14.1)	(85.9-89.5)	(45.7-47.3)	(9.1-10.4)	(0.7-2.0)
Trout	**1**	**6.2±1.50^{abc}**	**10.8±1.63^{abc}**	**89.2±1.63^{abc}**	**47.0±0.00^{a}**	**8.8±0.00^{ab}**	**1.9±0.29^{ab}**
		(6.2-6.2)	(10.8-10.8)	(89.2-89.2)	(47.0-47.0)	(8.8-8.8)	(1.9-1.9)
White shrimp	**17**	**8.9±0.36^{a}**	**12.3±0.40^{abc}**	**87.7±0.40^{abc}**	**43.8±0.56^{ab}**	**7.5±0.26^{bc}**	**1.7±0.07^{ab}**
		(6.6-12.0)	(9.0-14.9)	(85.1-91.0)	(40.7-49.3)	(6.1-9.7)	(1.2-2.3)
Black prawn	**2**	**8.2±1.06^{ab}**	**14.7±1.15^{a}**	**85.3±1.15^{c}**	**40.4±0.01^{b}**	**7.1±0.25^{c}**	**1.7±0.21^{ab}**
		(7.4-9.1)	(13.1-16.3)	(83.7-86.9)	(40.4-40.4)	(6.8-7.4)	(1.6-1.8)
Freshwater fish	**4**	**7.5±0.75^{ab}**	**11.1±0.82^{bc}**	**88.9±0.82^{ab}**	**46.9±0.58^{a}**	**8.7±0.22^{ab}**	**1.7±0.15^{ab}**
		(5.5-9.0)	(10.3-12.0)	(88.0-89.7)	(45.6-47.9)	(8.2-9.2)	(1.6-1.8)
Marine fish	**5**	**6.4±0.67^{bc}**	**13.1±0.73^{ab}**	**86.9±0.73^{bc}**	**43.7±0.92^{ab}**	**9.7±0.23^{a}**	**2.0±0.13^{a}**
		(3.8-7.5)	(10.8-14.4)	(85.6-89.2)	(40.4-45.9)	(8.8-10.1)	(1.6-2.1)

Values with different letters in a column are different from each other (α =0.05)

Table 4.2 Moisture and dry matter-based concentrations of ash, organic matter, carbon, nitrogen and phosphorus of fingerling feeds. Values represent mean and standard error with minimum and maximum concentration.

Feed type	N	Moisture	Dry matter (%)				
		(%)	Ash	OM	Carbon	Nitrogen	Phosphorus
Channel	**3**	**7.1±0.75^{bc}**	**8.3±1.21^{fg}**	**91.7±1.21^{ab}**	**45.3±0.63^{bc}**	**6.8±0.25^{abcd}**	**1.2±0.16^{de}**
		(6.5-9.4)	(6.6-9.3)	(90.7 -93.4)	(40.0-43.2)	(6.4-7.3)	(1.0-1.4)
Clarias	**14**	**7.9±0.33^{b}**	**10.9±0.56^{ef}**	**89.1±0.56^{bc}**	**42.4±0.24^{cd}**	**5.4±0.14^{cde}**	**1.2±0.07^{e}**
		(6.0-9.5)	(8.3-15.6)	(84.4 -91.7)	(40.8-43.7)	(4.1-5.8)	(0.9-1.8)
Pangasius	**2**	**10.0±0.89^{a}**	**18.6±1.49^{a}**	**81.4±1.49^{g}**	**38.9±0.03^{e}**	**4.4±0.84^{e}**	**1.7±0.20^{bcd}**
		(9.6-10.5)	(18.0-19.1)	(80.9 -82.0)	(38.8-38.9)	(3.5-5.2)	(1.5-1.9)
Snakehead	**2**	**7.1±0.89^{b}**	**15.1±1.49^{abc}**	**84.9±1.49^{efg}**	**40.9±1.11^{de}**	**7.3±0.01^{ab}**	**1.8±0.20^{abc}**
		(5.9-8.2)	(15.0-15.3)	(84.7 -85.0)	(40.8-41.1)	(7.3-7.3)	(1.8-1.8)
Tilapia	**11**	**8.3±0.38^{ab}**	**11.7±0.63^{de}**	**88.3±0.63^{cd}**	**42.9±0.66^{cd}**	**6.7±0.48^{abcd}**	**1.5±0.08^{cd}**
		(6.4-11.4)	(7.4-15.6)	(84.4 -92.6)	(38.3-45.9)	(3.3-8.0)	(1.0 2.1)
Carp	**1**	**8.4±1.25^{ab}**	**6.4±2.10^{g}**	**93.6±2.10^{a}**	**44.9±0.00^{bc}**	**4.8±0.00^{e}**	**0.6±0.28^{f}**
		(8.4-8.4)	(6.4-6.4)	(93.6 -93.6)	(44.9-44.9)	(4.8-4.8)	(0.6-0.6)
Asian sea bass	**1**	**7.2±1.25^{ab}**	**15.5±2.10^{abcd}**	**84.5±2.10^{defg}**	**42.7±0.00^{cd}**	**7.8±0.00^{ab}**	**2.3±0.28^{ab}**
		(7.2-7.2)	(15.5-15.5)	(84.5 -84.5)	(42.7-42.7)	(7.8-7.8)	(2.3-2.3)
Salmon	**2**	**3.2±0.89^{c}**	**9.4±1.49^{efg}**	**90.6±1.49^{abc}**	**49.0±0.28^{a}**	**8.2±0.33^{a}**	**1.4±0.20^{cde}**
		(2.7-3.7)	(8.5-10.3)	(89.7 -91.5)	(48.7-49.3)	(7.9-8.6)	(1.2-1.5)
Trout	**4**	**7.1±0.63^{b}**	**8.6±1.05^{fg}**	**91.4±1.05^{ab}**	**46.8±0.22^{ab}**	**8.0±0.21^{a}**	**1.6±0.14^{cd}**
		(5.6-8.5)	(8.4-8.9)	(91.1 -91.6)	(46.2-47.3)	(7.4-8.3)	(1.5-1.8)
White shrimp	**12**	**9.2±0.36^{a}**	**11.9±0.61^{cde}**	**88.1±0.61^{cde}**	**43.7±0.58^{bcd}**	**6.9±0.19^{abcd}**	**1.4±0.08^{cde}**
		(6.6-11.6)	(7.4-14.5)	(85.5 -92.6)	(40.6-46.6)	(6.2-7.9)	(1.0-1.9)
Black prawn	**3**	**8.8±0.72^{ab}**	**16.4±1.21^{ab}**	**83.6±1.21^{fg}**	**40.6±0.32^{de}**	**7.2±0.21^{abc}**	**2.3±0.16^{a}**
		(8.0-9.3)	(14.5-17.7)	(82.3 -85.5)	(39.9-40.9)	(6.8-7.6)	(2.2-2.5)
Freshwater fish	**1**	**9.4±1.25^{ab}**	**12.7±2.10^{bcdef}**	**87.3±2.10^{bcdef}**	**41.1±0.00^{de}**	**5.1±0.00^{de}**	**1.9±0.28^{abc}**
		(9.4-9.4)	(12.7-12.7)	(87.3 -87.3)	(41.1-41.1)	(5.1-5.1)	(1.9-1.9)
Marine fish	**1**	**8.3±1.25^{ab}**	**12.4±2.10^{bcdefg}**	**87.6±2.10^{abcdef}**	**45.0±0.00^{bc}**	**6.0±0.00^{bcde}**	**1.1±0.28^{def}**
		(8.3-8.3)	(12.4-12.4)	(87.6 -87.6)	(45.0-45.0)	(6.0-6.0)	(1.1-1.1)

Values with different letters in a column are different from each other (α =0.05)

Table 4.3 Moisture and dry matter-based concentrations of ash, organic matter, carbon, nitrogen and phosphorus of grower feeds. Values represent mean and standard error with minimum and maximum concentrations.

Feed type	N	Moisture (%)	Dry matter (%)				
			Ash	OM	Carbon	Nitrogen	Phosphorus
Channel	**6**	**7.1±0.75^{bc}**	**9.0±1.00^{c}**	**91.0±1.00^{a}**	**44.1±0.23^{cd}**	**6.3±0.42^{cde}**	**1.3±0.13^{abcd}**
		(6.5-11.9)	(6.6-11.3)	(88.7-93.4)	(43.5-45.1)	(5.4-7.7)	(1.0-1.8)
Clarias	**17**	**7.9±0.32^{bc}**	**10.6±0.60^{bc}**	**89.4±0.60^{ab}**	**42.4±0.31^{cdef}**	**4.8±0.13^{ef}**	**1.1±0.08^{d}**
		(6.3-9.9)	(8.4-17.2)	(82.8-91.7)	(37.8-43.6)	(3.5-5.8)	(0.8-1.6)
Pangasius	**4**	**9.3±0.67^{ab}**	**15.1±1.23^{a}**	**84.9±1.23^{c}**	**40.1±0.64^{f}**	**4.1±0.40^{f}**	**1.6±0.16^{ab}**
		(9.3-9.4)	(9.2-18.4)	(81.6-90.8)	(38.6-41.7)	(3.3-4.8)	(1.0-2.1)
Snakehead	**2**	**9.5±0.94^{a}**	**13.7±1.74^{ab}**	**86.3±1.74^{bc}**	**31.2± 0.07^{ef}**	**7.2±0.17^{abcd}**	**1.8±0.23^{a}**
		(8.5-10.5)	(13.6-13.8)	(86.2-86.4)	(41.1-41.2)	(7.0-7.3)	(1.8-1.9)
Tilapia	**31**	**8.3±0.24^{bc}**	**10.4±0.44^{bc}**	**89.6±0.44^{ab}**	**42.7±0.32^{cde}**	**5.0±0.26^{ef}**	**1.3±0.06^{cd}**
		(2.7-10.5)	(6.1-16.2)	(83.8-94.0)	(39.6-45.3)	(2.8-7.1)	(0.6-2.2)
Carp	**4**	**9.0±0.67^{abc}**	**11.0±1.23^{bc}**	**89.0±1.23^{ab}**	**42.4±1.68^{cdef}**	**5.6±0.50^{de}**	**1.2±0.16^{cd}**
		(6.3-12.3)	(8.7-13.3)	(86.7-91.3)	(37.8-45.1)	(4.3-6.7)	(0.6-1.8)
Asian sea bass	**2**	**7.9±0.94^{abcd}**	**15.8±1.74^{a}**	**84.2±1.74^{c}**	**41.9±0.89^{def}**	**7.0±0.18^{abcd}**	**1.8±0.23^{a}**
		(7.7-8.0)	(14.4-17.3)	(82.7-85.6)	(41.0-42.8)	(6.8-7.2)	(1.7-1.8)
Salmon	**4**	**4.1±0.67^{e}**	**9.7±1.23^{bc}**	**90.3±1.23^{ab}**	**48.6±1.56^{a}**	**8.0±0.45^{ab}**	**1.5±0.16^{abc}**
		(3.3-5.0)	(7.5-12.9)	(87.2-92.5)	(45.9-51.5)	(7.2-9.1)	(1.0-2.0)
Trout	**5**	**7.4±0.60^{cd}**	**8.4±1.10^{c}**	**91.6±1.10^{a}**	**46.7±0.94^{ab}**	**7.4±0.21^{abc}**	**1.5±0.14^{abcd}**
		(5.2-9.7)	(7.3-10.6)	(89.4-92.7)	(44.9-50.3)	(6.9-8.2)	(1.3-1.6)
White shrimp	**10**	**9.5±0.42^{a}**	**10.6±0.78^{bc}**	**89.4±0.78^{ab}**	**43.4±0.43^{cde}**	**5.9±0.36^{de}**	**1.2±0.10^{bcd}**
		(7.4-11.2)	(8.2-14.8)	(85.2-91.8)	(40.8-45.7)	(4.5-8.5)	(0.9-1.6)
Freshwater fish	**12**	**8.0±0.39^{bc}**	**10.1±0.71^{bc}**	**89.9±0.71^{ab}**	**44.6±0.72^{bc}**	**6.7±0.38^{bcd}**	**1.4±0.09^{abcd}**
		(6.2-9.3)	(7.9-14.6)	(85.4-92.1)	(41.3-49.2)	(3.8-7.9)	(1.0-2.0)
Marine fish	**3**	**6.1±0.77^{de}**	**9.9±1.42^{bc}**	**90.1±1.42^{ab}**	**47.2±1.01^{a}**	**8.5±0.41^{a}**	**1.7±0.19^{a}**
		(4.7-7.3)	(9.3-10.3)	(89.7-90.7)	(45.6-49.1)	(7.7-9.0)	(1.6-1.8)

Values with different letters in a column are different from each other (α =0.05)

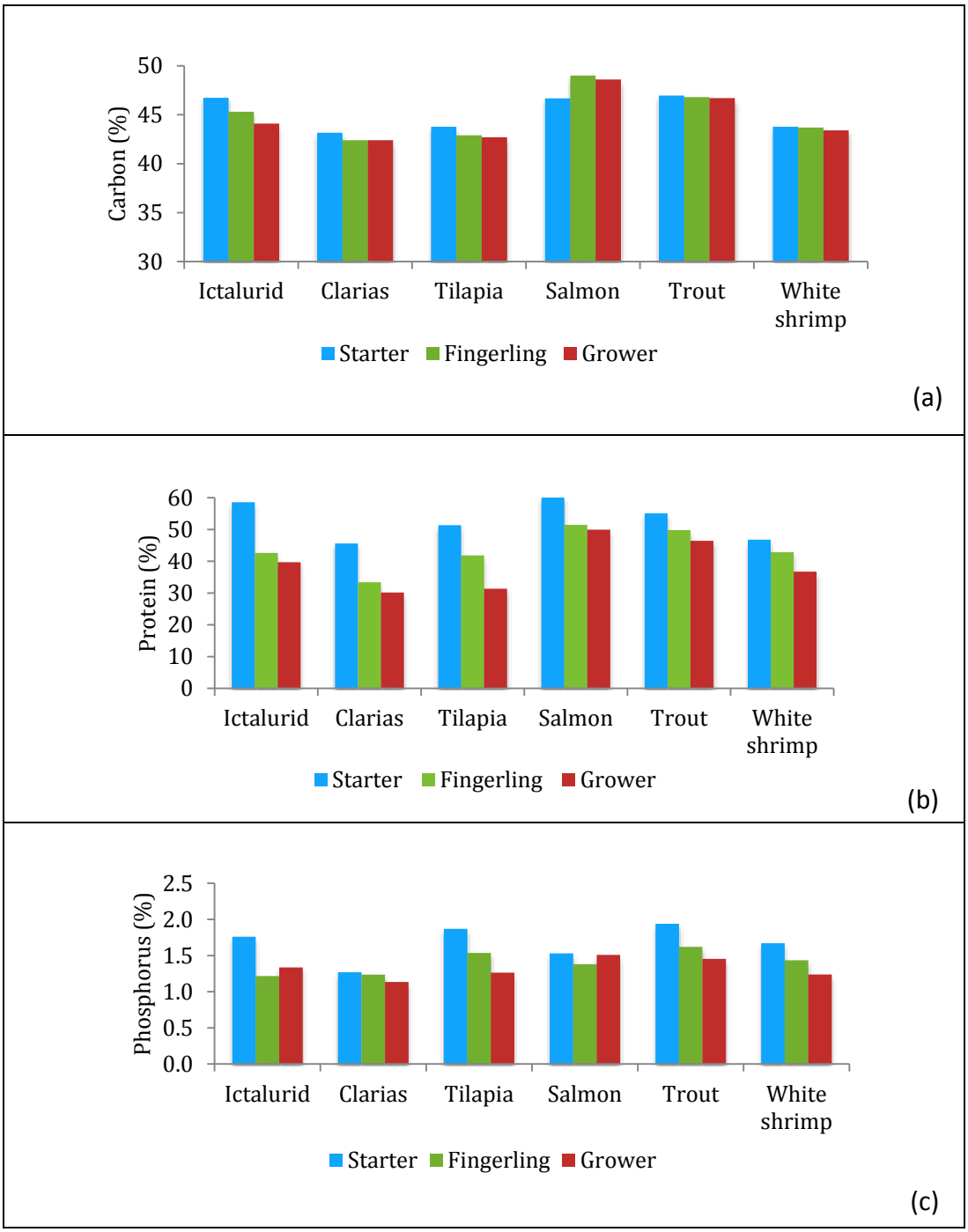

Figure 4.1 Concentrations of carbon, protein, and phosphorus in feeds for different growth stages of cultured species.

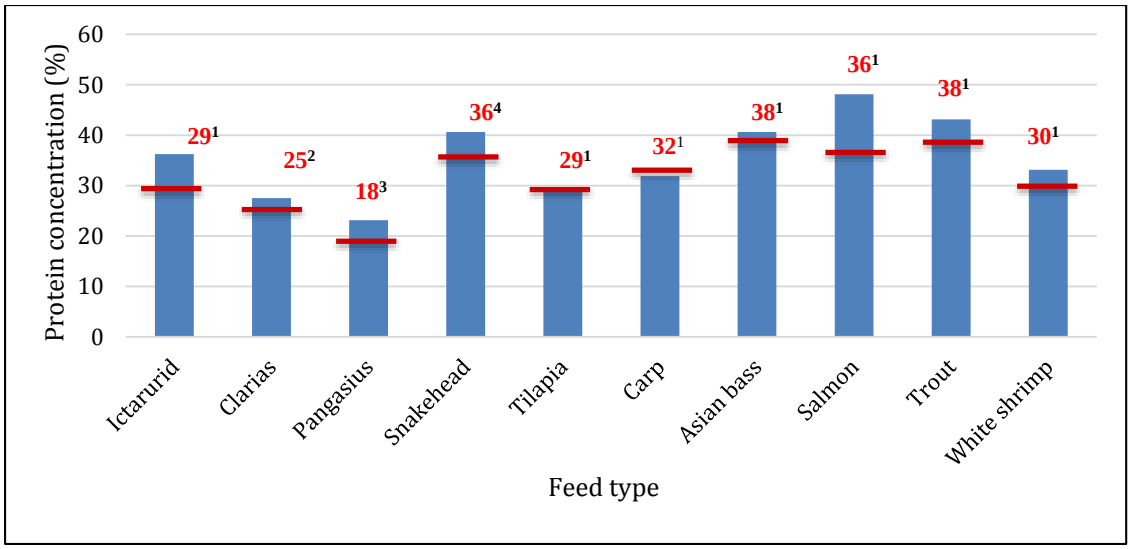

Figure 4.2 Protein concentration in feeds and protein requirement for each culture species

[1]NRC (2011).
[2]Boonyaratpalin (1988).
[3]Chuapohuk (1994).
[4]Boonyaratpalin (1981).

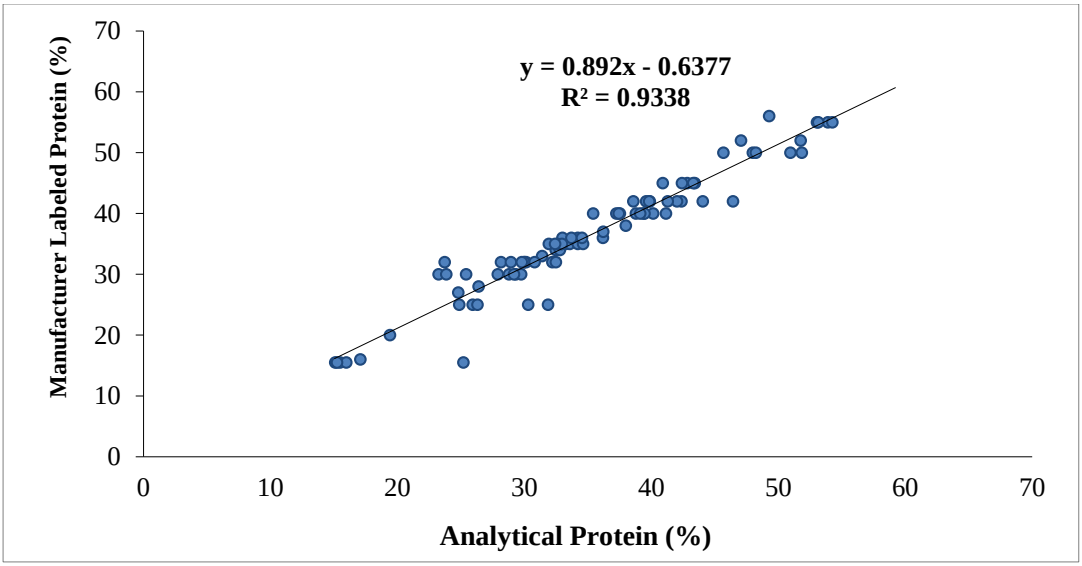

Figure 4.3 Regression between crude protein concentrations provided by manufacturer for feeds and crude protein concentrations estimated from measured concentrations of nitrogen in feed.

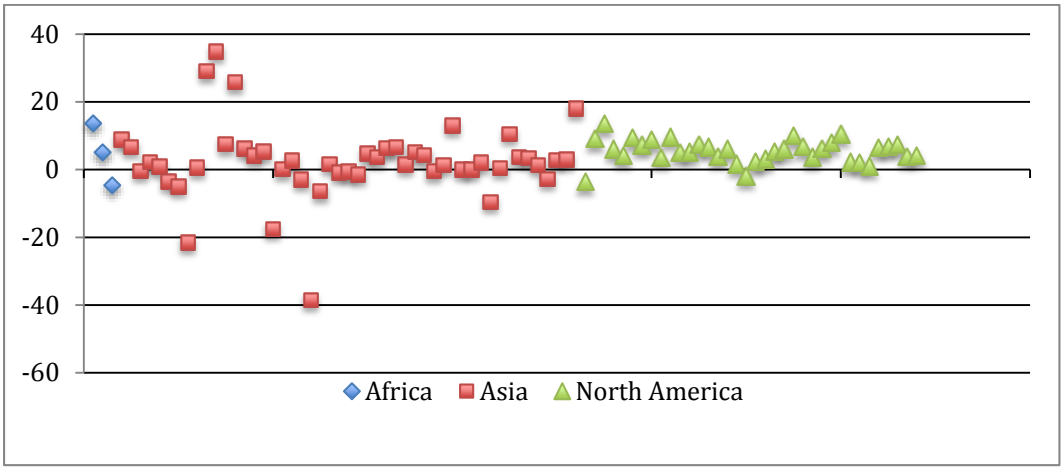

Figure 4.4 Differences of protein concentration between the levels provided by manufacturer and the levels estimated from measured concentrations of nitrogen in feed.

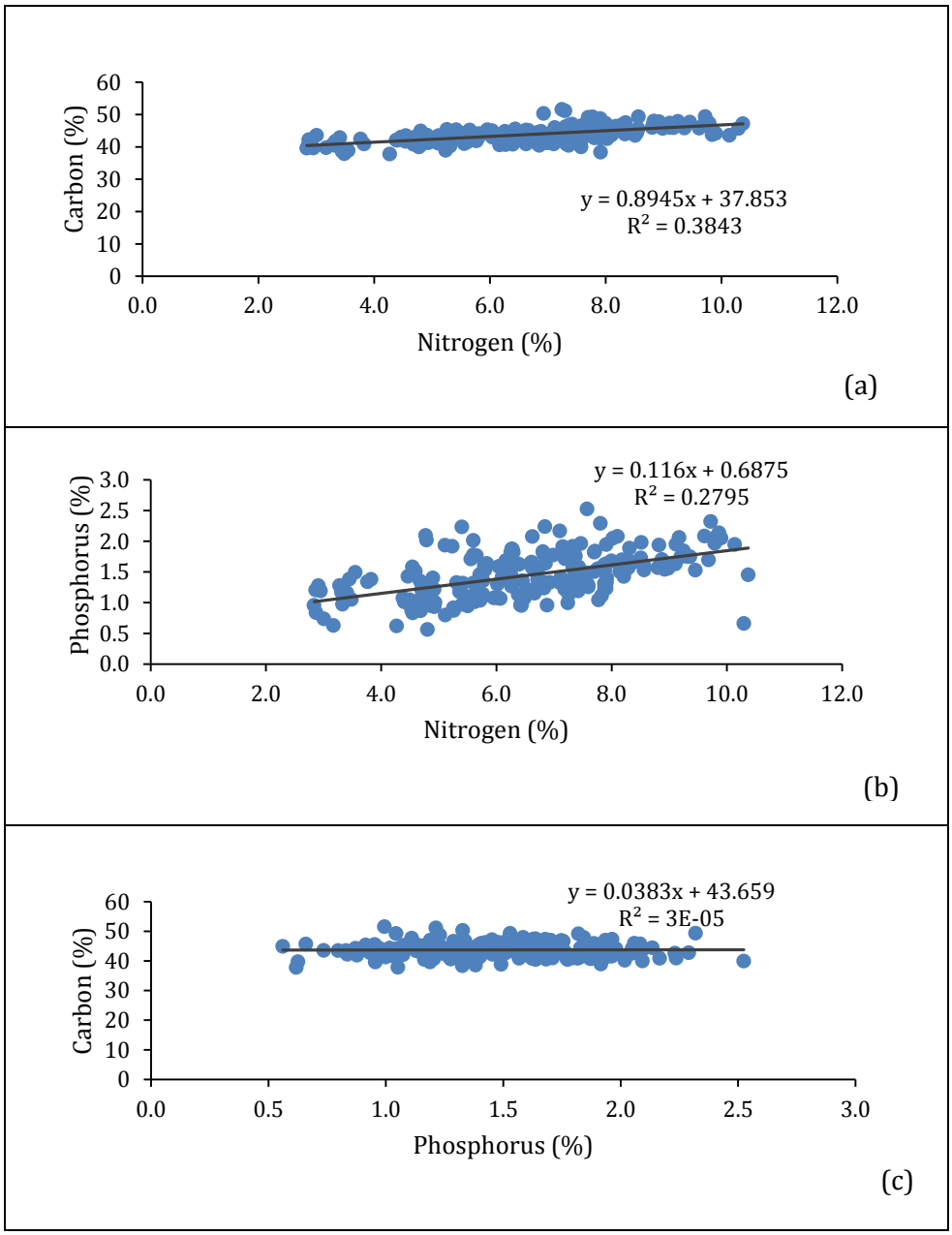

Figure 4.5 Relationships between carbon and nitrogen (a), phosphorus and nitrogen (b), and carbon and phosphorus (c) for feed samples (n=203).

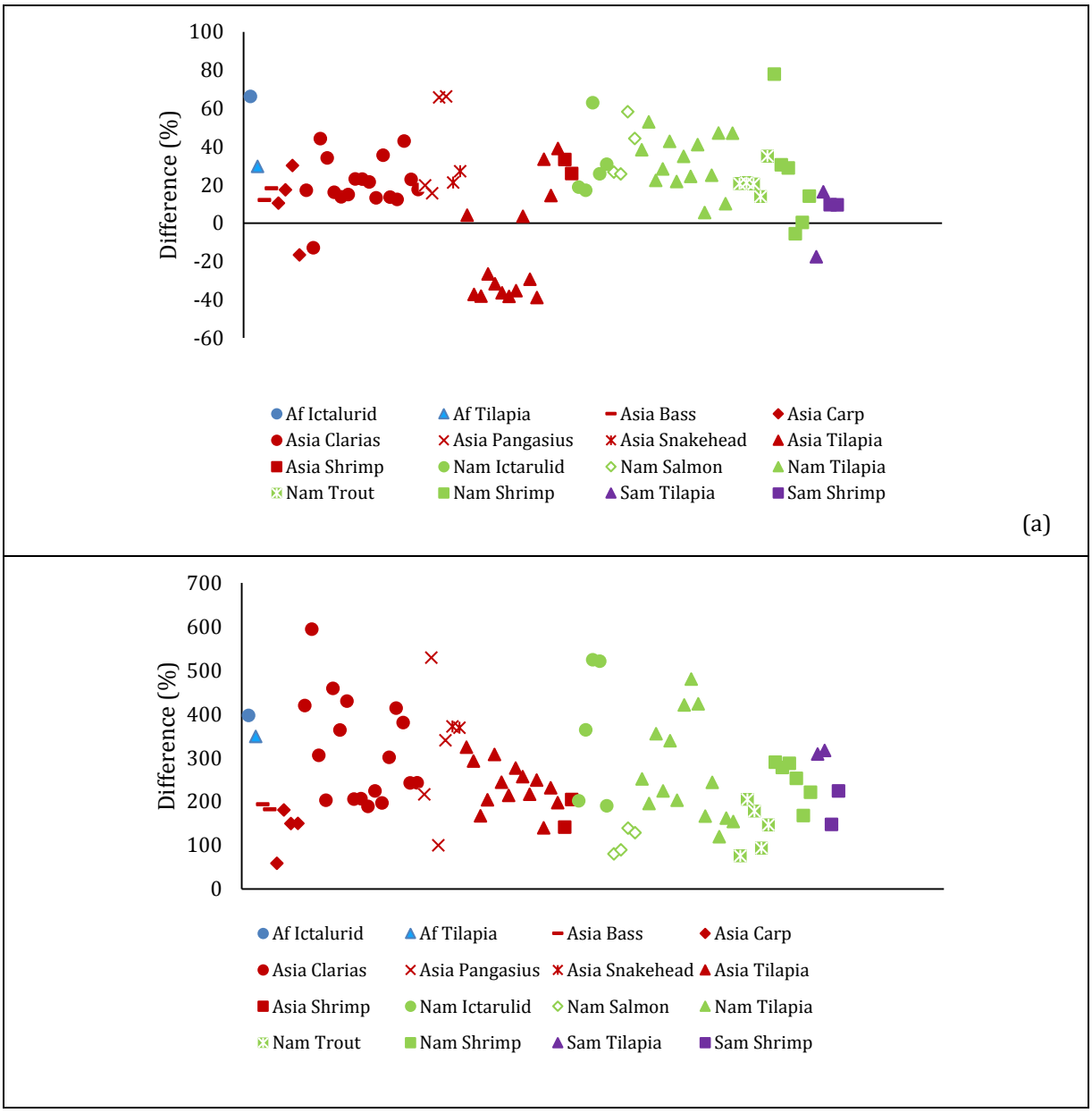

Figure 4.6 Percentage of the differences between protein and phosphorus concentrations in feeds and the requirement of cultured species in Africa (Af), Asia, North America (Nam), and South America (Sam).

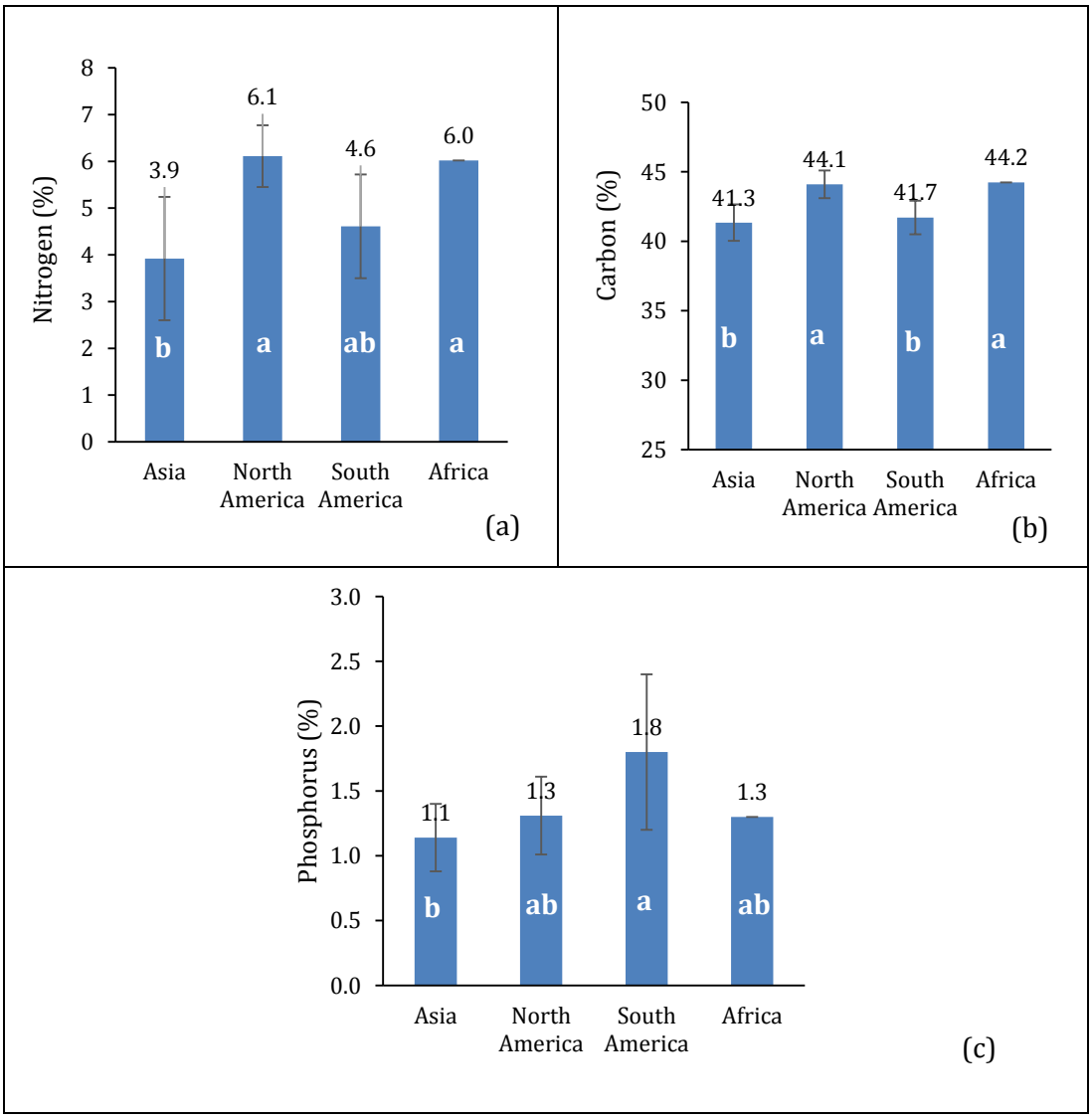

Figure 4.7 Nitrogen (a), C (b) and P (c) concentrations of tilapia grower feeds from three continents on an oven dry matter basis. Bar with same letters do not differ at P<0.05 according to LSD Ad Hoc Test.

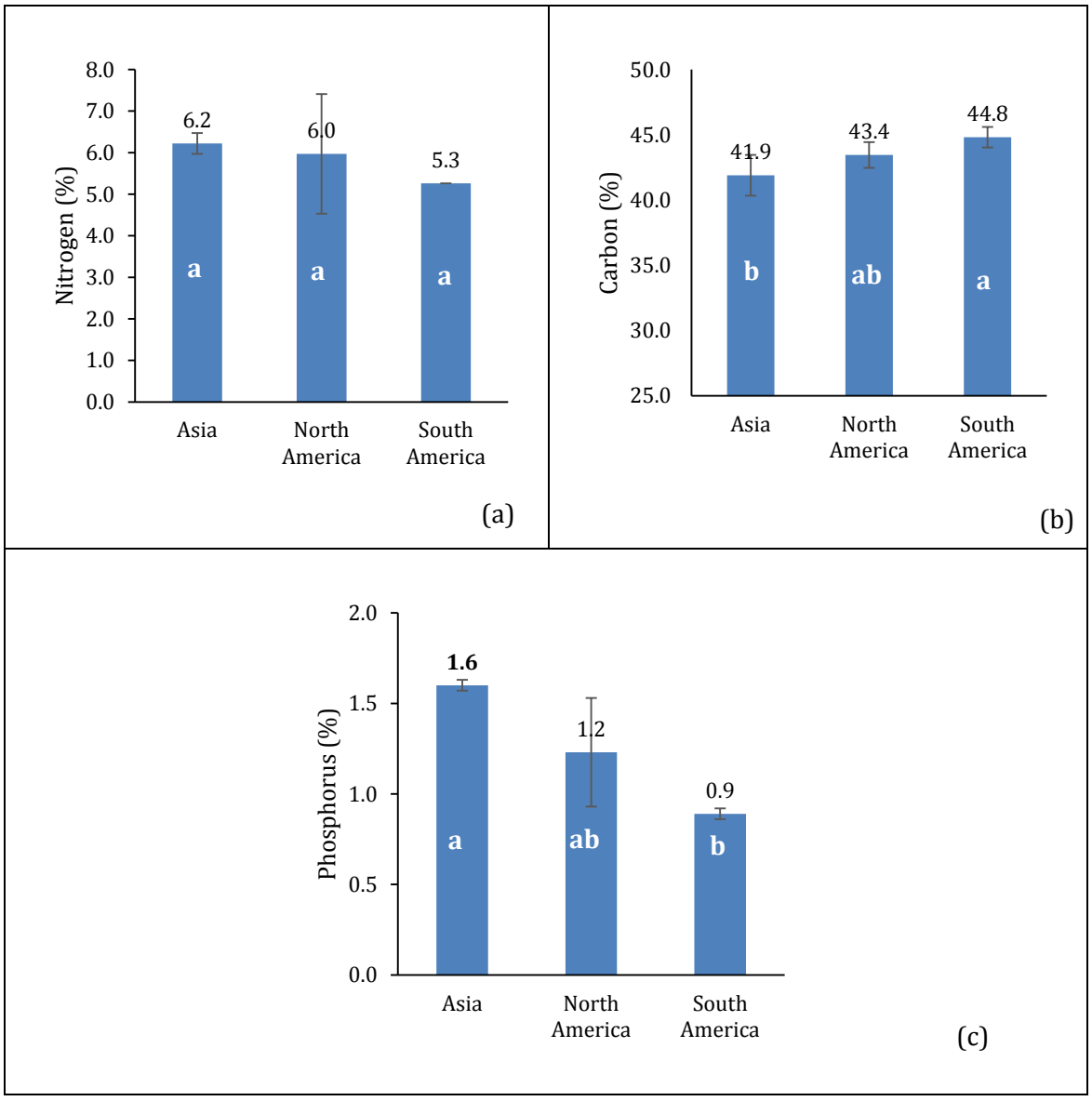

Figure 4.8 N (a), C (b) and P (c) concentrations of whiteleg shrimp grower feeds from three continents on an oven dry matter basis. Bar with same letters do not differ at P<0.05 according to LSD Ad Hoc Test.

Table 4.4 Moisture and dry matter-based concentrations of ash, organic matter, carbon, nitrogen and phosphorus of five species. Values represent mean and standard error with minimum and maximum concentrations.

Cultured species	N	Moisture	Ash	OM	Carbon	Nitrogen	Phosphorous
Channel	**3**	**65.9 ± 0.65^{c}**	**9.5 ± 1.79^{b}**	**90.5 ± 1.79^{a}**	**51.9 ± 1.39^{ab}**	**9.0 ± 0.46^{bc}**	**1.7 ± 0.21^{ab}**
		(65.1 - 66.9)	(8.4 - 10.7)	(89.3 - 91.6)	(48.9 - 57.8)	(7.9 - 9.7)	(1.6 - 1.7)
Tilapia	**5**	**73.1 ± 0.50^{b}**	**13.6 ± 1.38^{ab}**	**86.4 ± 1.38^{ab}**	**45.1 ± 1.07^{c}**	**9.0 ± 0.35^{bc}**	**1.8 ± 0.19^{a}**
		(71.9 - 75.0)	(8.7 - 19.8)	(80.2 - 91.3)	(43.6 - 46.8)	(8.5 - 9.4)	(1.4 - 2.9)
Salmon	**3**	**66.2 ± 0.65^{c}**	**9.3 ± 1.79^{b}**	**90.7 ± 1.79^{a}**	**52.1 ± 1.39^{a}**	**8.3 ± 0.46^{c}**	**1.2 ± 0.21^{b}**
		(65.8 - 66.5)	(8.6 - 9.9)	(90.1 - 91.4)	(51.4 - 52.7)	(8.1 - 8.4)	(1.1 - 1.2)
Trout	**3**	**76.5 ± 0.65^{a}**	**12.0 ± 1.79^{ab}**	**88.0 ± 1.79^{ab}**	**50.2 ± 1.39^{ab}**	**9.7 ± 0.46^{ab}**	**1.7 ± 0.21^{ab}**
		(76.0 - 77.0)	11.4 - 12.9)	(87.1 - 88.6)	(49.1 - 51.7)	(9.5 - 9.9)	(1.7 - 1.7)
White shrimp	**4**	**72.7 ± 0.56^{b}**	**10.6 ± 1.55^{b}**	**89.4 ± 1.55^{a}**	**41.5 ± 1.20^{d}**	**9.1 ± 0.39^{bc}**	**1.3 ± 0.19^{ab}**
		(70.4 - 74.0)	(9.0 - 12.5)	(87.5 - 91.0)	(39.9 - 43.8)	(8.3 - 11.1)	(1.2 - 1.4)
Black prawn	**1**	**72.9 ± 1.12^{b}**	**19.7 ± 3.10^{a}**	**80.3 ± 3.10^{b}**	**45.9 ± 2.40^{bcd}**	**11.4 ± 0.79^{a}**	**1.0 ± 0.37^{b}**

Values with different letters in a column are different from each other (α =0.05)

Table 4.5 Typical feed conversion ratios (FCR), feed biological oxygen demand (BOD_f) and acidity potential of feeds. Carbon (C), nitrogen (N), and phosphorus (P) concentration in feeds and cultured animals based on grower feeds.

Species	FCR	Feed (% air dry weight)			Feed BOD_f (kg/kg feed)	Cultured species (% live weight)			Acidity Potential (kg $CaCO_3$/kg)
		C	N	P		C	N	P	
Channel	**2.0**[1]	**40.1±0.54**^{c}	**5.8±0.43**^{bc}	**1.2±0.14**^{a}	**1.08±0.029**^{ab}	**17.7±1.25**^{a}	**3.1±0.16**^{a}	**0.6±0.06**^{a}	**3.4±0.25**^{abc}
		(38.3-42.1)	(4.8-7.1)	(0.9-1.7)	(1.00-1.18)	(16.2-20.2)	(2.8-3.3)	(0.5-0.6)	(2.8-4.1)
Tilapia	**1.7**[2]	**39.1±0.31**^{c}	**4.6±0.24**^{c}	**1.1±0.05**^{a}	**1.04±0.015**^{b}	**12.5±0.96**^{bc}	**2.5±0.14**^{b}	**0.5±0.05**^{ab}	**2.7±0.14**^{c}
		(36.3-44.0)	(2.6-6.5)	(0.6-2.0)	(0.90-1.19)	(11.7-13.1)	(2.3-2.6)	(0.4-0.8)	(1.5-3.8)
Salmon	**1.1**[3]	**46.7±1.65**^{a}	**7.7±0.40**^{a}	**1.4±0.22**^{a}	**1.08±0.021**^{ab}	**15.4±1.10**^{ab}	**2.6±0.16**^{ab}	**0.4±0.06**^{b}	**3.7±0.19**^{ab}
		(43.7-49.8)	(7.0-8.7)	(1.0-1.8)	(1.04-1.13)	(10.9-17.6)	(2.2-2.8)	(0.3-0.4)	(3.4-4.2)
Trout	**1.2**[4]	**43.2±1.17**^{b}	**6.9±0.21**^{ab}	**1.3±0.05**^{a}	**1.16±0.028**^{a}	**11.8±1.10**^{c}	**2.3±0.16**^{b}	**0.4±0.06**^{ab}	**3.9±0.13**^{a}
		(41.1-47.7)	(6.6-7.7)	(1.2-1.5)	(1.10-1.25)	(11.3-12.4)	(2.2-2.4)	(0.4-0.4)	(3.7-4.4)
White shrimp	**1.5**[5]	**39.3±0.42**^{c}	**5.3±0.35**^{bc}	**1.1±0.10**^{a}	**1.03±0.019**^{b}	**11.3±0.96**^{c}	**2.5±0.14**^{b}	**0.4±0.05**^{b}	**3.0±0.20**^{bc}
		(36.8-41.0)	(4.0-7.9)	(0.8-1.5)	(0.94-1.17)	(10.5-12.4)	(2.2-3.0)	(0.3-0.4)	(2.3-4.5)

Values with different letters in a column are different from each other (α =0.05)

[1]Robinson and Li (2010).

[2]Chiu et al. (2013), Pelletier and Tyedmers (2010).

[3]Karalazos et al (2011).

[4]Aubin et al. (2009), Boissey et al. (2011).

[5]Bauer et al (2012).

Table 4.6 Feed biochemical oxygen demand (BOD_f) and system loads of nitrogen and phosphorus from feed to produce of 1 kg of five common aquaculture species.

Species	BOD (kg O_2/ harvest kg)	Nutrient retention (%)		Nutrient load (kg/harvest ton)	
		Nitrogen	Phosphorus	Nitrogen	Phosphorus
Channel	2.1±0.06[a]	26.6±1.37[a]	23.2±0.38[ab]	94.0±7.11[a]	22.1±2.39[a]
	(2.00-2.37)	(23.8-28.1)	(22.5-23.8)	(77.3-116.3)	(16.3-30.5)
Tilapia	1.77±0.02[b]	31.5±0.94[a]	25.1±4.73[ab]	64.7±3.38[b]	18.1±0.84[ab]
	(1.54-2.03)	(28.8-33.1)	(20.2-39.2)	(37.0-91.4)	(9.0-31.9)
Salmon	1.19±0.02[e]	30.9±2.3[a]	22.6±2.04[ab]	57.2±2.90[b]	11.5±1.84[c]
	(1.15-1.24)	(26.4-33.4)	(18.5-24.8)	(52.5-64.4)	(7.5-14.9)
Trout	1.39±0.03[d]	27.7±0.67[a]	25.0±0.31[a]	65.1±2.11[b]	14.0± 0.56[bc]
	(1.32-1.50)	(26.6-28.9)	(24.5-25.5)	(62.2-73.3)	(12.8-15.5)
White shrimp	1.55±0.03[c]	31.1±2.53[a]	21.7±0.93[b]	63.8±4.23[b]	15.2± 1.28[bc]
	(1.41-1.76)	(27.3-38.1)	(19.7-24.2)	(48.2-95.3)	(10.7-20.0)

Values with different letters in a column are different from each other (α =0.05)

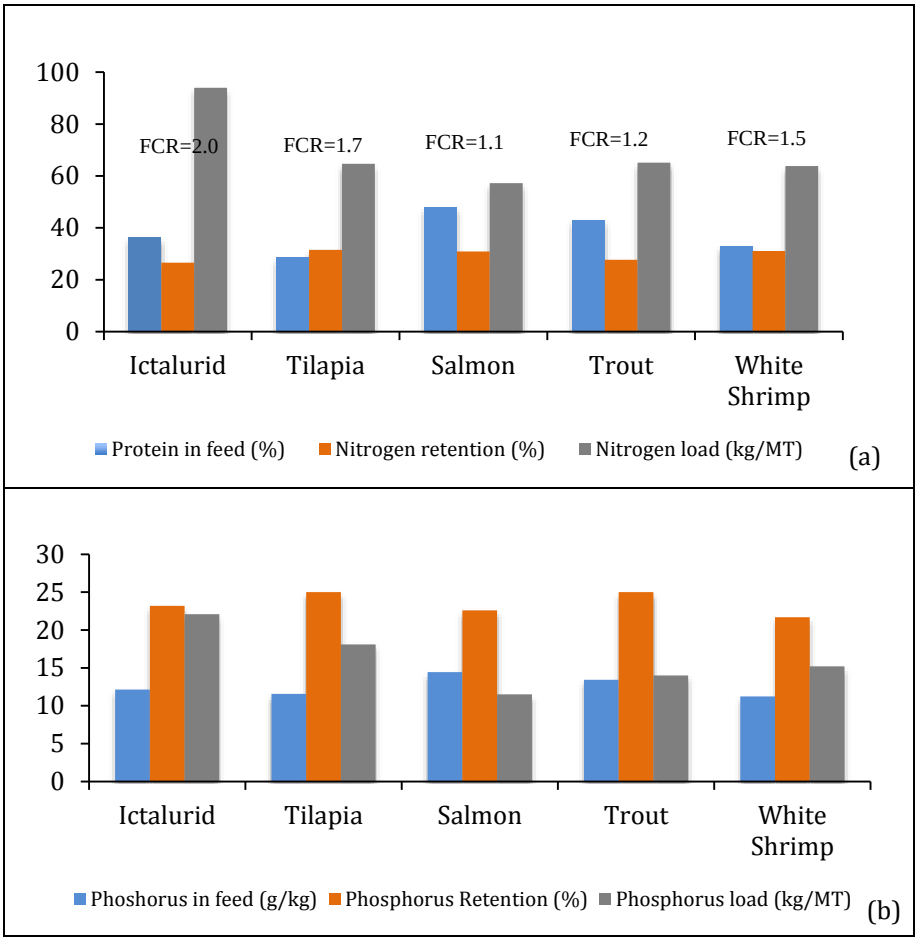

Figure 4.9 Comparison of N and P concentrations in feeds, nutrient retention, and nutrient loads for each cultured species.

Table 4.7 Feed biochemical oxygen demand (BOD_f), acidity potential of feed and system loads of nitrogen and phosphorus from feed to produce of 1 kg of five common aquaculture species based on FCR=1.

Species	Feed BOD (kg/kg feed)	Acidity Potential (kg $CaCO_3$/kg)	Nitrogen load (kg/t harvest)	Phosphorus load (kg/t harvest)
Channel	0.54	16.80	47.00	11.05
Tilapia	0.61	16.00	38.06	10.65
Salmon	0.98	33.73	52.00	10.45
Trout	0.97	32.25	54.25	11.67
White shrimp	0.69	20.27	42.53	10.13

References

Ackefors, H., Enell, M., 1990. Discharge of nutrients from Swedish fish farming to adjacent sea areas. Ambio. 19, 28-35.

Anderson, T.A., De Silva, S.S., 1997. Strategies for low-pollution feeds and feeding. Aquacult. Asia 2, 18-25.

Aubin, J., Papatryphon, E., van der Werf, H.M.G., Chatzifotis, S., 2009. Assessment of the environmental impact of carnivorous finfish production systems using life cycle assessment. J. Clean. Prod. 17, 354–361.

Bauer, W., Prentice-Hernandez, C., Tesser, M.B., Wasielesky, W. Jr., Poersch, L.H.S., 2012. Substitution of fishmeal with microbial floc meal and soy protein concentrate in diets for the pacific white shrimp *Litopeneaus vannamei*. Aquaculture 342, 112-116.

Bergheim, A., Hustveit, H., Kittelsen, A., Selmer-Olsen, A., 1982. Estimated pollution loadings from Norwegian fish farms. I. Investigation 1978-79. Aquaculture. 28, 346-361.

Bermudes, M., Glencross, B.D., Austen, K., Hawkins, W., 2010. Effect of high water temperatures on nutrient and energy retention in barramundi (*Lates calcarifer*). Aquaculture. 306, 160-166.

Beveridge, M.C.M., 1985. Cage and pen fish farming. Carrying capacity models and environmental impact. FAO Fish. Tech. Pap. 255, 131.

Boissy, J., Aubin, J., Drissi, A., VanderWerf, H.M.G., Bell, G.J., Kaushik, S., 2011. Environmental impacts of plant-based salmonid diets at feed and farm scales. Aquaculture 321, 61–70.

Bonsdorff, E., Blomqvist, E.M., Mattila, J., Norkko, A., 1996. Long-term changes and coastal eutrophication; examples from Aland Islands and Archipelago Sea, northern Baltic Sea, Oceanol. Acta 20, 319-329.

Bonsdorff, E., Blomqvist, E.M., Mattila, J., Norkko, A., 1997. Coastal eutrophication: causes consequences and perspectives in the archipelago areas of the northern Baltic Sea. Estuarine, Coastal Shelf Sci. 44, 63-72.

Boonyaratpalin, M., 1988. Catfish feed. National Inland Fisheries Institute. Expansion Paper No. 528, Department of Fisheries, Bangkok, Thailand. Pp. 17.

Boonyaratpalin, M., 1981. Lipid requirements of snakehead fingerlings. Progress Report of the Regional Project RAS/76/003, Network of Aquaculture Centers in Asia, Bangkok, Thailand, pp. 18.

Boyd, C.E., 2009. Estimating mechanical aeration requirement in shrimp ponds from oxygen demand of feed. In: Browdy, C.L., Jory, D.E. (Eds.), The rising tide proceedings of the special session on sustainable shrimp farming. World Aquaculture Society, Baton Rouge, LA.

Boyd, C.E., 2006. Estimates of bottom soil and effluent load of phosphorus at a semi-intensive marine shrimp farming. J World Aquacult. Soc. 37, 41-47.

Boyd, C.E., 1978. Effluents from catfish ponds during fish harvest. J. Env. Qual. 7, 59-63.

Boyd, C.E., Gross, A., 1999. Biochemical oxygen demand in channel catfish Ictalurus punctatus pond waters. J. World Aquacult. Soc. 30, 349-356.

Boyd, C.E., Queiroz, J., 2001. Nitrogen and phosphorus loads by system, USEPA should consider system variables in setting new effluent rules. Global Aquaculture Advocate 4, 84-86.

Boyd, C., Tucker, C.S., 2004. Handbook for aquaculture water quality. Craftmaster Printers, Inc., Auburn, Alabama, pp. 439.

Boyd, C., Tucker, C.S., 1998. Pond aquaculture water quality management: waste management, Kluwer Academic Publishers. 541-573.

Bromley, P.J., Smart, G., 1981. The effects of the major food categories on growth, composition and food conversion in rainbow trout (*Salmo gairdneri Richardson*). Aquaculture 23, 325–336.

Bureau, D.P., Kaushik, S.J., Cho, C.Y., 2002. Bioenergetics. In: Halver, J.E., Hardy, R.W. (Eds), Fish Nutrition in Academic Press. San Diego, California, pp. 1-56.

Burford, M.A., Williams, K.C., 2001. The fate of nitrogenous waste from shrimp feeding. Aquaculture. 198, 79-93.

Cho, C.Y., Bureau, D.P., 1997. Reduction of waste output from salmonids aquaculture though feeds and feeding. Prog. Fish Cult. 59, 155-160.

Chiu, A., Li, L., Guo, S., Bai, J., Fedor, C., Naylor, R.L., 2013. Feed and fishmeal use in the production of carp and tilapia in China. Aquaculture 414–415, 127–134.

Christensen, K.D., Horsted, J. 1991. Miljobelastning fra havbrug og saltvandsdambrug, DFH Rapport. 397, pp. 90.

Chuapohuk, W., 1994. Development of a low protein high energy practical diet for Sutchi's catfish, Pangasius sutchi Fowler. Thai J. Agri. Sci. 27, 19-25.

Cowey, C.B., Walton, M.J., 1989. Intermediary metabolism. In: Halver, J.E. (Ed.), Fish Nutrition, Second Edition in Academic Press. New York, pp. 259-329.

Edwards, D.J., 1978. Salmon and Trout Farming in Norway. Fishing News Books Ltd., Farnham, UK, pp. 195.

Enell, M., 1987. Environmental impact of cage fish farming with special reference to phosphorus and nitrogen loadings. Common Meeting of the International Council for Exploration of Sea, CMICES/F, pp. 44.

Food and Agriculture Organization of the United Nations (FAO), 2012. The state of world fisheries and aquaculture. FAO Fisheries and Aquaculture Department, Food and agriculture organization of the United Nations, Rome.

Gavine, F.M., Phillipss, M.J., Murray, A. 1995. Influence of improved feed quality and food conversion ratio on phosphorus loadings from cage culture of rainbow trout, *Oncorhynchus mykiss* (Walbaum), in freshwater lakes. Aquacult. Res. 26, 483-495.

Gowen, R.J., Bradbury, N.B., 1987. The ecological impact of salmonid farming in coastal waters. In: Barnes, M. (Ed.), Oceanography and Marine biology: An Annual Review in Oceanogr. Mar. Biol. Ann. Rev. 25, 563-75.

Gowen, R.J., Bradbury, N.B., Brown, J.R., 1985. The ecological impact of salmon farming in Scottish coastal waters: a preliminary appraisal. Common Meeting of the international Council for Exploration of Sea, CM-ICES/F, pp. 35.

Grisdale-Helland, B., Helland, S.J., 1997. Replacement of protein by fat and carbohydrate in diets for Atlantic salmon (*Salmo salar*) at the end of the freshwater stage. Aquaculture 152, 167-180.

Hall, P.O.J., Holby, O., Kollberg, S., Samuelson, M.O., 1992. Chemical flux and mass balances in a marine fish cage farm. 4. Nitrogen. Mar. Ecol Prog. Set. 89, 81-91.

Hall, P.O.J., Anderson, L.G., Holby, O., KoUberg, S., Samuelson, M.O., 1990. Chemical flux and mass balances in a marine fish cage farm. I. Carbon. Mar. Ecol. Prog. Ser. 61, 61-73.

Handy, R.D., Poxton, M.G., 1993. Nitrogen pollution in mariculture: toxicity and excretion of nitrogenous compounds by marine fish. Reviews in Fish Biology and Fisheries 3, 205–241.

Holby, O., Hall, P.O.J., 1991. Chemical flux and mass balances in a marine fish cage farm. II. Phosphorus. Mar. Ecol. Prog. Ser. 70, 263-272.

Helland, S.J., Hatlen, B., Grisdale-Helland, B., 2010. Energy, protein and amino acid requirements for maintenance and efficiency of utilization for growth of Atlantic salmon post-smolts determined using increasing ration levels. Aquculture 305, 150-158.

Islam, M.S. 2005. Nitrogen and phosphorus budget in coastal and marine cage aquaculture and impacts of effluent loading on ecosystem: review and analysis towards model development. Mar. Pollut. B. 50, 48–61.

Jackson, M.L., 1958. Soil chemical analysis. Prentice-Hall, Inc., Englewood Cliffs, N.J. pp. 498.

Karalazos, V., Bendiksen, E.A., Bell, J.G., 2011. Interactive effects of dietary protein/lipid level and oil source on growth, feed utilization and nutrient and fatty acid digestibility of Atlantic salmon. Aquaculture 311, 193-200.

Langaker R. 1988. Forureining fra smolt og settefiskanlegg. Hovedoppgave ved Inst. For Akvakulturforskning. As-NLH, pp. 76p.

Leung, K.M.Y., Chu, J.C.W., Wu, R.S.S., 1999. Nitrogen budget for the areolated grouper *Epinephelus areolatus* cultured under laboratory conditions and in open-sea cages. Mar. Ecol. Prog. Ser. 186, 271–281.

Lupatsch, I., Kissil, G.W., 2005. Feed formulations based on energy and protein demands in white grouper (*Epinephelus aneneus*). Aquaculture 248, 83-95.

Lupatsch, I., Kissil, G.W., Sklan, D., Pfeffer, E., 2001. Effects of varying dietary protein and energy supply on growth, body composition and protein utilization in gilthead seabream (*Sparus aurata L.*). Aquacult. Nutr. 7, 71-80.

Mariotti, F.D., Tome, D., Mirand, P.P., 2008. Converting nitrogen into protein beyond 6.25 and Jone's Factors. Crit. Rev. Food Sci. Nutr. 48, 177-184.

Muller, F., Varadi, L., 1980. The results of cage fish culture in Hungary. Aquacult. Hung. 2, 154-167.

NRC., 1993. Nutrient Requirements of Fish in National Academies Press. Washington, DC.

Pelletier, N., Tyedmers, P., 2010. Life cycle assessment of frozen tilapia fillets from Indonesian lake-based and pond-based intensive aquaculture systems. J. Ind. Ecol. 14, 467–481.

Penczak, T., Galicka, W., Molinski, M., Kusto, E., Zalewski, M., 1982. The enrichment of a mesotrophic lake by carbon, phosphorus and nitrogen from the cage aquaculture of rainbow trout, *Salmo gairdneri*. J. Appl. Ecol. 19, 371–393.

Persson, G., 1987. Sambandet mellan foda, production och fororening vid odling av stor regnbage (*salmo gairdneri*). Naturvardsverket Rapport. 3382, pp. 76.

Phillips, M.J., Beveridge, M.C.M., Ross, L.G., 1985. The environmental impact of salmonid cage culture on inland fisheries: present status and future trends. J. Fish Biol. 27, 123–127.

Pillay, T.V.R., 2004. Aquaculture and the environment: Nature of environmental impacts. Blackwell Publishing, 7-19.

Robinson, E.H., Li, M.H., 2010. Channel catfish, *Ictalurus puntatus*, size and feed conversion ratio. J. World Aquacult. Soc. 41, 829-833.

Rosamond, L.N., Rebecca, J.G., Jurgenne, H.P., Nils, K., Malcolm, C.M.B., Jason, C., Carl, F., Jane, L., Harold, M., Max, T., 2000. Effect of aquaculture on world fish supplies. Nature. 405, 1017-1024.

Tan, R.K.H., Dominy, W.G., 1997. Commercial pelleting of crustacean feeds. In: D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds.), Crustacean Nutrition. Advances in World Aquaculture Society, Baton Rouge, Louisiana, pp. 520-549.

Wiesmann, H., Scheid, H.S., Pfeffer, M.E., 1988. Water pollution with phosphorus of dietary origin by intensively fed rainbow trout (*Salmo gairdneri*). Aquaculture 69, 263-270.

Wong, F.J., Chou, F., 1989. Dietary protein requirement of early grow-out seabass (*Lates calcarifer* Bloch) and some observations on the performance of two practical formulated

feeds. In: Bahru, J., (Ed), Report of the Workshop on Shrimp and Finfish Feed Development. Malaysia, pp. 91-102.

Chapter 5 Mineral Nutrient Concentrations in Selected Aquaculture Species and their Feeds: Environmental Considerations

5.1 Abstract

Minerals and vitamin premixes are generally added into aquatic diets for maximizing quality and quantity of the production. Aquatic diets are supplemented with high levels of minerals, generally present in quantities greater than what can be assimilated by cultured species. The excess dietary minerals may be absorbed and accumulated in the animal tissues or dispersed into the environment. It is worth considering whether trace metals in aquatic feeds can negatively impact the environment, animal health and consumers. Moreover, information on mineral nutritional requirement of aquatic animals and mineral composition of aquatic feeds is limited as compared to those for terrestrial animals consequently additional data on this topic would be helpful. The objective of this study was to quantify the trace mineral concentrations in various types of feeds from major aquacultural countries. A total of 203 manufactured feeds for cultured species (channel catfish, tilapia, salmon, trout, carp, pangasius, snakehead, Asian sea bass, Pacific white shrimp and black tiger shrimp) were collected from countries including the United States, Canada, Ecuador, China, Brazil, Vietnam, Thailand, Guatemala and Ghana. All feed samples were analyzed for mineral, moisture and ash content. Concentrations of macro and micro-minerals were generally found to be higher in feed samples than in bodies of cultured species. Also, P, Mg, Cu and Zn concentrations of the feeds were found to be higher than the animals' requirement. System loads for macro-minerals S, Ca, and K were found to be highest

for channel catfish (2.43, 28.76, and 20.19 kg/t, respectively). System loads for micro-minerals Zn, As and Mo were found to be highest in the case of channel catfish species (239.3, 1.59 and 4.69 g, respectively). Tilapia also exhibited the highest system loads for the following nutrients: 879.2 g Fe/t, 161.7 g Mn/t, 2.01 g Pb/t, 6.50 g Cr/t and 2.68 g Se/t production. In case of white shrimp, highest system loads for Cu nutrients were found for every ton produced 36.2 g/t. Salmon exhibited highest system load for Cd (2.05 kg/t), and Co (4.11 kg/t) nutrients. Concentration increases in the water of the culture system, which possibly could result from addition of the various elements in feed did not appear potentially harmful.

5.2 Introduction

Mineral nutrients are inorganic elements necessary for normal bodily functions of living things. Thus, the presence of minerals in formulated aquaculture feeds is indispensable (Watanabe et al., 1997). Minerals can be divided into two categories, namely, macro-minerals and micro-minerals. Common macro-minerals are calcium, magnesium, sodium, potassium, chloride and phosphorus. These elements have many functions in aquatic animal physiology, but two of the most important are regulation of osmotic balance in fish and shrimp, and bone formation and integrity in fish (Lall, 2002; Roy and Lall, 2006). Common micro-minerals include chromium, copper, iodine, selenium and zinc. Some essential minerals are toxic if their concentrations are too high, and most non-nutrient trace elements also can be toxic at elevated concentrations (Tacon and de Silva, 1983; Mance, 1987; Langston, 1990; Merian, 1991; Bryan and Langston, 1992).

Aquatic feeds are composed of animal ingredients, plant ingredients, mineral and vitamin premixes, as well as other additives. In mineral premixes, zinc, copper, iron, manganese, cobalt and other minerals are included to promote optimal animal growth (NRC, 1993). However, information on mineral and nutritional composition of aquatic feeds and animals is still limited as compared to that of terrestrial, livestock animals (Cheng et al., 2005; Wang et al., 2006). The investigation of mineral nutrient requirements of aquatic species is complicated by the fact that aquatic species can absorb minerals from the surrounding water in addition to absorbing them from their diets (Cowey and Sargent, 1979). Minerals absorbed by the gills can sometimes meet an animal's mineral requirement even if its diet is deficient in one or more nutrients (Shearer, 1989). Cheng et al. (2013) reported that pellet feeds contained higher concentrations of metals than found in zooplankton, mud carp, and trash fish. Where the amounts and concentrations of minerals in the

water are not optimal, feeds can be an important source of trace metals to farmed fish (Zhou and Wong, 2000; Lacerda et al., 2006).

Excessive amounts of dietary metals may be accumulated in aquatic animal tissues, excreted into the water or adsorbed by sediments (Chen et al., 2000; Casado-Martinez et al., 2010). This can deleteriously affect the health of aquatic organisms as a result of toxicity from excessive environmental concentrations or bioaccumulation in the food chain (Fernandes et al., 2007). Toxicity may develop in cultured animals if excessive amounts of trace elements are assimilated. High levels of certain metals in animal tissue pose a health risk for humans who consume such contaminated products (Fernandes et al., 2008). The adverse human health effects associated with exposure to heavy metals are diverse and include, but are not limited to, neurotoxic and carcinogenic effects (Cheng et al., 2013). A number of studies have investigated heavy metal concentrations in some wild and cultured fish species (Dalman et al., 2006; Storelli et al., 2007; Ikem and Egilla, 2008), wild and cultured mussel (Yap et al., 2004; Cardellicchio et al., 2008) and wild white shrimp (Paez-Osuna and Ruiz-Fernandez, 1995; Wei et al., 2002; Ip et al., 2005) to determine whether trace metal levels in animals are within permissible limits for human consumption.

The purpose of the present study was to accumulate data on the elemental composition of aquaculture feeds and aquaculture species. Data were analyzed to estimate the contribution of aquaculture feeds to mineral inputs into culture systems and natural waters receiving effluents for these systems.

5.3 Materials and Methods

5.3.1 Sample Collection and Preparation

A total of 203 feed samples were collected for different species as follows: channel catfish, *Ictalurus punctatus*, clarias catfish, *Clarias microcephalus*× *C. gariepinus*; pangasius catfish, *Pangasius hypophthamus*; tilapia, *Oreochromis niloticus*; snakehead, *Channa micropeltes*; Asian sea bass, *Lates calcarifer*; common carp, *Cyprinus carpio*; salmon, *Salmo salar*; trout, *Oncorhynchus mykiss*; whiteleg shrimp, *Litopenaeus vannamei*; black tiger prawn, *Penaeus monodon*. The samples were obtained from different countries: United States; Canada; Ecuador; China; Brazil; Vietnam; Thailand; Guatemala; Ghana. The feeds were categorized into three groups, 'starter', 'fingerling' and 'grower' feeds based on the growth stage of the cultured species for which they were intended. Catfish, tilapia, salmon, trout and whiteleg shrimp samples (whole body) were procured from fish markets in Alabama and Georgia, USA. Three individual animals of each species were obtained for analysis.

Feed samples were pulverized with a mortar and pestle. Fish and shrimp samples were chopped into small pieces and then were ground using a food processor (Black and Decker, Maryland, USA). Samples were dried in a convection oven at 105ºC before grinding them again using with an IKA Economical Analytical Mill (Cole-Parmer, 27 Vernon Hills, IL, USA). Moisture content of both fish and feed samples was calculated using Equation 5.1 by recording their original weight (wet) and dried weight (dry). For moisture content, samples in ceramic crucibles were placed in convection oven at 105°C for 8 hr. Crucibles and contents were then transferred to desiccators until they were at room temperature before weighing. The crucibles and dry samples were then incinerated in a furnace (62700 Barnstead Thermolyne, Dubuque, Iowa, USA) at 500°C for 8 hr and ash concentrations were determined using Equation 5.2.

$$Moisture\ content\ (\%) = \frac{Wd-Wc}{Ww} \times 100 \quad (5.1)$$

$$Ash\ content\ (\%) = \frac{Wa-Wc}{Wd-Wc} \quad (5.2)$$

where: Ww = wet weight of the sample (g)

Wd = weight of sample and crucible after drying (g)

Wa = weight of sample and crucible after ashing (g)

Wc = weight of crucible (g)

The loss of volatile matter from the dry samples during ignition allowed an estimate of total organic matter in samples by using Equation 5.3.

$$\text{Organic matter} = 100\% - \%\ \text{Ash} \quad (5.3)$$

5.3.2 Quantification of Macro and Micro-minerals

Ash samples were digested and solubilized using an acid solution (1 N HNO_3 plus 1 N HCl in 1:1 ratio). Ash in crucibles was treated with 5 ml of the acid solution. Crucibles were held on a hot plate until nearly dry. The residues were quantitatively transferred into a 100-ml volumetric flask that was then brought to volume with glass-distilled water. The resulting solutions were filtered through Whatman Number 42, acid-washed filter paper. Concentrations of arsenic (As), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu) iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), lead (Pb), sulfur (S), selenium (Se) and zinc (Zn) were determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES).

5.3.3 Statistical Analysis

All data were statistically analyzed using Multivariate analysis of variance (MANOVA) to determine significant differences ($p < 0.05$) among treatments, which was followed by the Fisher's Least Significant Difference test to determine significant differences among treatment means. All statistical analyses were carried out using SAS (V9.2 SAS Institute, Cary, NC, USA).

5.4 Results

5.4.1 Ash and Macro-mineral Analysis in Feed Samples

The estimated ash and macromineral element concentrations measured in feed samples are summarized in Table 5.1. The average concentrations of ash for feed ranged from 8.7% for trout feed to 16.2% for pangasius catfish feed. Concentrations of macroelements in individual feeds exhibited the following ranges: S, 1.01-4.58 g/kg (0.101 to 0.458%); P, 10.34-20.68 g/kg; Ca, 13.67-85.99 g/kg; Mg, 1.68-9.18 g/kg; K, 7.37-39.57 g/kg; Na, 2.91-15.45 g/kg. Snakehead feeds had the highest concentrations of macro-minerals as compared to other feeds. The lowest concentrations for P, Ca, and Na were found in common carp feeds. The average concentration of P was similar for all fish feeds and ranged from 10.34 to 19.3 g/kg. Although feed for whiteleg shrimp was similar in P concentrations to fish feed, black tiger prawn feed had the highest mean P concentration 20.7 g/kg. The lowest concentrations for S were found in salmon feeds. Lowest Mg and K concentrations were found in trout feed. Ash and macromineral element concentrations in starter, fingerling and grower feeds are summarized separately in Tables 5.2, 5.3, and 5.4, respectively.

The result from PCA analysis demonstrated that PC1 was able to separate ($\alpha = 0.05$) the concentrations of Ca, K, and S in snakehead and pangasius feeds from the other feeds (Figure 5.4a). The concentrations of Ca, K, and S in some of the feeds from Asia and South America were

greater than those in feeds from Africa and North America (Figure 5.5a). In addition, PC3 was able to separate ($\alpha = 0.05$) the concentration of P in black tiger prawn and Asian sea bass feeds from clarias and carp feeds (Figure 5.4b). However, PC3 was unable to separate the concentration of P in feeds from different continents (Figure 5.5b).

5.4.2 Ash and Macro-mineral Analysis in Cultured Species

Ash and macromineral concentrations in five cultured species are summarized in Table 5.5. Concentrations of macroelements in cultured species ranged as follows: ash, 9.3-19.7%; S, 0.36-6.90 g/kg (0.036-0.690%); P, 9.60-18.11 g/kg; Ca, 6.48-54.47 g/kg; Mg, 0.84-3.00 g/kg; K, 5.12-10.60g/kg; Na, 0.65-9.40 g/kg. Black tiger prawn and whiteleg shrimp had higher concentrations of S, Ca, Mg, K, and Na than fish. While, channel catfish had the lowest concentrations of Ca, Mg, K and Na. Tilapia whole body samples exhibited the highest concentration of P (18.11 g/kg), while the black tiger prawn samples had the lowest average P concentration (9.60 g/kg).

5.4.3 Micro-mineral Analysis in Feeds

Concentrations of micro-minerals in all feed samples are summarized in Tables 5.6. Concentrations of microelements in feeds ranged as follows: Fe, 347.1-2326.3 mg/kg; Mn, 54.9-226.7 mg/kg; Zn, 81.0-616.6 mg/kg; Cu, 12.2-45.8 mg/kg; As, 0.07-1.30 mg/kg; Pb, 0.14-2.33 mg/kg; Cd, 0.18-3.10 mg/kg; Co, 0.43-3.29 mg/kg; Cr, 1.98-25.69 mg/kg; Mo, 0.11-5.63 mg/kg; Se, 0.29-2.40 mg/kg. Differences in mineral concentrations in feeds were found for all microelements. Variation among feeds was especially high for Cr, Cu, Fe, Mn, and Zn. Micro-mineral concentrations for starter, fingerling and grower feeds were tabulated in Tables 5.7, 5.8, and 5.9, respectively.

5.4.4 Micro-mineral Analysis in Cultured Species

Micromineral concentrations in five cultured species are summarized in Table 5.10. Concentrations of Fe, Mn, Zn and Cu in cultured species were as follows: Fe, 20.7-307.0 mg/kg; Mn, 2.4-31.7 mg/kg; Zn, 11.9-148.3 mg/kg; Cu, 0.2-90.1 mg/kg. Concentrations of As and Se were found in whiteleg shrimp at levels of 0.55 mg/kg and 0.38 mg/kg, respectively. Concentrations of Pb (1.7 mg/kg) and Cd (28.8 mg/kg) were detected only in black tiger prawn and channel catfish, respectively.

5.4.5 Comparison of Mineral elements in Feeds

Concentrations of P, Mg, Cu and Zn in different feed types and the required amounts for optimal growth of aquatic animals are shown in Figure 5.2. Concentrations of nutrients in feeds generally exceeded the required levels. This assures that nutrients will be provided in adequate amounts, but it leads to greater nutrient loads and increased likelihood of pollution problems. Required levels of P in feeds were found to vary from 0.3 to 1.5% (Lall, 2002), but higher levels were found in feeds for pangasius, snakehead, Asian sea bass, Atlantic salmon and rainbow trout. Required P level for whiteleg shrimp should be 1.3% (Pan et al., 2005), but in our study, the P levels in whiteleg shrimp feed exceeded this value.

The Mg requirement in feeds was reported to be between 0.25 and 0.70 g/kg for fish species (Lall, 2002) and 2.6 to 3.5 g/kg for shrimp species (Change et al., 2005). However, in feeds studied here, Mg levels exceeded the required value for fish and shrimp species. Copper concentration in a typical feed should vary from 1.5 to 5.0 mg/kg for fish species (Ogino and Yang, 1980) and from 15 to 34 mg/kg for shrimp species (Davis et al., 1993a; Lee and Shiau, 2002). Zinc level requirement was reported to be 15 to 30 mg/kg for aquatic species (Ogino and Yang, 1979; Davis et al., 1993b; Shiau and Jiang, 2006; Lin et al., 2008b). In the present study, all feeds were found

to have Zn levels in excess of the requirement. Concentrations of mineral elements in feeds did not show any trends related to stages of cultured species for which intended (Figure 5.3)

5.4.6 Nutrient Loading

System nutrient loads for macro-minerals that would result from use of the feeds studied here for production of 1 tonne (t) of different cultured species at typical FCRs are given in Table 5.11. System loads for S, Ca, and K were highest for channel catfish (2.43, 28.76, and 20.19 kg/t, respectively). Tilapia exhibited the greatest system load for Mg and Na (5.67 and 6.30 kg/t) (Figure 5.6).

System loads for micre-minerals that would result from production of 1 t of each of five cultured species are given in Table 5.12. System loads for Zn, As and Mo were highest for channel catfish (239.3, 1.59 and 4.69 kg/t, respectively). Tilapia exhibited the largest system loads for Fe, 879.2 g/t; Mn, 161.7 g/t; Pb, 2.01 g/t; Cr, 6.50 g/t and Se, 2.68 g/t. The greatest system load for Cu 36.2 g/t was found for whiteleg shrimp production. Salmon exhibited the highest system load for Cd (2.05 g/t) and Co (4.11 g/t) (Figure 5.6).

The result from PCA analysis demonstrated that PC1 and PC2 were able to separate (α = 0.05) Ca, K, S, and Mo loads of channel production from trout and salmon production (Figure 5.7a). It was possible to separate (α = 0.05) load of Se for salmon production from production of the other species with PC3, and PC4 separated Pb load for tilapia production from those of the other species (Figure 5.7b).

5.5 Discussion

5.5.1 Macro-minerals

The high degree of variability in concentrations of minerals in aquaculture feeds results from a number of factors such as differences in the mineral composition of the basic plant and

animal meals used in diet formulation, the addition of specific macro and trace mineral premixes, and contaminants present in feed ingredients. The biological availability of macro trace elements varies depending on the ingredients used in diets. A number of factors affecting bioavailability to include the concentrations and form of mineral nutrients, digestibility of the diet, particle size, nutrient interaction which can be either synergistic of antagonistic, physiological and conditions of animals, concentration of minerals in water, and the species under consideration. Although the mineral requirements of larval fish have not been reported, it is likely that their requirement for certain minerals, especially those which are not absorbed from the surrounding water is higher than that of adult fish because of their high metabolic activity and growth rate.

There are a number of problems related to the quantification of mineral requirements, including identification of the potential contribution of minerals from the water, leaching of minerals from the diets, availability of proper test diets that have low concentrations of targeted minerals, and limited data on mineral bioavailability.

Data on mineral nutrition has been reported for many animals, but such information for aquaculture species is limited. The knowledge of mineral nutrition for aquaculture species is comparatively complicated because both dietary intake and waterborne mineral uptake have to be considered in evaluating the mineral budgets. According to a previous study, the concentrations of macro-minerals in manufactured feeds seem to have a wide range. It is interesting to note that the concentration of most elements – particularly Fe, Mn, and Zn in this study were considerably higher than normally recommended. Tacon and Silva (1983) indicated that the known macro-mineral requirements of several common aquatic animals were greater than concentrations typically suggested in dietary requirements of rations.

Fish in freshwater containing 1 to 3 mg Mg/L have been shown to require 0.25 to 0.70 g Mg/kg of diet (Lall, 2002). Whereas, Mg concentrations in the feeds of this study were above the required levels, ranging from 1.68 g/kg for rainbow trout diets to 9.18 g/kg for snakehead diets. Shearer (1989) stated that rainbow trout could absorb Mg from water containing 1.3 mg/L to meet their metabolic requirement. Seawater typically contains a high Mg concentration (1,350 mg/L), thus marine species may not require Mg from the diet (Dall and Moriarty, 1983). Atlantic salmon cultured in water containing 54 mg Mg/L needed about 0.1 g Mg/kg in their diet (El-Mowafi and Maage, 1998). The average concentration of Mg found in juvenile salmon feed samples (1.89 g/kg or 0.189%) was almost five times as high as their requirement (0.4%) (Table 5.1, Figure 5.1). Chen et al. (2005) indicated that the Mg requirement of juvenile *L. vannamei* reared in low salinity (2%) water was 2.6 to 3.5 g/kg diet – accordant with the level we observed in *L. vannamei* feed.

Calcium and P are directly associated with the development and maintenance of the sketetal system in fish and they participate in several physiological processes (NRC, 1993). In general, the Ca requirement of most fish can be met by absorption from the surrounding water of from feed ingredients (Lall, 2002). Robinson et al. (1986, 1987) found that fingerling tilapia and channel catfish reared in Ca-free water required 7.0 and 4.5 g Ca/kg, respectively, in diets. Mean Ca concentrations of 21.5 g/kg were found in tilapia feed while the corresponding concentration for channel catfish feed was 20.3 g/kg which are considerably greater than the calcium requirements of these two species (Table 5.1).

The uptake of Ca from seawater was insufficient to meet the Ca requirement of red sea bream, and this species required 3.4 g Ca/kg in its diet (Sakamoto and Yione, 1973, 1976). Hossain and Furuichi (1999, 2000a, b, c) also reported that a dietary Ca supplement was essential for redlip mullet, scorpion fish, and Japanese flounder, but not for black sea bream. In addition to being

essential, dietary Ca interacts with other essential dietary elements including P, Mg and Zn (Nakamura, 1982; Hardy and Shearer, 1985; Gatlin and Phillips, 1989; Vielma and Lall, 1998a). For example, Nakamura (1982) found that excess dietary Ca inhibited P absorption in common carp, and an inhibitory effect by dietary Ca supplement on Mg deposition in vertebrae and scales has been observed in Atlantic salmon (Vielma and Lall, 1998a).

The concentration of P in natural waters is generally very low in comparison to Ca concentration (Boyd, 2000). Hence, P uptake from water by aquatic animals is unlikely to meet their requirements for this nutrient (Lall, 2002). It is necessary for aquatic feeds to be supplemented with P because of the low concentration of this element in both freshwater and seawater (Lall, 2002). This makes dietary P potentially more critical than other mineral macro-minerals for both fish and shrimp. In most fish species, P deficiency signs present poor growth, reduced feed efficiency, low bone mineralization, skeletal deformities, and low ash but high lipid content in whole body samples (Tacon, 1992; Lall, 2002). Dietary P requirements have been reported for many aquatic species to include common carp (Ogino and Takeda, 1976), rainbow trout (Ogino et al., 1979), Atlantic salmon (Ketola, 1975; Lall and Bishop, 1977; Vielma and Lall, 1998b), sunshine bass (Brown et al., 1993), and haddock (Roy and Lall, 2003). The dietary P requirements of fish species have been documented to range from 3 to 15 g/kg diet (Lall, 2002). Gatlin (2000) indicated that dietary phosphorus requirements have been reported to range from 3 to 9 g/kg for various fish species and from 3 to 20 g/kg for crustaceans. There is some evidence that scale fish (or those with more structural tissue) have a higher dietary phosphorus requirement than do scaleless fish. Available P requirement values as low as 3 g/kg diet had been found in subadult (Eya and Lovell, 1997) and fingerling channel catfish (*Ictalurus punctatus*) (Wilson et al., 1982).

A value of 5.6 g/kg diet was reported in rainbow trout (*Oncorhynchus mykiss*) by Rodehutscord et al. (1995). This level was greatly lower than the level of P found in trout feed (15.7 P/kg diet).

Phosphorus requirements for crustacean were much higher than the values reported above for various fish species. For instance, Pan et al. (2005) conducted an experiment on graded monobasic calcium phosphate in *Litopenaeus vannamei* diets, and reported a total P concentration of 13.3 g/kg was required for optimal weight gain and feed conversion efficiency. This recommended level is close to the average concentration of P in our feed samples (15.0 g P/kg diet) (Table 5.1). Cheng et al. (2006) indicated that the dietary P requirement of *L. vannamei* was dependent on Ca content of the diet.

Sodium and K are essential elements for physiological processes including acid:base balance and osmoregulation (Lall, 2002). Shiau and Lu (2004) demonstrated that juvenile hybrid tilapia raised in freshwater require approximately 1.5 g Na/kg diet, while in the present study, tilapia feeds contained an average of 4.8 Na g/kg (Table 5.1). No dietary sodium requirement of fish was apparent in seawater (Shiau and Lu, 2004). There were no effects of sodium chloride supplementation on channel catfish in freshwater (Murray and Andrews, 1979) as well as on Atlantic salmon raised in freshwater or seawater (Shaw et al., 1975). However, supplementation of sodium chloride at the level of 45 to 116 g/kg diet inhibited feed efficiency of rainbow trout (Salman and Eddy, 1988). Shiau and Hsieh (2001a) indicated that juvenile hybrid tilapia cultured in freshwater required 2 to 3 K g/kg diet for optimal growth. Dietary K requirement estimated for juvenile channel catfish was 2.6 g/kg. Black tiger prawn reared in water of 20 ppt salinity had a K requirement of 12 g/kg diet (Shiau and Hsieh, 2001b). Roy et al. (2007) reported that dietary supplementation of K at 10 g/kg improved weight gain of *L. vannamei* in low salinity (4 ppt) water. The average K concentration in feeds for juvenile tilapia, channel catfish, black tiger prawn and

whiteleg shrimp were 12.4, 11.3, 13.1 and 12.0 g/kg, respectively (Table 5.1). Concentrations of K in channel catfish and tilapia feeds of this study were considerably higher than recommended requirements (Figure 5.1).

5.5.2 Micro-minerals

Feeds contain small quantities of many trace minerals, several of which function as micro-minerals. Copper is an essential element for aquatic animals because it affects the activity of several important enzymes. A level of 1.5 to 5.0 mg Cu/kg diet was suitable for several fish including common carp, rainbow trout (Ogino and Yang, 1980), channel catfish (Gatlin and Wilson, 1986a) and hybrid tilapia (Shiau and Ning, 2003). Lorentzen et al. (1998) reported the requirement of dietary Cu in Atlantic salmon to be 5 to 10 mg/kg. The result of the present study demonstrated that the Cu concentration in fish feeds ranged from 12.2 mg/kg for rainbow trout to 35.7 mg/kg for snakehead (Table 5.6). All the feeds were considerably higher in Cu than the reported required level. This could be problematic as high levels may cause physiological problems. Copper concentrations of more than 15 mg/kg diet as $CuSO_4 \cdot 5H_2O$ (4 mg Cu/kg) led to a decrease in growth of channel catfish (Murai et al., 1981).

The dietary Cu requirement of crustaceans cannot be met by the low concentration of Cu in seawater (0.5 μg Cu/L) (Bjerregaar and Vislie, 1986). Davis et al. (1993a) reported a dietary Cu deficiency in *L. vannamei* fed a diet containing 34 mg/kg Cu. The dietary Cu requirement of *P. monodon* was reported to be 15 to 21 mg/kg (Lee and Shiau, 2002). Copper is a component of hemocynin, the equivalent of hemoglobin in shrimp blood. Copper also serves as a cofactor in many enzyme systems. The higher Cu requirement of crustaceans is well known, and in this study too, Cu concentrations in shrimp feed were found to be approximately two times greater than those in fish feed (Table 5.6, Figure 5.2).

Iron is a trace element essential for the production of hemoglobin in blood of vertebrate animals. The deficiency sign of Fe, hypochromic microcytic anemia, has been reported for several fish species such as brook trout (Kawatsu, 1972), common carp (Sakamoto and Yone, 1978) and red sea bream (Sakamoto and Yone, 1976). However, depression of growth was not observed in Fe-deficient fish. Gatlin and Wilson (1986b) indicated that juvenile channel catfish required a minimum of 30 mg Fe/kg diet for best growth. A much higher requirement of 497.9 mg Fe/kg was found in juvenile channel catfish feed. Excessive levels of Fe in diets can be toxic to cultured species. For example, Deshimura and Yone (1987) described that excessive Fe supplementation seems to have potentially adverse effects on growth of the shrimp *M. japonicas*. Moreover, Fe-catalyzed lipid oxidation increases with Fe supplementation, and may have adversely effect on feed stability (Sutton et al., 2006). Desjardins et al. (1987) reported that supplementation of a trout diet with ferrous sulfate significantly increased the oxidation process in that diet. The Fe concentrations in feeds varied from 347.1 mg/kg for salmon feed to 2,326.3 mg/kg for pangasius catfish feed, and the concentrations of Fe in all feed categories (Table 5.6) were found to be higher than what is normally recommended.

Manganese serves as a cofactor in several enzyme systems. Ogino and Yang (1980) stated that dietary Mn concentrations of 12.0 to 13.0 mg/kg were recommended for rainbow trout and common carp. Gatlin and Wilson (1984a) reported that 2.4 mg Mn/kg diet was sufficient for normal growth of channel catfish. A requirement of 7.0 Mn/kg was recommended for hybrid tilapia (Lin et al., 2008a). Pan et al. (2008) estimated a requirement of 14.0 mg Mn/kg diet for juvenile gibel carp (*Carassius auratus*). Information on Mn requirements of crustaceans is limited. Kanazawa et al. (1984) described that supplementation of 10 and 100 mg Mn/kg diet did not enhance the growth of *M. japonicas*. However, significant absorption from the water is unlikely

because seawater typically contains only around 10 μg/L of Mn. Therefore, a dietary source of Mn could be crucial for marine fish and shrimp. The results from this study indicated that concentrations of Mn ranged from 54.9 mg/kg for black tiger prawn feed to 226.7 mg/kg for snakehead feed (Table 5.6). These concentrations were greater than the usually reported Mn concentrations in fish diets.

Selenium is an important trace element that functions as a component of the glutathione peroxidase enzyme system. A deficiency of dietary Se has been reported to result in growth depression. Lall (2002) demonstrated that a combined deficiency of Se and vitamin E was required to prevent muscular dystrophy in channel catfish. A dietary Se level of 8.0 mg/kg was required for coho salmon (*Oncorhynchus kisutch*) (Felton et al., 1996). Concentrations of 0.25 mg/kg and 0.15 to 0.38 mg/kg of Se in diets were recommended for maximum growth of channel catfish (Gatlin and Wilson, 1984b) and rainbow trout (Hilton et al., 1980), respectively. Information on dietary Se requirements of crustaceans are very limited. But, Davis (1990) found that juvenile *L. vannamei* had maximum growth when fed diets containing 0.2 to 0.4 mg Se/kg. The U.S. Food and Drug Administration allows Se supplementation of 0.3 mg Se/kg from sodium selenite and sodium silenate in aquatic feeds. Nevertheless, the toxicity of Se to various cultured species already was well established before its dietary requirement was recognized (Davis, 1990). Chronic toxicity of Se has been shown in several fish species at dietary levels of 13.0 to 15.0 mg/kg from sodium selenite resulting in a reduction in growth and an increase in mortality (Gatlin and Wilson, 1984b; NRC, 2005). The threshold of Se toxicity for white sturgeon (*Acipenser transmontanus*) fed diets that contained selenomethionine was estimated to be 10 to 20 mg/kg (Tashjian et al., 2006). However, Hardy et al. (2010) found that cutthroat trout (*Oncorhynchus clarki bouvieri*) fed diets containing selenomethionine up to 10 mg Se/kg did not show any signs of toxicity. It has been

reported that threshold levels of 3.0 to 4.0 mg Se/kg diet led to adverse effects on fish reared in water containing 2.0 to 5.0 μg Se/L (NRC, 2005). The concentration of Se in water is generally less than 0.1 μg/L. The margin between the nutritive requirements of Se and the toxic level is very narrow, causing difficulty in the determination of exact amounts of this mineral to add to aquatic feed (Poston et al., 1976). The mean Se concentrations ranged from 0.29 mg/kg for Atlantic salmon feed to 2.4 mg/kg for black tiger shrimp feeds, which is acceptable - sufficient, but not toxic (Table 5.6).

Zinc is required for normal growth and development of aquaculture species (NRC, 1980). Dietary requirements of Zn for a variety of freshwater fish follow: 20 mg Zn/kg diet for channel catfish (Gatlin and Wilson, 1983; Scarpa and Galtin, 1992), red drum (Gatlin et al., 1991) and blue tilapia (McClain and Gatlin, 1988), 15.0 to 30.0 mg Zn/kg diet for rainbow trout (Ogino and Yang, 1979) and 26.0 to 29.0 mg Zn/kg diet for hybrid tilapia (Lin et al., 2008b). Davis et al. (1993b) conducted an experiment on *L. vannamei* and reported a level of 33.0 mg Zn/kg diet could maintain normal tissue mineralization, although growth was not affected by this concentration of dietary Zn. Similarly, a requirement of 32.0 to 34.0 mg Zn/kg diet was recommended for *P. monodon* (Shiau and Jiang, 2006). In a study by Jeng and Sun (1981), supplementation of dietary Zn at the level of 300.0 mg/kg from $ZnSO_4 \cdot 7H_2O$ resulted in slower growth and lower feed efficiency in common carp. However, the mean concentrations of Zn in feeds that ranged from 81.0 mg/kg carp feed to 616.6 mg/kg for snakehead feed were considerably in excess of dietary requirements (Figure 5.2).

Information on other trace minerals in aquatic animal diets is limited. Cobalt is a component of vitamin B_{12}. Ghosh (1975) demonstrated that cobalt chloride addition at 0.6 to 1.0 mg/L in water improved growth and survival of mullet (*Mugil parsia*). Molybdenum has been

shown to enhance growth and survival of carp (George, 1970 cited by Lall, 2002). Other inorganic elements including As, Ba, Br, and Cd potentially are required by the cultured species; however, requirements for these elements have been difficult to establish. Moreover, some minerals such as As and Hg may accumulate in animal tissues, leading to adverse effects on human consumers. Tacon and de Silvil (1983) studied several types of feed, and the highest concentrations of Fe (540 mg/kg in salmon grower diets); Zn (555 mg/kg in eel growth diets, 1,162 mg/kg in larval diets, and Cu (47 mg/kg in trout and salmon starter diets, and 98 mg/kg in eel grower diets) might lead to concerns over accumulation in culture animals and possible toxic effects on consumers.

There is limited information on trace metal concentrations in aquatic animals. Yang et al. (2014) provided data on metal pollution in common fish species of the Tibetan Plateau. They reported metal concentrations in fish muscles as follows: Cr, 0.09-0.74 mg/kg; Mn, 0.38-4.49 mg/kg; Ni, 0.06-0.91 mg/kg; Cu, 1.00-32.2 mg/kg; Zn, 13.1-102.5 mg/kg; As, 0.12-3.10 mg/kg; Pb, 0.46-3.22 mg/kg; Ba, 0.60-4.93 mg/kg; Se, 0.77-9.38 mg/kg. Cheng et al. (2013) reported live weight concentrations of Cd, Pb, Cr, Zn, Cu, Ni, and Mn in different freshwater fish species, and reported that Northern snakehead had higher concentrations of all metals except Zn, than other fish species ($P<0.05$). The lowest concentrations of Cd (0.08 ± 0.01 mg/kg, Pb (0.12 ± 0.02 mg/kg, Ni (0.15 ± 0.03 mg/kg and Cu (0.58 ± 0.05 mg/kg were observed in bighead carp ($P<0.05$). A number of studies have revealed that concentrations of minerals in animal flesh were influenced by several factors including seasons; biological differences such as species, size, age, sex and sexual maturity; food source and composition; and environment factors such as water chemistry, salinity, temperature and contaminants (Bodsha and Sainsbyry, 1978; Farmer et al., 1979; Lal, 1995). The Food and Agricultural Organization (1983) suggests limits on levels for Cd and Pb in fish flesh of 0.5 mg/kg and for Cu and Zn of 30 mg/kg. Trace minerals present in the flesh of

cultured species can be categorized into two types; essential: Mn, Fe, Co, Cu, Zn, Ni, Mo and Cr; non-essential: Al, Ti, V, Ag, Pb and Cr. The primary functions of essential minerals include skeleton structure, components of hormones, enzymes, and enzyme activators, regulation of acid-base equilibrium and maintain colloidal system (Khan et al., 1987; Kirkpatrick and Coffin, 1974; Lal, 1995).

5.5.3 Environmental Issues

Channel catfish production had the greatest system loads of nearly all mineral elements (Tables 5.11 and 5.12), and it will be used as an example for estimating water quality effects. In a 1.5-m deep (15,000 m^3/ha) channel catfish pond with production of 6,000 kg/ha, the potential increase in aqueous concentrations of elements would be as follows: S, 1.02 mg/L; Ca, 11.1 mg/L; Mg, 2.23 mg/L; K, 8.07 mg/L; Na, 1.84 mg/L; Fe, 0.232 mg/L; Mn, 0.055 mg/L; Zn, 0.087 mg/L; Cu, 0.010 mg/L; As, 0.008 mg/L; Pb, 0.0025 mg/L; Cd, 0.0025 mg/L; Co, 0.0006 mg/L; Cr, 0.0014 mg/L; Mo, 0.017 mg/L, Se, 0.0006 mg/L. Calcium and Mg are sources of hardness in water, and the factors relating these two ions to hardness are 2.5 and 4.12, respectively (Boyd, 2000). The potential increase in hardness would be 36.94 mg/L.

Aside from P loads (see Chapter 3), there is little concern about increases in macro-mineral elements and hardness in water because these variables do not cause eutrophication or toxicity. The concentrations of micre-minerals are not great, and references to allowable concentrations of these elements reported by USEPA (1986) suggest that such increases in waters with normal ambient concentrations would be tolerable – at least over a single growing season.

The system loads of elements are larger than environmental loads in most culture systems. In ponds, mineral nutrients may exceed the equilibrium concentration of a controlling mineral and precipitate from the water – this is especially likely to occur for Ca, Mg, and metallic micro-

minerals such as Cu, Zn, Fe, Mn, Pb, Cd, Co and Cr (Boyd, 2000). Certain quantities of the elements are contained in uneaten feed, feces, and remains of plankton that settle to the bottom to become sediment organic matter. These elements are sequestered until released by microbial decomposition. Moreover, cation exchange sites in clay and other sediment colloids can remove both macro element and micro-mineral cations from the water (Boyd et al., 2007a; Pine and Boyd, 2010; McNevin et al., 2004). Potassium and possibly other cations also may be strongly fixed with the interlayers of 2:1 clay minerals in sediment (Boyd et al., 2007b).

There appears to be little likelihood of environmental problems with culture systems or in water bodies receiving aquaculture effluents as a result of mineral element pollution from feeds. The exception, of course, is P that is notorious for stimulation of phytoplankton productivity in pond culture and in receiving water bodies (Boyd and Tucker, 2014). Nevertheless, the conservation of mineral nutrients through providing concentration no greater than necessary in feeds and managing feed efficiently to achieve a good FCR are good practices.

5.6 Conclusion

- Mean ash concentrations on a dry matter basis ranged from 8.7% (trout) to 16.2% (pangasius) for all feeds, whereas mean ash concentrations for whole body samples of cultured species varied from 9.3% (salmon) to 19.7% (black prawn).
- Mean macro-mineral concentrations varied in feeds (dry weight basis) as follows: S, 1.01-4.58%; P, 10.34-20.68%; Ca, 13.67-85.99%; Mg, 1.68-9.18%; K, 7.37-39.57%; Na, 2.91-15.45%.
- Concentrations of macro and micro-minerals generally were higher in feed than minimum recommended levels suggested for feeds for each species. Though this avoids nutrient deficiencies in species, it leads to greater amounts of nutrients released as waste.

- System loads for S, Ca and K were highest for channel catfish (2.55, 27.75 and 20.18 kg/t, respectively). Whiteleg shrimp exhibited the highest system load for Na (5.92 kg/t) whereas highest system load for Mg was found for Tilapia (5.67 kg/t).
- System loads for Zn, As and Mo were highest for channel catfish (239.3, 1.59 and 4.69 g/t, respectively). Tilapia also exhibited highest system loads for the following nutrients: Fe, 879.2 g/t; Mn, 161.7 g/t; Pb, 2.01 g/t; Cr, 6.50 g/t; Se, 2.68 g/t. Whiteleg shrimp had the highest system load for Cu (36.2 g/t), while salmon had the highest system loads for Cd (2.05 g/t) and Co (4.11 g/t).
- Most elements in feeds were at concentrations above suggested animal requirement levels, but the proportions of feed nutrients that were bioavailable was not known. Data on bioavailability of micro-minerals in aquaculture feeds would complement the present study.

Table 5.1 Ash and macro-mineral concentrations (dry matter basis) in feeds for different species. Entries represent mean and standard error with minimum and maximum concentrations.

Feed type	Number (N)	Ash (%)	Macro-minerals (g/kg feed)					
			S	P	Ca	Mg	K	Na
Channel	**13**	**9.4 ± 0.49^{de}**	**1.40 ± 1.06^{ef}**	**14.37 ± 0.98^{def}**	**20.32 ± 2.87^{cde}**	**2.82 ± 0.26^{de}**	**11.23 ± 0.57^{d}**	**3.34 ± 0.43^{f}**
		(7 - 12)	(0.91 - 2.13)	(9.53 - 20.59)	(6.12 - 41.56)	(1.71 - 4.49)	(8.67 - 14.78)	(1.59 - 6.70)
Clarias	**32**	**10.8 ± 0.42^{bc}**	**1.29 ± 0.64^{f}**	**11.84 ± 0.44^{g}**	**15.24 ± 0.86^{e}**	**3.49 ± 0.16^{bc}**	**12.48 ± 0.27^{c}**	**4.99 ± 0.39^{cd}**
		(8 - 17)	(0.90 - 2.816)	(8.31 - 17.78)	(6.17 - 23.50)	(1.79 - 5.59)	(9.74 - 16.22)	(1.60 - 10.32)
Pangasius	**6**	**16.2 ± 1.49^{a}**	**2.56 ± 0.33^{b}**	**16.45 ± 1.79^{abcde}**	**66.70 ± 15.54^{ab}**	**8.47 ± 1.62^{a}**	**25.10 ± 4.81^{b}**	**9.49 ± 2.80^{bc}**
		(9 - 19)	(1.40 - 3.66)	(9.73 - 20.93)	(6.14 - 101.22)	(3.26 - 11.67)	(8.95 - 35.55)	(1.68 - 22.10)
Tilapia	**44**	**10.7 ± 0.40^{c}**	**1.48 ± 0.11^{ef}**	**13.60 ± 0. 55^{ef}**	**21.47 ± 2.23^{cd}**	**3.92 ± 0.381^{b}**	**12.38 ± 0.87^{cd}**	**4.76 ± 0.52^{cde}**
		(6 - 16)	(0.33 - 3.73)	(6.26 - 22.31)	(4.26 - 92.49)	(1.59 - 15.28)	(6.89 - 38.28)	(1.21 - 14.27)
Snakehead	**4**	14.4 ± **0.42^{a}**	4.58 ± **0.16^{a}**	18.20 ± **0.34^{ab}**	85.99 ± **1.32^{a}**	9.18 ± **0.29^{a}**	39.57 ± **0.82^{a}**	15.45 ± **0.97^{a}**
		(14 - 15)	(4.26 - 5.0)	(17.70 - 19.15)	(82.66 - 88.37)	(8.48 - 9.85)	(37.74 - 41.66)	(13.15 - 17.14)
Asian sea bass	**3**	**15.7 ± 0.83^{a}**	**1.51 ± 0.07^{de}**	**19.33 ± 1.80^{ab}**	**38.65 ± 2.13^{b}**	**3.09 ± 0.15^{cd}**	**10.01 ± 1.25^{cde}**	**12.10 ± 1.87^{ab}**
		(14 - 17)	(1.39 - 1.64)	(17.13 - 22.90)	(34.83 - 42.18)	(2.80 - 3.31)	(7.50 - 11.39)	(9.42 - 15.69)
Carp	**5**	**10.1 ± 1.35^{bcde}**	**1.45 ± 0.20cd^{ef}**	**10.34 ± 2.17^{fg}**	**13.67 ± 4.73^{cde}**	**2.98 ± 0.39b^{cde}**	**9.64 ± 1.22^{def}**	**2.91 ± 1.10^{def}**
		(6 - 13)	(1.04 - 2.06)	(5.61 - 17.63)	(6.48 - 32.22)	(1.68 - 3.86)	(6.98 - 13.87)	(1.38 - 7.30)
Salmon	**12**	**10.6 ± 0.54^{bcd}**	**1.01 ± 0.06^{g}**	**15.00 ± 1.18^{cdef}**	**23.23 ± 1.96^{c}**	**1.89 ± 0.09^{fg}**	**8.06 ± 0.30^{ef}**	**5.12 ± 0.57^{cd}**
		(8 - 14)	(0.60 - 1.31)	(6.60 - 19.64)	(6.98 - 33.10)	(1.47 - 2.45)	(5.86 - 9.36)	(1.20 - 7.31)
Trout	**10**	**8.7 ± 0.38^{e}**	**1.18 ± 0.12^{fg}**	**15.68 ± 0.60^{bcd}**	**18.17 ± 1.23^{de}**	**1.68 ± 0.10^{g}**	**7.37 ± 0.43^{f}**	**3.54 ± 0.39^{ef}**
		(7 - 11)	(0.74 - 2.05)	(13.27 - 19.35)	(14.71 - 28.06)	(1.40 - 2.53)	(5.80 - 10.86)	(2.17 - 6.65)
White shrimp	**39**	**11.8 ± 0.33^{b}**	**1.63 ± 0.08^{cde}**	**14.88 ± 0.54^{de}**	**21.81 ± 1.26^{c}**	**3.03 ± 0.07^{d}**	**12.02 ± 0.17^{cd}**	**6.08 ± 0.57^{c}**
		(7 - 15)	(0.77 - 3.46)	(8.70 - 23.20)	(7.51 - 38.85)	(2.38 - 4.13)	(10.09 - 14.72)	(0.56 - 14.23)
Black prawn	**5**	**15.7 ± 0.84^{a}**	**1.90 ± 0.18^{bcd}**	**20.68 ± 1.61^{a}**	**38.43 ± 3.39^{b}**	**2.82 ± 0.11^{d}**	**13.08 ± 1.33^{cd}**	**9.56 ± 0.76^{b}**
		(13 - 18)	(1.53 - 2.47)	(16.38 - 25.24)	(28.65 - 46.62)	(2.46 - 3.12)	(9.71 - 16.40)	(7.56 - 11.40)
Freshwater fish	**17**	**10.5 ± 0.47^{cd}**	**1.93 ± 0.20^{bc}**	**14.71 ± 0.79^{def}**	**36.25 ± 6.07^{b}**	**3.86 ± 0.65^{bcd}**	**13.11 ± 1.67^{cd}**	**5.16 ± 0.64^{cd}**
		(8 - 15)	(0.95 - 3.48)	9.99 - 20.13)	(7.74 - 89.46)	(1.62 - 10.01)	(5.78 - 24.88)	(2.23 - 10.86)
Marine fish	**9**	**11.9 ± 0.63^{bc}**	**1.51 ± 0.81^{de}**	**17.72 ± 1.13^{abc}**	**21.44 ± 1.77^{cd}**	**2.30 ± 0.20^{ef}**	**8.50 ± 0.49^{ef}**	**6.21 ± 0.87^{c}**
		(9 - 14)	(1.18 - 1.86)	(10.79 - 21.35)	(13.27 - 31.63)	(1.59 - 3.73)	(6.20 - 11.04)	(1.70 - 8.98)

Values with different letters in a column are different from each other (α =0.05)

Table 5.2 Ash and macro-mineral concentrations (dry matter basis) in starter feeds for different species. Values represent mean and standard error with minimum and maximum concentrations.

Feed type	Number of Samples	Ash	Macro-minerals (g/kg feed)					
	(N)	(%)	S	P	Ca	Mg	K	Na
Channel	**4**	**10.8 ± 0.82^{c}**	**1.38 ± 0.29^{ab}**	**17.57 ± 1.47^{ab}**	**30.22 ± 4.38^{abc}**	**1.98 ± 0.31^{bc}**	**9.16 ± 1.29^{ab}**	**5.11 ± 1.32^{ab}**
		(9 - 12)	(0.91 - 2.13)	(15.29 - 20.59)	(21.01 - 41.56)	(1.71 - 2.10)	(8.67 - 9.76)	(4.00 - 6.70)
Clarias	**1**	**12.9 ± 1.63^{abc}**	**1.41 ± 0.58^{ab}**	**12.74 ± 2.94^{b}**	**23.19 ± 8.77^{abc}**	**2.92 ± 0.61^{abc}**	**12.27 ± 2.58^{ab}**	**9.52 ± 2.63^{ab}**
		(12.9-12.9)	(1.41-1.41)	(12.74-12.74)	(23.19-23.19)	(2.92-2.92)	(12.27-12.27)	(9.52-9.52)
Tilapia	**2**	**9.9 ± 1.15^{c}**	**1.28 ± 0.41^{ab}**	**18.72 ± 2.08^{ab}**	**19.21 ± 6.20^{c}**	**1.99 ± 0.43^{bc}**	**9.38 ± 1.83^{ab}**	**3.40 ± 1.86^{b}**
		(9 - 10)	(0.62 - 1.93)	(16.66 - 20.78)	(18.60 - 19.81)	(1.69 - 2.30)	(6.89 - 11.87)	(3.26 - 3.54)
Salmon	**6**	**11.6 ± 0.67^{bc}**	**0.93 ± 0.24^{b}**	**15.33 ± 1.20^{b}**	**22.67 ± 3.58^{c}**	**1.96 ± 0.25^{c}**	**8.77 ± 1.05^{b}**	**5.65 ± 1.07^{ab}**
		(11 - 14)	(0.60 - 1.17)	(6.60 - 19.64)	(6.98 - 33.10)	(1.47 - 2.33)	(8.25 - 9.36)	(1.20 - 7.30)
Trout	**1**	**10.8 ± 1.63^{abc}**	**1.30 ± 0.58^{ab}**	**19.35 ± 2.94^{ab}**	**19.28 ± 8.77^{bc}**	**1.71 ± 0.61^{abc}**	**7.27 ± 2.58^{ab}**	**6.65 ± 2.63^{ab}**
		(10.8-10.8)	(1.30-1.30)	(19.35-19.35)	(19.28-19.28)	(1.71-1.71)	(7.27-7.27)	(6.65-6.65)
White shrimp	**17**	**12.3 ± 0.40^{abc}**	**1.68 ± 0.14^{a}**	**16.73 ± 0.71^{ab}**	**23.72 ± 2.13^{c}**	**2.97 ± 0.15^{a}**	**12.04 ± 0.63^{a}**	**6.98 ± 0.64^{ab}**
		(9 - 15)	(0.93 - 3.46)	(12.16 - 23.20)	(7.51 - 38.85)	(2.40 - 3.58)	(10.23 - 14.02)	(3.25 - 14.23)
Black prawn	**2**	**14.7 ± 1.15^{a}**	**1.55 ± 0.41^{ab}**	**17.06 ± 2.08^{ab}**	**37.64 ± 6.20^{ab}**	**2.61 ± 0.43^{abc}**	**10.95 ± 1.83^{ab}**	**9.45 ± 1.86^{a}**
		(13 - 16)	(1.53 - 1.57)	(16.38 - 17.74)	(28.65 - 46.62)	(2.46 - 2.76)	(9.71 - 12.18)	(8.03 - 10.87)
Freshwater fish	**4**	**11.1 ± 0.82^{bc}**	**1.87 ± 0.29^{a}**	**16.81 ± 1.47^{ab}**	**40.77 ± 4.38^{a}**	**2.85 ± 0.31^{ab}**	**12.54 ± 1.29^{a}**	**5.15 ± 1.32^{ab}**
		(10 - 12)	(0.95 - 3.11)	(15.85 - 18.43)	(32.27 - 59.37)	(1.76 - 5.53)	(6.92 - 23.85)	(3.59 - 8.55)
Marine fish	**5**	**13.1 ± 0.73^{ab}**	**1.61 ± 0.26^{ab}**	**19.58 ± 1.31^{a}**	**23.73 ± 3.92^{bc}**	**2.22 ± 0.27^{bc}**	**7.81 ± 1.15^{b}**	**7.87 ± 1.18^{ab}**
		(11 - 14)	(1.38 - 1.86)	(15.67 - 21.35)	(19.25 - 31.62)	(1.84 - 2.39)	(6.20 - 9.46)	(4.53 - 8.98)

Values with different letters in a column are different from each other (α =0.05)

Table 5.3 Ash and macro-mineral concentrations (dry matter basis) in fingerling feeds for different species. Values represent mean and standard error with minimum and maximum concentrations.

Feed type	N	Ash	Macro-minerals (g/kg feed)					
		(%)	S	P	Ca	Mg	K	Na
Channel	**3**	**8.3 ± 1.27^{fg}**	**1.41 ± 0.25^{cde}**	**12.17 ± 1.66^{fgh}**	**13.94 ± 3.41^{de}**	**3.21 ± 0.58^{cd}**	**12.28 ± 1.01^{bc}**	**2.75 ± 1.594^{d}**
		(7 - 9)	(1.07 - 1.67)	(9.53 - 13.75)	(6.11 - 21.65)	(2.19 - 4.49)	(10.73 - 14.78)	(1.59 - 4.38)
Clarias	**14**	**10.9 ± 0.59^{defg}**	**1.33 ± 0.11^{de}**	**12.37 ± 0.77^{g}**	**16.81 ± 1.58^{de}**	**3.69 ± 0.27^{bc}**	**12.85 ± 0.46^{b}**	**4.57 ± 0.74^{cd}**
		(8 - 16)	(1.02 - 1.75)	(9.44 - 17.78)	(10.87 - 23.50)	(2.31 - 5.59)	(10.63 - 16.22)	(2.08 - 7.65)
Pangasius	**2**	**18.6 ± 0.38^{a}**	**3.22 ± 0.71^{ab}**	**17.03 ± 1.36^{bcde}**	**98.59 ± 11.01^{a}**	**11.35 ± 3.90^{ab}**	**32.48 ± 9.19^{a}**	**15.19 ± 3.58^{ab}**
		(18 - 19)	(2.78 - 3.65)	(14.91 - 19.16)	(95.94 - 101.22)	(11.15 - 11.55)	(29.40 - 35.55)	(8.28 - 22.10)
Snakehead	**2**	**15.1 ± 0.38^{c}**	**4.80 ± 0.71^{a}**	**17.96 ± 13.58^{bc}**	**88.11 ± 11.01^{a}**	**9.186 ± 3.90^{abcde}**	**39.43 ± 9.19^{a}**	**17.07 ± 3.58^{a}**
		(15 - 15)	(4.61 - 5.00)	(17.70 - 18.23)	(87.85 - 88.37)	(8.98 - 9.39)	(38.97 - 39.89)	(17.00 - 17.14)
Tilapia	**11**	**11.4 ± 0.42^{de}**	**1.74 ± 0.13^{c}**	**15.26 ± 0.82^{cdef}**	**22.72 ± 1.86^{c}**	**3.28 ± 0.32^{c}**	**10.48 ± 0.55^{cd}**	**5.37 ± 0.86^{cd}**
		(7 - 16)	(1.07 - 3.73)	(9.70 - 20.48)	(6.85 - 55.01)	(1.78 - 15.28)	(7.72 - 38.28)	(2.11 - 12.64)
Carp	**1**	**6.4 ± 2.20^{g}**	**1.05 ± 0.43^{cde}**	**5.61 ± 2.88^{h}**	**6.48 ± 5.91^{e}**	**1.68 ± 1.01^{cde}**	**6.98 ± 1.74^{de}**	**1.80 ± 2.76^{cd}**
		(6-6)	(1.05-1.05)	(5.61-5.61)	(6.48-6.48)	(1.68-1.68)	(6.98-6.98)	(1.80-1.80)
Asian sea bass	**1**	**15.5 ± 2.20^{abcd}**	**1.51 ± 0.43^{cde}**	**22.90 ± 2.88^{ab}**	**38.95 ± 5.91^{b}**	**3.31 ± 1.01^{bcde}**	**11.13 ± 1.74^{bcd}**	**9.42 ± 2.76^{abc}**
		(15-15)	(1.51-1.51)	(22.90-22.90)	(38.95-38.95)	(3.31-3.31)	(11.13-11.13)	(9.42-9.42)
Salmon	**2**	**9.4 ± 1.56^{efg}**	**1.20 ± 0.30^{cde}**	**13.79 ± 2.03^{cdefg}**	**22.457 ± 4.18^{cd}**	**1.68 ± 0.71^{de}**	**7.94 ± 1.23^{de}**	**4.67 ± 1.95^{cd}**
		(9 - 10)	(1.09 - 1.31)	(12.30 - 15.29)	(19.95 - 24.96)	(1.65 - 1.71)	(7.67 - 8.23)	(3.77 - 5.56)
Trout	**4**	**8.6 ± 1.10^{fg}**	**0.96 ± 0.21^{e}**	**16.20 ± 1.44^{cdef}**	**16.95 ± 2.96^{cde}**	**1.62 ± 0.50e**	**6.92 ± 0.87^{e}**	**3.59 ± 1.38^{cd}**
		(8 - 9)	(0.74 - 1.26)	(14.78 - 17.56)	(16.09 - 18.39)	(1.50 - 1.77)	(5.80 - 7.52)	(2.84 - 4.25)
White shrimp	**12**	**11.9 ± 0.64^{de}**	**1.57 ± 0.12^{cd}**	**14.34 ± 0.83^{defg}**	**21.14 ± 1.71^{cd}**	**3.03 ± 0.29^{cd}**	**12.03 ± 0.50^{b}**	**6.51 ± 0.80^{c}**
		(7 - 14)	(0.77 - 2.27)	(9.57 - 18.67)	(7.89 - 30.33)	(2.38 - 3.64)	(10.09 - 14.72)	(1.62 - 13.29)
Black prawn	**3**	**17.2 ± 0.37^{b}**	**1.90 ± 0.37^{bcd}**	**23.26 ± 1.23^{a}**	**39.59 ± 5.21^{b}**	**2.81 ± 0.98^{cde}**	**11.30 ± 1.71^{bcd}**	**8.72 ± 2.18^{abc}**
		(15 - 18)	(1.74 - 2.47)	(21.66 - 25.24)	(32.78 - 44.19)	(2.79 - 3.12)	(11.12 - 16.40)	(7.56 - 11.40)
Freshwater fish	**1**	**12.7 ± 2.20^{cdef}**	**3.48 ± 0.43^{a}**	**19.34 ± 2.88^{abcd}**	**89.46 ± 5.91^{a}**	**10.01 ± 1.01^{a}**	**24.83 ± 1.74^{a}**	**6.51 ± 2.76^{bcd}**
		(12-12)	(3.48-3.48)	(19.34-19.34)	(89.46-89.46)	(10.01-10.01)	(24.83-24.83)	(6.51-6.51)
Marine fish	**1**	**12.4 ± 2.20^{cdefg}**	**1.76 ± 0.43^{bcde}**	**10.79 ± 2.87^{efgh}**	**13.27 ± 5.91^{cde}**	**3.72 ± 1.01^{bcde}**	**11.04 ± 1.74^{bcd}**	**1.70 ± 2.76^{cd}**
		(12-12)	(1.76-1.76)	(10.97-10.97)	(13.27-13.27)	(3.72-3.72)	(11.04-11.04)	(1.07-1.07)

Values with different letters in a column are different from each other (α =0.05)

Table 5.4 Ash and macro-mineral concentrations (dry matter basis) in grower feeds for different species. Values represent mean and standard error with minimum and maximum concentrations.

Feed type	Number of Samples	Ash	Macro-minerals (g/kg feed)					
	(N)	(%)	S	P	Ca	Mg	K	Na
Channel	**6**	**9.0 ± 1.00^{c}**	**1.41 ± 0.22^{cd}**	**13.33 ± 1.28^{abcd}**	**16.92 ± 5.55^{ce}**	**3.19 ± 0.62^{bcd}**	**12.09 ± 1.55^{bcd}**	**2.47 ± 1.19^{b}**
	(7 - 11)	(1.03 - 2.04)	(9.78 - 18.37)	(7.77 - 28.02)	(2.00 - 4.17)	(8.89 - 13.90)	(1.85 - 3.73)	
Clarias	**17**	**10.6 ± 0.59^{bc}**	**1.25 ± 0.13^{d}**	**11.36 ± 0.76^{d}**	**13.49 ± 3.30^{e}**	**3.36 ± 0.37^{bc}**	**12.18 ± 0.92^{c}**	**5.08 ± 0.70^{b}**
	(8 - 17)	(0.90 - 2.82)	(8.31 - 15.81)	(6.17 - 21.26)	(1.79 - 5.04)	(9.74 - 14.40)	(1.60 - 10.32)	
Pangasius	**4**	**15.1 ± 1.36^{a}**	**2.23 ± 0.31^{b}**	**16.16 ± 2.05^{abc}**	**50.76 ± 13.62^{ab}**	**7.03 ± 1.46^{a}**	**21.41 ± 4.48^{b}**	**6.63 ± 1.78^{b}**
	(9 - 18)	(1.40 - 2.97)	(9.73 - 20.93)	(6.14 - 87.94)	(3.264 - 11.67)	(8.95 - 35.00)	(1.68 - 10.71)	
Snakehead	**2**	**13.7 ± 1.92^{ab}**	**4.36 ± 0.43^{a}**	**18.44 ± 2.91^{ab}**	**83.87 ± 19.27^{a}**	**9.17 ± 2.06^{a}**	**39.70 ± 6.34^{a}**	**13.83 ± 2.52^{a}**
	(14 - 14)	(4.26 - 4.46)	(17.72 - 19.15)	(82.66 - 85.08)	(8.48 - 9.85)	(37.74 - 41.66)	(13.15 - 14.52)	
Tilapia	**31**	**10.4 ± 0.44^{bc}**	**1.35 ± 0.10^{d}**	**12.64 ± 0.56^{bcd}**	**20.28 ± 2.44^{ce}**	**3.90 ± 0.27^{b}**	**12.37 ± 0.68^{c}**	**4.58 ± 0.52^{b}**
	(6 - 16)	(0.33 - 3.55)	(6.26 - 22.31)	(4.26 - 92.49)	(1.59 - 9.23)	(7.01 - 29.06)	(1.21 - 14.27)	
Carp	**4**	**11.0 ± 1.22^{bc}**	**1.56 ± 0.27^{bcd}**	**11.53 ± 1.56^{cd}**	**15.47 ± 6.80^{ce}**	**3.30 ± 0.76^{bcd}**	**10.31 ± 1.90^{cde}**	**3.19 ± 1.45^{b}**
	(9 - 13)	(1.04 - 2.06)	(6.18 - 17.63)	(8.13 - 32.22)	(2.59 - 3.86)	(7.93 - 13.87)	(1.38 - 7.30)	
Asian sea bass	**2**	**15.8 ± 1.72^{a}**	**1.51 ± 0.38^{bcd}**	**17.54 ± 2.21^{a}**	**38.50 ± 9.62^{bc}**	**2.98 ± 1.07^{bcd}**	**9.45 ± 2.69^{cde}**	**13.43 ± 2.06^{a}**
	(14 - 17)	(1.39 - 1.64)	(17.13 - 17.95)	(34.83 - 42.18)	(2.80 - 3.17)	(7.51 - 11.39)	(11.17 - 15.69)	
Salmon	**4**	**9.7 ± 1.22^{bc}**	**1.03 ± 0.27^{d}**	**15.11 ± 1.56^{abc}**	**24.45 ± 6.80^{bce}**	**1.88 ± 0.76^{cd}**	**7.06 ± 1.90^{e}**	**4.57 ± 1.45^{b}**
	(8 - 13)	(0.87 - 1.24)	(9.94 - 19.49)	(17.88 - 32.79)	(1.52 - 2.45)	(5.86 - 8.48)	(3.21 - 7.31)	
Trout	**5**	**8.4 ± 1.09^{c}**	**1.33 ± 0.24^{cd}**	**14.53 ± 1.40^{abc}**	**18.93 ± 6.08^{cde}**	**1.72 ± 0.68^{d}**	**7.76 ± 1.70^{de}**	**2.87 ± 1.30^{b}**
	(7 - 11)	(0.98 - 2.05)	(13.27 - 16.05)	(14.71 - 28.06)	(1.40 - 2.53)	(6.41 - 10.86)	(2.17 - 3.45)	
Whiteleg shrimp	**10**	**10.6 ± 0.78^{bc}**	**1.63 ± 0.17^{bcd}**	**12.23 ± 1.01^{cd}**	**18.96 ± 4.47^{ce}**	**3.03 ± 0.50^{bcd}**	**11.87 ± 1.26^{cd}**	**4.02 ± 0.94^{b}**
	(8 - 15)	(0.99 - 2.55)	(8.70 - 16.30)	(8.79 - 33.54)	(2.43 - 4.13)	(10.18 - 13.60)	(0.56 - 10.36)	
Freshwater fish	**12**	**10.1 ± 0.72^{bc}**	**1.85 ± 0.16^{bc}**	**13.76 ± 0.93^{abc}**	**32.80 ± 4.20^{bd}**	**3.65 ± 0.47^{bc}**	**12.14 ± 1.18^{c}**	**4.96 ± 0.86^{b}**
	(8 - 15)	(1.06 - 3.21)	(9.99 - 20.13)	(7.74 - 88.53)	(1.62 - 9.72)	(5.78 - 24.88)	(2.23 - 10.86)	
Marine fish	**3**	**9.9 ± 1.41^{bc}**	**1.25 ± 0.31^{cd}**	**16.93 ± 1.81^{a}**	**20.33 ± 7.85^{bce}**	**1.96 ± 0.87^{cd}**	**8.81 ± 2.20^{cde}**	**4.95 ± 1.68^{b}**
	(9 - 10)	(1.18 - 1.37)	(15.57 - 18.20)	(15.71 - 24.80)	(1.59 - 2.15)	(8.40 - 9.41)	(3.87 - 5.58)	

Values with different letters in a column are different from each other (α =0.05)

Table 5.5 Ash and macro-mineral concentrations (dry matter basis) in whole bodies (n=3) for different species. Values represent mean and standard error with minimum and maximum concentrations.

Cultured specie	Ash content	Macro-minerals (g/kg)					
	(%)	S	P	Ca	Mg	K	Na
Channel	**9.5 ± 1.79^{b}**	**0.40 ± 0.54^{c}**	**16.57 ± 2.14^{ab}**	**6.48 ± 2.77^{f}**	**0.84 ± 0.013^{f}**	**5.12 ± 0.30^{d}**	**0.65 ± 0.39^{e}**
	(8.4 - 10.7)	(0.38 - 0.408)	(16.48 - 16.70)	(6.25 - 6.73)	(0.83 - 0.84)	(4.97 - 5.27)	(0.60 - 0.70)
Tilapia	**13.6 ± 1.38^{ab}**	**0.84 ± 0.46^{c}**	**18.11 ± 1.85^{a}**	**29.92 ± 2.77^{c}**	**1.47 ± 0.01^{d}**	**7.56 ± 0.26^{c}**	**3.09 ± 0.34^{c}**
	(8.7 - 19.8)	(0.83 - 0.86)	(14.21 - 29.15)	(22.54 - 50.80)	(1.45 - 1.50)	(7.06 - 8.65)	(2.45 - 4.85)
Salmon	**9.3 ± 1.79^{b}**	**0.36 ± 0.54^{c}**	**11.60 ± 2.14^{b}**	**15.20 ± 2.77^{e}**	**1.13 ± 0.01^{e}**	**5.33 ± 0.30^{d}**	**1.85 ± 0.39^{de}**
	(8.6 - 9.9)	(0.36 - 0.37)	(11.46 - 11.78)	(14.99 - 15.41)	(1.13 - 1.13)	(5.22 - 5.39)	(1.75 - 1.90)
Trout	**12.0 ± 1.79^{ab}**	**0.65 ± 0.54^{c}**	**17.16 ± 2.14^{ab}**	**21.79 ± 2.77^{d}**	**1.62 ± 0.01^{c}**	**8.83 ± 0.30^{b}**	**2.28 ± 0.39^{cd}**
	(11.4-12.9)	(0.65 - 0.66)	(17.14 - 17.18)	(21.75 - 21.81)	(1.61 - 1.62)	(8.73 - 8.91)	(2.27 - 2.29)
Whiteleg shrimp	**10.6 ± 1.55^{b}**	**3.51 ± 0.46^{b}**	**13.39 ± 1.85^{ab}**	**54.47 ± 2.77^{a}**	**2.74 ± 0.01^{b}**	**8.47 ± 0.26^{b}**	**5.09 ± 0.34^{b}**
	(9.0 - 12.5)	(2.55 - 6.30)	(12.16 - 13.92)	(26.13 - 64.82)	(2.68 - 2.77)	(8.10 - 9.55)	(4.08 - 5.46)
Black tiger prawn	**19.7 ± 3.10^{a}**	**6.90 ± 0.93^{a}**	**9.60 ± 3.71^{ab}**	**32.90 ± 2.77^{b}**	**3.00 ± 0.023^{a}**	**10.60 ± 0.52^{a}**	**9.40 ± 0.68^{a}**

Values with different letters in a column are different from each other (α=0.05)

Table 5.6 Micro-mineral concentrations (dry matter basis) in feeds for different species. Values represent mean and standard error with minimum and maximum concentrations.

Feed type	Micre-minerals (mg/kg feed)										
	Fe	Mn	Zn	Cu	As	Pb	Cd	Co	Cr	Mo	Se
Channel	**497.9 ± 100.9^{cde}**	**78.0 ± 5.6^{d}**	**152.5 ± 18.7^{bc}**	**16.7 ± 3.65^{cde}**	**0.96 ± 0.22^{ab}**	**0.37 ± 0.11^{de}**	**1.41 ± 0.16^{cde}**	**0.97 ± 0.14^{deg}**	**1.98 ± 0.11^{d}**	**1.96 ± 0.36^{bc}**	**1.40 ± 0.36^{abc}**
	(177.01 - 1258.1)	(41.70 - 103.11)	(56.23 - 273.58)	(9.33 - 60.12)	(0.00 - 1.86)	(0.00 - 1.17)	(0.00 - 2.20)	(0.26 - 2.10)	(1.47 - 2.60)	(0.24 - 5.07)	(0.00 - 4.49)
Clarias	**365.3 ± 44.4^{de}**	**95.3 ± 5.5^{bc}**	**135.0 ± 9.8^{c}**	**18.6 ± 3.02^{cde}**	**0.22 ± 0.11^{de}**	**2.33 ± 0.22^{a}**	**0.18 ± 0.10^{h}**	**0.47 ± 0.12^{f}**	**5.83 ± 2.04^{cd}**	**1.19 ± 0.13^{de}**	**2.16 ± 0.25^{a}**
	(120.13 - 1490.4)	(59.14 - 218.20)	(43.90 - 245.78)	(7.38 - 71.84)	(0.00 - 2.77)	(0.00 - 4.52)	(0.00 - 2.50)	(0.00 - 2.84)	(2.15 - 63.26)	(0.09 - 3.12)	(0.25 - 5.44)
Pangasiu	**2326.3 ± 379.2^{a}**	**221.9 ± 37.3^{a}**	**283.1 ± 81.5^{bc}**	**20.7 ± 2.58^{c}**	**0.46 ± 0.20^{bcde}**	**1.42 ± 0.50^{abc}**	**2.14 ± 0.43^{bc}**	**1.67 ± 0.24^{bc}**	**15.85 ± 2.54^{b}**	**2.14 ± 0.39^{bc}**	**0.45 ± 0.31^{de}**
	(971.42 - 3735.3)	(99.30 - 326.20)	(64.58 - 536.38)	(15.22 - 30.67)	(0.00 - 1.16)	(0.12 - 2.59)	(0.73 - 3.13)	(1.17 - 2.84)	(7.59 - 21.47)	(1.04 - 3.18)	(0.00 - 1.85)
Tilapia	**619.5 ± 104.4^{bc}**	**102.9 ± 8.0^{bc}**	**168.1 ± 16.2^{bc}**	**15.7 ± 1.46^{cde}**	**0.45 ± 0.076^{cd}**	**1.23 ± 0.19^{b}**	**1.21 ± 0.11^{def}**	**0.93 ± 0.11^{eg}**	**4.24 ± 0.46^{c}**	**1.77 ± 0.21^{c}**	**1.58 ± 0.21^{ab}**
	(174.53 - 4507.9)	(38.59 - 286.47)	(51.99 - 578.87)	(5.13 - 55.24)	(0.00 - 1.86)	(0.00 - 5.37)	(0.00 - 3.06)	(0.00 - 3.38)	(1.06 - 17.17)	(0.00 - 5.92)	(0.00 - 5.16)
Snakehead	**2272.2 ± 269.0^{a}**	**226.7 ± 9.6^{a}**	**616.6 ± 118.7^{a}**	**35.7 ± 3.67^{b}**	**0.61 ± 0.40^{abcde}**	**2.32 ± 0.28^{a}**	**3.10 ± 0.16^{a}**	**1.93 ± 0.20^{b}**	**25.69 ± 1.24^{a}**	**5.63 ± 0.29^{a}**	**0.54 ± 0.54^{bcde}**
	(1719.7 - 2877.3)	(208.54 - 247.43)	(395.89 - 836.02)	(29.23 - 42.84)	(0.00 - 1.71)	(1.89 - 3.11)	(2.75 - 3.50)	(1.55 - 2.36)	(22.89 - 28.41)	(5.02 - 6.24)	(0.00 - 2.17)
Asian sea bass	**593.5 ± 107.7^{bcd}**	**65.7 ± 11.7^{de}**	**210.1 ± 56.7^{bc}**	**17.1 ± 1.80^{cd}**	**0.30 ± 0.11^{de}**	**1.25 ± 0.15^{b}**	**1.10 ± 0.15^{ef}**	**2.12 ± 0.63^{abcde}**	**4.36 ± 0.84^{c}**	**0.73 ± 0.21^{ef}**	**1.82 ± 0.24^{a}**
	(432.93 - 798.23)	(42.67 - 81.15)	(97.10 - 274.51)	(13.56 - 19.01)	(0.11 - 0.50)	(1.08 - 1.55)	(0.80 - 1.28)	(0.88 - 2.97)	(3.15 - 5.97)	(0.36 - 1.09)	(1.45 - 2.27)
Carp	**1244.3 ± 668.0^{abcde}**	**91.5 ± 33.3^{bcde}**	**81.0 ± 18.1^{d}**	**12.5 ± 2.74^{de}**	**0.07 ± 0.07^{e}**	**0.71 ± 0.60^{bcde}**	**0.32 ± 0.32^{gh}**	**0.43 ± 0.25^{fg}**	**4.05 ± 1.32^{cd}**	**0.25 ± 0.25^{fg}**	**0.40 ± 0.39^{cde}**
	(280.06 - 3885.7)	(26.85 - 220.34)	(27.67 - 139.51)	(4.85 - 21.44)	(0.00 - 0.34)	(0.00 - 3.10)	(0.00 - 1.59)	(0.00 - 1.08)	(1.51 - 9.11)	(0.00 - 1.25)	(0.00 - 1.96)
Salmon	**347.1 ± 29.0^{e}**	**59.4 ± 3.2^{e}**	**169.1 ± 11.8^{b}**	**15.0 ± 2.27^{cde}**	**0.11 ± 0.05^{e}**	**0.14 ± 0.09^{e}**	**1.86 ± 0.07^{b}**	**2.94 ± 0.43^{a}**	**2.13 ± 0.62^{de}**	**0.11 ± 0.05^{g}**	**0.29 ± 0.14^{e}**
	(141.34 - 456.09)	(44.42 - 85.98)	(74.10 - 222.39)	(9.83 - 39.44)	(0.00 - 0.44)	(0.00 - 1.03)	(1.16 - 2.11)	(0.49 - 5.42)	(0.97 - 8.89)	(0.00 - 0.60)	(0.00 - 1.44)
Trout	**358.3 ± 32.0^{e}**	**75.9 ± 12.7^{cde}**	**152.6 ± 14.7^{bc}**	**12.2 ± 1.25^{e}**	**0.43 ± 0.25^{bcde}**	**0.35 ± 0.14^{de}**	**1.72 ± 0.10^{bc}**	**0.50 ± 0.09^{f}**	**3.23 ± 0.64^{cd}**	**1.02 ± 0.27^{de}**	**1.09 ± 0.20^{bcd}**
	(263.77 - 577.83)	(30.90 - 128.54)	(61.71 - 254.20)	(9.06 - 19.61)	(0.00 - 2.59)	(0.00 - 1.24)	(0.91 - 2.11)	(0.32 - 1.14)	(1.70 - 8.10)	(0.31 - 3.17)	(0.18 - 1.89)
Whiteleg shrimp	**601.9 ± 73.2^{bc}**	**86.1 ± 19.0^{bcde}**	**168.4 ± 29.1^{bc}**	**45.8 ± 3.18^{a}**	**0.67 ± 0.13^{bc}**	**0.65 ± 0.14^{cd}**	**0.96 ± 0.14^{fg}**	**1.11 ± 0.36^{cdef}**	**5.40 ± 2.34^{cd}**	**1.45 ± 0.26^{cd}**	**0.84 ± 0.19^{cd}**
	(202.44 - 2885.2)	(35.46 - 794.44)	(45.49 - 1156.5)	(15.50 - 108.62)	(0.00 - 2.51)	(0.00 - 3.08)	(0.00 - 3.04)	(0.00 - 13.38)	(0.34 - 93.87)	(0.00 - 7.21)	(0.00 - 4.11)
Black tiger prawn	**565.2 ± 88.2^{bc}**	**54.9 ± 7.3^{e}**	**125.3 ± 23.6^{bcd}**	**43.3 ± 9.35^{ab}**	**0.48 ± 0.15^{bcd}**	**0.48 ± 0.18^{cde}**	**1.39 ± 0.23^{bcdef}**	**3.29 ± 1.45^{abcdef}**	**3.51 ± 0.38^{ce}**	**1.27 ± 0.25^{cde}**	**2.40 ± 0.47^{a}**
	(398.92 - 805.86)	(37.94 - 72.79)	(52.15 - 198.43)	(24.28 - 66.15)	(0.08 - 0.92)	(0.04 - 1.04)	(0.84 - 2.00)	(0.63 - 6.85)	(2.31 - 4.64)	(0.89 - 2.20)	(0.85 - 3.63)
Freshwater fish	**848.2 ± 144.2^{b}**	**129.6 ± 20.9^{b}**	**203.3 ± 37.1^{bc}**	**38.9 ± 6.84^{ab}**	**1.30 ± 0.26^{a}**	**1.66 ± 0.35^{ab}**	**1.54 ± 0.29^{bcdef}**	**1.41 ± 0.19^{bcd}**	**3.59 ± 0.59^{ce}**	**2.98 ± 0.45^{b}**	**1.02 ± 0.26^{bcd}**
	(242.17 - 2496.1)	(52.59 - 307.22)	(52.83 - 584.97)	(6.67 - 109.83)	(0.00 - 3.07)	(0.00 - 4.83)	(0.13 - 3.60)	(0.50 - 3.06)	(1.57 - 9.89)	(0.61 - 7.20)	(0.00 - 3.35)
Marine fish	**685.5 ± 155.8^{bc}**	**68.5 ± 3.5^{de}**	**152.7 ± 20.8^{bc}**	**19.8 ± 4.78^{cde}**	**0.13 ± 0.07^{e}**	**0.60 ± 0.23^{cde}**	**1.65 ± 0.23^{bcd}**	**1.60 ± 0.45^{bcde}**	**2.31 ± 0.19^{d}**	**1.31 ± 0.82^{bcdefg}**	**0.63 ± 0.32^{cde}**
	(240.74 - 1752.8)	(58.46 - 90.61)	(110.33 - 308.51)	(10.38 - 56.53)	(0.00 - 0.68)	(0.00 - 1.97)	(0.00 - 2.28)	(0.11 - 4.30)	(1.52 - 3.27)	(0.00 - 7.78)	(0.00 - 2.64)

Values with different letters in a column are different from each other (α=0.05)

Table 5.7 Micro-mineral concentrations (dry matter basis) in starter feeds for different species. Values represent mean and standard error with minimum and maximum concentrations.

Feed type	Micre-minerals (mg/kg feed)										
	Fe	Mn	Zn	Cu	As	Pb	Cd	Co	Cr	Mo	Se
Channel	**897.2 ± 195.74^{ab}**	**78.9 ± 12.17^{bc}**	**224.2 ± 43.20^{ab}**	**24.3 ± 6.74^{bc}**	**0.92 ± 0.433^{ab}**	**0.51 ± 0.465^{ab}**	**1.34 ± 0.524^{abc}**	**1.42 ± 0.572^{abc}**	**2.15 ± 0.799^{ab}**	**1.10 ± 1.225^{ab}**	**2.55 ± 0.448^{ab}**
	(315.41 - 1258.1)	(49.90 - 96.98)	(172.50 - 273.58)	(12.18 - 60.12)	(0.01 - 1.58)	(0.00 - 0.88)	(0.00 - 1.91)	(1.16 - 2.10)	(1.76 - 2.60)	(0.24 - 2.68)	(1.24 - 4.49)
Clarias	**414.9 ± 701.69^{abc}**	**80.7 ± 4.65^{b}**	**245.8 ± 75.74^{ab}**	**18.9 ± 21.22^{abc}**	**0.00 ± 0.459^{b}**	**1.41 ± 1.133^{ab}**	**0.00 ± 0.367^{c}**	**2.84 ± 0.406^{a}**	**3.78 ± 1.686^{ab}**	**0.30 ± 0.591^{b}**	**5.26 ± 1.686^{a}**
	(414.88 - 414.88)	(80.69 - 80.69)	(245.78 - 245.78)	(18.95 - 18.95)	(0.00 - 0.00)	(1.41 - 1.41)	(0.00 - 0.00)	(2.84 - 2.84)	(3.78 - 3.78)	(0.30 - 0.30)	(5.26 - 5.26)
Tilapia	**662.9 ± 276.82^{abc}**	**53.6 ± 17.20^{bcd}**	**260.3 ± 61.10^{ab}**	**17.1 ± 9.53^{bc}**	**0.16 ± 0.612^{ab}**	**0.43 ± 0.657^{ab}**	**1.59 ± 0.741^{ab}**	**1.09 ± 0.810^{abc}**	**3.63 ± 1.130^{ab}**	**0.99 ± 1.733^{ab}**	**2.05 ± 0.634^{abc}**
	(486.01 - 839.75)	(48.49 - 58.62)	(178.23 - 342.46)	(12.31 - 21.82)	(0.01 - 0.30)	(0.00 - 0.85)	(1.24 - 1.94)	(0.46 - 1.73)	(2.76 - 4.50)	(0.62 - 1.36)	(1.91 - 2.19)
Salmon	**327.1 ± 159.82^{c}**	**60.7 ± 9.93^{bcd}**	**171.0 ± 35.28^{ab}**	**16.7 ± 5.50^{c}**	**0.16 ± 0.353^{b}**	**0.00 ± 0.380^{b}**	**1.82 ± 0.428^{ab}**	**2.33 ± 0.467^{ab}**	**1.54 ± 0.653^{b}**	**0.12 ± 1.000^{b}**	**0.11 ± 0.366^{d}**
	(141.34 - 444.08)	(54.01 - 64.93)	(74.10 - 204.63)	(9.83 - 39.44)	(0.00 - 0.44)	(0.00 - 0.00)	(1.16 - 2.11)	(0.49 - 3.95)	(0.97 - 2.11)	(0.00 - 0.60)	(0.00 - 0.29)
Trout	**263.8 ± 391.48^{bc}**	**107.6 ± 24.33^{abc}**	**153.0 ± 86.41^{ab}**	**19.5 ± 13.47^{abc}**	**0.00 ± 0.865^{ab}**	**0.13 ± 0.930^{ab}**	**2.11 ± 1.047^{a}**	**0.32 ± 1.145^{bc}**	**2.50 ± 1.598^{ab}**	**0.31 ± 2.451^{b}**	**0.54 ± 0.896^{bcd}**
	(263.77 - 263.77)	(107.59 - 107.59)	(152.99 - 152.99)	(19.54 - 19.54)	(0.00 - 0.00)	(0.13 - 0.13)	(2.11 - 2.11)	(0.32 - 0.32)	(2.50 - 2.50)	(0.31 - 0.31)	(0.54 - 0.54)
Whiteleg shrimp	**639.8 ± 115.49^{bc}**	**48.9 ± 1.61^{d}**	**141.7 ± 19.61^{b}**	**43.7 ± 3.85^{a}**	**0.59 ± 0.141^{ab}**	**0.91 ± 0.245^{ab}**	**1.54 ± 0.122^{ab}**	**1.04 ± 0.134^{c}**	**3.40 ± 0.397^{a}**	**1.32 ± 0.202^{ab}**	**0.67 ± 0.267^{cd}**
	(325.09 - 2885.2)	(41.93 - 115.28)	(55.22 - 279.17)	(15.50 - 78.35)	(0.00 - 2.51)	(0.00 - 3.08)	(0.00 - 2.23)	(0.00 - 3.36)	(0.60 - 8.32)	(0.00 - 7.21)	(0.00 - 4.11)
Black tiger prawn	**433.8 ± 496.17^{abc}**	**42.1 ± 3.29^{d}**	**153.9 ± 53.56^{ab}**	**51.3 ± 15.01^{ab}**	**0.22 ± 0.325^{b}**	**0.62 ± 0.801^{ab}**	**0.85 ± 0.259^{bc}**	**1.05 ± 0.287^{c}**	**2.85 ± 1.192^{ab}**	**1.55 ± 0.418^{ab}**	**1.49 ± 1.193^{abcd}**
	(431.81 - 435.72)	(37.94 - 46.32)	(109.28 - 198.43)	(36.52 - 66.15)	(0.08 - 0.36)	(0.19 - 1.04)	(0.84 - 0.85)	(0.63 - 1.48)	(2.31 - 3.38)	(0.89 - 2.20)	(0.85 - 2.13)
Freshwater fish	**1346.5 ± 195.74^{a}**	**123.1 ± 12.17^{a}**	**281.4 ± 43.20^{a}**	**20.5 ± 6.74^{bc}**	**1.38 ± 0.433^{a}**	**1.69 ± 0.465^{a}**	**1.38 ± 0.524^{abc}**	**1.46 ± 0.572^{abc}**	**3.86 ± 0.799^{a}**	**1.92 ± 1.225^{a}**	**0.94 ± 0.448^{cd}**
	(564.85 - 2496.1)	(104.09 - 176.07)	(174.42 - 488.37)	(13.49 - 36.85)	(0.23 - 2.49)	(0.00 - 4.15)	(0.58 - 3.60)	(0.71 - 2.69)	(1.87 - 8.29)	(0.61 - 3.64)	(0.00 - 1.82)
Marine fish	**714.7 ± 261.27^{abc}**	**62.9 ± 2.32^{c}**	**145.5 ± 34.69^{b}**	**15.1 ± 8.34^{c}**	**0.26 ± 0.222^{b}**	**0.96 ± 0.484^{ab}**	**1.65 ± 0.181^{ab}**	**1.17 ± 0.200^{c}**	**2.44 ± 0.746^{ab}**	**0.40 ± 0.293^{b}**	**1.36 ± 0.614^{bcd}**
	(378.62 - 897.14)	(58.46 - 67.36)	(110.33 - 308.51)	(10.38 - 18.32)	(0.00 - 0.68)	(0.00 - 1.97)	(1.53 - 2.27)	(1.09 - 1.23)	(1.94 - 3.27)	(0.17 - 7.78)	(0.12 - 2.64)

Values with different letters in a column are different from each other (α=0.05)

Table 5.8 Micro-mineral concentrations (dry matter basis) in fingerling feeds for different species. Values represent mean and standard error with minimum and maximum concentrations.

Feed type	Micre-minerals (mg/kg feed)										
	Fe	Mn	Zn	Cu	As	Pb	Cd	Co	Cr	Mo	Se
Channel	**309.9 ± 278.89^{de}**	**89.9 ± 26.06^{b}**	**95.4 ± 43.27^{de}**	**12.0 ± 4.82^{de}**	**1.17 ± 0.359^{a}**	**0.45 ± 0.205^{cd}**	**1.12 ± 0.402^{bcd}**	**0.67 ± 0.399^{cd}**	**2.08 ± 1.441^{c}**	**1.88 ± 0.789^{b}**	**1.05 ± 0.412^{bcd}**
	(247.50 - 415.43)	(67.20 - 103.11)	(56.23 - 126.74)	(9.33 - 13.66)	(0.07 - 1.75)	(0.00 - 1.17)	(1.08 - 1.14)	(0.33 - 0.90)	(1.81 - 2.51)	(1.46 - 2.17)	(0.38 - 1.99)
Clarias	**349.5 ± 37.46^{e}**	**93.9 ± 40.71^{b}**	**127.6 ± 59.98^{cde}**	**15.3 ± 6.13^{de}**	**0.23 ± 0.187^{b}**	**2.69 ± 0.376^{b}**	**0.18 ± 0.240^{d}**	**0.37 ± 0.833^{cde}**	**3.14 ± 5.196^{c}**	**1.14 ± 0.187^{b}**	**1.87 ± 0.311^{b}**
	(190.42 - 647.59)	(59.14 - 137.73)	(43.90 - 190.30)	(7.38 - 68.14)	(0.00 - 2.77)	(0.63 - 4.52)	(0.00 - 2.50)	(0.00 - 1.22)	(2.24 - 4.29)	(0.25 - 3.12)	(0.69 - 3.15)
Pangasius	**3072.0 ± 341.57^{a}**	**254.3 ± 31.91^{a}**	**254.6 ± 52.99^{bc}**	**23.0 ± 5.90^{de}**	**0.61 ± 0.439^{ab}**	**2.56 ± 0.251^{b}**	**2.56 ± 0.492^{a}**	**2.20 ± 0.489^{bc}**	**18.03 ± 1.765^{b}**	**2.47 ± 0.967^{ab}**	**0.00 ± 0.505^{d}**
	(2408.8 - 3735.3)	(236.57 - 272.09)	(190.12 - 319.12)	(15.42 - 30.67)	(0.30 - 0.93)	(2.54 - 2.59)	(2.54 - 2.59)	(1.57 - 2.84)	(16.68 - 19.39)	(1.76 - 3.18)	(0.00 - 0.00)
Tilapia	**2717.9 ± 341.57^{ab}**	**243.0 ± 31.91^{a}**	**821.7 ± 52.99^{a}**	**42.0 ± 5.90^{bc}**	**0.00 ± 0.439^{b}**	**1.92 ± 0.251^{b}**	**3.07 ± 0.492^{a}**	**2.27 ± 0.489^{bc}**	**27.72 ± 1.765^{a}**	**5.14 ± 0.967^{a}**	**1.08 ± 0.505^{bcd}**
	(2558.5 - 2877.3)	(238.47 - 247.43)	(807.35 - 836.02)	(41.17 - 42.84)	(0.00 - 0.00)	(1.89 - 1.95)	(2.99 - 3.14)	(2.18 - 2.36)	(27.02 - 28.41)	(5.02 - 5.27)	(0.00 - 2.17)
Snakehead	**583.6 ± 65.42^{d}**	**111.6 ± 16.64^{b}**	**190.8 ± 27.46^{bcd}**	**16.9 ± 3.04^{de}**	**0.27 ± 0.195^{b}**	**0.60 ± 0.132^{c}**	**1.23 ± 0.227^{bc}**	**0.96 ± 0.258^{cd}**	**4.68 ± 0.939^{c}**	**1.13 ± 0.289^{b}**	**0.79 ± 0.245^{cd}**
	(219.97 - 2145.4)	(60.21 - 272.45)	(60.49 - 375.09)	(5.13 - 29.37)	(0.00 - 1.57)	(0.00 - 3.93)	(0.00 - 2.65)	(0.00 - 3.35)	(1.06 - 12.95)	(0.00 - 5.35)	(0.00 - 4.78)
Asian sea bass	**456.0 ± 483.05^{cde}**	**26.9 ± 45.13^{b}**	**27.7 ± 74.94^{e}**	**4.8 ± 8.35^{e}**	**0.00 ± 0.621^{b}**	**0.00 ± 0.354^{d}**	**0.00 ± 0.696^{d}**	**0.00 ± 0.691^{d}**	**1.51 ± 2.496^{c}**	**0.00 ± 1.367^{b}**	**0.00 ± 0.714^{cd}**
	(456.00 - 456.00)	(26.85 - 26.85)	(27.67 - 27.67)	(4.85 - 4.85)	(0.00 - 0.00)	(0.00 - 0.00)	(0.00 - 0.00)	(0.00 - 0.00)	(1.51 - 1.51)	(0.00 - 0.00)	(0.00 - 0.00)
Carp	**549.3 ± 140.15^{de}**	**81.1 ± 152.32^{ab}**	**274.5 ± 224.44^{bcde}**	**18.9 ± 22.94^{bcde}**	**0.50 ± 0.699^{ab}**	**1.08 ± 1.408^{abcd}**	**1.28 ± 0.898^{abcd}**	**2.97 ± 3.117^{abcd}**	**5.97 ± 19.441^{abc}**	**0.75 ± 0.698^{b}**	**1.73 ± 1.163^{b}**
	(549.25 - 549.25)	(81.15 - 81.15)	(274.51 - 274.51)	(18.88 - 18.88)	(0.50 - 0.50)	(1.08 - 1.08)	(1.28 - 1.28)	(2.97 - 2.97)	(5.97 - 5.97)	(0.75 - 0.75)	(1.73 - 1.73)
Salmon	**310.2 ± 341.57^{de}**	**50.1 ± 31.91^{b}**	**150.8 ± 52.99^{cde}**	**12.3 ± 5.90d^{e}**	**0.11 ± 0.439^{b}**	**0.11 ± 0.251^{cd}**	**1.82 ± 0.492^{ab}**	**2.89 ± 0.489^{b}**	**1.27 ± 1.765^{c}**	**0.15 ± 0.967^{b}**	**0.56 ± 0.505^{cd}**
	(246.55 - 373.83)	(44.42 - 55.88)	(137.30 - 164.37)	(11.75 - 12.87)	(0.00 - 0.22)	(0.00 - 0.22)	(1.80 - 1.84)	(2.70 - 3.08)	(1.19 - 1.34)	(0.00 - 0.30)	(0.00 - 1.13)
Trout	**382.5 ± 241.53^{de}**	**73.9 ± 22.56^{b}**	**128.2 ± 37.47^{cde}**	**10.1 ± 4.17^{e}**	**0.20 ± 0.311^{b}**	**0.44 ± 0.177^{cd}**	**1.89 ± 0.348^{ab}**	**0.48 ± 0.346^{cde}**	**4.92 ± 1.248^{c}**	**0.73 ± 0.684^{b}**	**1.34 ± 0.357^{bc}**
	(295.53 - 465.65)	(30.90 - 124.01)	(61.71 - 158.25)	(9.06 - 10.76)	(0.00 - 0.44)	(0.00 - 1.00)	(1.82 - 1.96)	(0.35 - 0.88)	(3.06 - 8.10)	(0.35 - 0.99)	(0.34 - 1.89)
Whiteleg shrimp	**507.3 ± 64.83^{d}**	**83.0 ± 15.62^{b}**	**160.4 ± 25.79^{cde}**	**41.7 ± 2.86^{b}**	**0.58 ± 0.186^{ab}**	**0.11 ± 0.123^{d}**	**0.68 ± 0.216^{cd}**	**0.36 ± 0.241^{cde}**	**2.84 ± 0.879^{c}**	**1.54 ± 0.283^{b}**	**0.19 ± 0.232^{d}**
	(202.44 - 1362.0)	(35.46 - 794.44)	(56.57 - 1156.5)	(18.26 - 108.62)	(0.00 - 2.23)	(0.00 - 1.89)	(0.00 - 3.04)	(0.00 - 13.38)	(0.81 - 93.87)	(0.00 - 2.78)	(0.00 - 1.41)
Black tiger prawn	**453.8 ± 129.66^{de}**	**71.3 ± 31.23^{b}**	**129.1 ± 51.58^{cde}**	**26.9 ± 5.72^{cd}**	**0.69 ± 0.372^{ab}**	**0.24 ± 0.247^{cd}**	**1.78 ± 0.432^{ab}**	**6.66 ± 0.483^{a}**	**3.63 ± 1.758^{c}**	**1.23 ± 0.566^{b}**	**3.01 ± 0.463^{a}**
	(398.92 - 805.86)	(45.29 - 72.79)	(52.15 - 139.26)	(24.28 - 64.89)	(0.32 - 0.92)	(0.04 - 0.74)	(1.56 - 2.00)	(0.70 - 6.85)	(3.41 - 4.64)	(0.93 - 1.37)	(2.38 - 3.63)
Freshwater fish	**806.5 ± 483.05^{cde}**	**291.8 ± 45.13^{a}**	**336.7 ± 74.94^{b}**	**74.2 ± 8.35^{a}**	**0.00 ± 0.621^{b}**	**3.78 ± 0.354^{a}**	**2.80 ± 0.696^{a}**	**2.09 ± 0.691^{bc}**	**6.48 ± 2.496^{c}**	**2.30 ± 1.367^{ab}**	**0.00 ± 0.714^{d}**
	(806.54 - 806.54)	(291.75 - 291.75)	(336.74 - 336.74)	(74.22 - 74.22)	(0.00 - 0.00)	(3.78 - 3.78)	(2.80 - 2.80)	(2.09 - 2.09)	(6.48 - 6.48)	(2.30 - 2.30)	(0.00 - 0.00)
Marine fish	**1752.8 ± 483.05^{bc}**	**90.6 ± 45.13^{b}**	**147.3 ± 74.94^{bcde}**	**24.4 ± 8.35^{bcde}**	**0.00 ± 0.621^{b}**	**0.46 ± 0.354^{cd}**	**0.00 ± 0.696^{d}**	**0.11 ± 0.691^{d}**	**3.07 ± 2.496^{c}**	**0.00 ± 1.367^{b}**	**0.00 ± 0.714^{d}**
	(1752.8 - 1752.8)	(90.61 - 90.61)	(147.28 - 147.28)	(24.44 - 24.44)	(0.00 - 0.00)	(0.46 - 0.46)	(0.00 - 0.00)	(0.11 - 0.11)	(3.07 - 3.07)	(0.00 - 0.00)	(0.00 - 0.00)

Values with different letters in a column are different from each other (α=0.05)

Table 5. 9 Micro-mineral concentrations (dry matter basis) in grower feeds for different species. Values represent mean and standard error with minimum and maximum concentrations.

Feed type	Micre-minerals (mg/kg feed)										
	Fe	Mn	Zn	Cu	As	Pb	Cd	Co	Cr	Mo	Se
Channel	**325.7 ± 48.53^{d}**	**71.6 ± 8.42^{de}**	**133.3 ± 21.37^{bc}**	**14.0 ± 0.72^{e}**	**0.88 ± 0.38^{abcd}**	**0.23 ± 0.11^{d}**	**1.60 ± 0.20^{bcd}**	**0.83 ± 0.18^{def}**	**1.82 ± 0.16^{f}**	**2.57 ± 0.60^{bc}**	**0.82 ± 0.42^{bcde}**
	(177.01 - 536.18)	(41.70 - 102.51)	(97.17 - 237.66)	(11.24 - 16.39)	(0.00 - 1.86)	(0.00 - 0.65)	(0.99 - 2.20)	(0.26 - 1.31)	(1.47 - 2.38)	(1.21 - 5.07)	(0.00 - 2.82)
Clarias	**375.3 ± 77.45^{cd}**	**97.3 ± 9.33^{bc}**	**134.5 ± 13.46^{bc}**	**21.4 ± 4.55^{cde}**	**0.22 ± 0.13^{cde}**	**2.09 ± 0.33^{ab}**	**0.18 ± 0.13^{h}**	**0.41 ± 0.15^{f}**	**8.16 ± 3.81^{bcdef}**	**1.29 ± 0.17^{d}**	**2.22 ± 0.397^{a}**
	(120.13 - 1490.4)	(63.24 - 218.20)	(44.05 - 235.35)	(7.53 - 71.84)	(0.00 - 1.95)	(0.00 - 4.28)	(0.00 - 1.75)	(0.00 - 2.60)	(2.15 - 63.26)	(0.09 - 2.62)	(0.25 - 5.44)
Pangasius	**1953.5 ± 383.62^{a}**	**205.7 ± 56.26^{ab}**	**297.3 ± 125.31^{ab}**	**19.6 ± 2.36^{d}**	**0.38 ± 0.27^{bcde}**	**0.84 ± 0.55^{bcd}**	**1.93 ± 0.65^{abcdef}**	**1.40 ± 0.08^{c}**	**14.76 ± 3.83^{b}**	**1.98 ± 0.52^{bcd}**	**0.68 ± 0.44^{cde}**
	(971.42 - 2641.2)	(99.30 - 326.20)	(64.58 - 536.38)	(15.22 - 25.05)	(0.00 - 1.16)	(0.12 - 2.46)	(0.73 - 3.13)	(1.17 - 1.52)	(7.59 - 21.47)	(1.04 - 2.92)	(0.00 - 1.85)
Tilapia	**592.2 ± 137.28^{bcd}**	**104.7 ± 9.15^{bc}**	**157.1 ± 20.52^{b}**	**15.9 ± 1.90^{de}**	**0.54 ± 0.09^{b}**	**1.28 ± 0.23^{c}**	**1.19 ± 0.12^{defg}**	**0.89 ± 0.11^{de}**	**4.18 ± 0.55^{c}**	**1.96 ± 0.24^{c}**	**1.72 ± 0.25^{ab}**
	(174.53 - 4507.9)	(38.59 - 286.47)	(51.99 - 578.87)	(8.07 - 55.24)	(0.00 - 1.86)	(0.00 - 5.37)	(0.00 - 3.06)	(0.26 - 3.38)	(1.33 - 17.17)	(0.00 - 5.92)	(0.00 - 5.16)
Snakehead	**1826.4 ± 106.77^{a}**	**210.4 ± 1.82^{a}**	**411.5 ± 15.59^{a}**	**29.4 ± 0.14^{bc}**	**1.22 ± 0.49^{abc}**	**2.71 ± 0.39^{a}**	**3.13 ± 0.37^{a}**	**1.59 ± 0.03^{b}**	**23.66 ± 0.77^{a}**	**6.12 ± 0.11^{a}**	**0.00 ± 0.03^{e}**
	(1719.7 - 1933.2)	(208.54 - 212.17)	(395.89 - 427.07)	(29.23 - 29.50)	(0.73 - 1.71)	(2.32 - 3.11)	(2.75 - 3.50)	(1.55 - 1.62)	(22.89 - 24.43)	(6.01 - 6.24)	(0.00 - 0.00)
Asian sea bass	**615.6 ± 182.65^{bcd}**	**58.0 ± 15.35^{e}**	**177.9 ± 80.79^{bc}**	**16.3 ± 2.73^{de}**	**0.21 ± 0.09^{de}**	**1.34 ± 0.21^{bc}**	**1.00 ± 0.20^{efg}**	**1.69 ± 0.81^{bcdef}**	**3.56 ± 0.41^{cd}**	**0.72 ± 0.36^{def}**	**1.86 ± 0.41^{abc}**
	(432.93 - 798.23)	(42.67 - 73.37)	(97.10 - 258.67)	(13.56 - 19.01)	(0.11 - 0.30)	(1.13 - 1.55)	(0.80 - 1.21)	(0.88 - 2.51)	(3.15 - 3.97)	(0.36 - 1.09)	(1.45 - 2.27)
Carp	**1441.4 ± 824.01^{ab}**	**107.7 ± 37.60^{bcde}**	**94.3 ± 15.85^{c}**	**14.4 ± 2.54^{de}**	**0.09 ± 0.08^{e}**	**0.89 ± 0.74^{bcd}**	**0.40 ± 0.40^{gh}**	**0.53 ± 0.29^{ef}**	**4.69 ± 1.49c^{def}**	**0.31 ± 0.31^{ef}**	**0.50 ± 0.49^{de}**
	(280.06 - 3885.7)	(64.42 - 220.34)	(65.58 - 139.51)	(9.39 - 21.44)	(0.00 - 0.34)	(0.00 - 3.10)	(0.00 - 1.59)	(0.00 - 1.08)	(2.78 - 9.11)	(0.00 - 1.25)	(0.00 - 1.96)
Salmon	**395.6 ± 38.13^{cd}**	**62.1 ± 9.28^{e}**	**175.2 ± 21.51^{b}**	**13.8 ± 0.87^{e}**	**0.04 ± 0.02^{e}**	**0.37 ± 0.24^{d}**	**1.94 ± 0.06^{b}**	**3.90 ± 0.53^{a}**	**3.44 ± 1.82^{cdef}**	**0.07 ± 0.02^{f}**	**0.44 ± 0.34^{de}**
	(284.64 - 456.09)	(46.26 - 85.98)	(133.45 - 222.39)	(11.92 - 15.62)	(0.00 - 0.09)	(0.00 - 1.03)	(1.80 - 2.08)	(3.09 - 5.42)	(1.26 - 8.89)	(0.00 - 0.11)	(0.00 - 1.44)
Trout	**357.8 ± 57.32^{cd}**	**71.1 ± 17.14^{cdef}**	**172.1 ± 21.49^{b}**	**12.5 ± 1.84^{e}**	**0.70 ± 0.48^{abcde}**	**0.33 ± 0.24^{d}**	**1.50 ± 0.15^{cde}**	**0.54 ± 0.15^{ef}**	**2.03 ± 0.12^{ef}**	**1.40 ± 0.48^{cde}**	**0.99 ± 0.29^{bcd}**
	(264.87 - 577.83)	(33.83 - 128.54)	(142.13 - 254.20)	(9.75 - 19.61)	(0.00 - 2.59)	(0.00 - 1.24)	(0.91 - 1.77)	(0.34 - 1.14)	(1.70 - 2.39)	(0.53 - 3.17)	(0.18 - 1.86)
Whiteleg shrimp	**452.3 ± 30.33^{bc}**	**78.8 ± 8.43^{cde}**	**158.7 ± 25.69^{b}**	**44.9 ± 4.51^{a}**	**0.65 ± 0.22^{abcd}**	**0.26 ± 0.15^{d}**	**0.97 ± 0.19^{fg}**	**1.01 ± 0.46^{bcdef}**	**2.40 ± 0.48^{def}**	**1.47 ± 0.46^{cd}**	**1.05 ± 0.39^{bcd}**
	(306.32 - 662.19)	(51.94 - 148.96)	(45.49 - 290.74)	(29.65 - 78.36)	(0.00 - 2.15)	(0.00 - 1.29)	(0.00 - 2.04)	(0.00 - 4.77)	(0.34 - 4.92)	(0.00 - 5.21)	(0.00 - 4.08)
Freshwater fish	**685.6 ± 132.73^{b}**	**118.3 ± 25.62^{bcd}**	**166.1 ± 43.85^{bc}**	**42.0 ± 8.55^{ab}**	**1.38 ± 0.32^{a}**	**1.47 ± 0.37^{bc}**	**1.49 ± 0.33^{bcdef}**	**1.34 ± 0.23^{bcd}**	**3.26 ± 0.67^{cde}**	**3.40 ± 0.57^{b}**	**1.13 ± 0.34^{bcd}**
	(242.17 - 1611.8)	(52.59 - 307.22)	(52.83 - 584.97)	(6.67 - 109.83)	(0.00 - 3.07)	(0.00 - 4.83)	(0.13 - 3.32)	(0.50 - 3.06)	(1.57 - 9.89)	(0.74 - 7.20)	(0.00 - 3.35)
Marine fish	**358.5 ± 62.07^{cd}**	**69.1 ± 5.89^{de}**	**154.0 ± 12.58^{b}**	**27.7 ± 14.40^{abcde}**	**0.03 ± 0.03^{e}**	**0.13 ± 0.06^{d}**	**2.02 ± 0.22^{bc}**	**2.85 ± 1.05^{abcd}**	**1.84 ± 0.16^{f}**	**0.93 ± 0.29^{de}**	**0.13 ± 0.12^{e}**
	(240.74 - 451.33)	(60.89 - 80.54)	(129.11 - 169.70)	(12.85 - 56.53)	(0.00 - 0.08)	(0.00 - 0.20)	(1.58 - 2.28)	(0.80 - 4.30)	(1.52 - 2.06)	(0.48 - 1.47)	(0.00 - 0.37)

Values with different letters in a column are different from each other (α=0.05)

Table 5.10 Micre-mineral concentrations in whole bodies for different species. Values represent mean and standard error together with minimum and maximum concentrations.

Cultured specie	Micro-minerals (mg/kg feed)										
	Fe	Mn	Zn	Cu	As	Pb	Cd	Co	Cr	Mo	Se
Channel	**68.4 ± 81.06^{a}**	**4.0 ± 2.98^{b}**	**11.9 ± 16.77^{c}**	**0.2 ± 3.62^{c}**	ND	ND	**28.85 ± 1.918**	ND	ND	ND	ND
	(67.76 - 69.10)	(3.70 - 4.21)	(8.91 - 15.50)	(0.15 - 0.16)			(20.97 - 37.22)				
Tilapia	**155.6 ± 70.20^{a}**	**5.1 ± 2.58^{b}**	**63.4 ± 14.52^{b}**	**2.1 ± 3.13^{c}**	ND	ND	ND	**0.25 ± 0.126^{a}**	ND	**9.39 ± 1.668^{a}**	ND
	(33.98 - 514.50)	(0.06 - 20.00)	(58.18 - 77.00)	(0.23 - 7.00)				(0.00 - 1.00)		(5.60 - 13.18)	
Salmon	**20.7 ± 81.06^{a}**	**2.4 ± 2.98^{b}**	**45.2 ± 16.77^{bc}**	**0.6 ± 3.62^{c}**	ND	ND	ND	ND	ND	ND	ND
	(9.83 - 36.57)	(1.73 - 2.95)	(39.03 - 51.32)	(0.56 - 0.72)							
Trout	**58.0 ± 81.06^{a}**	**6.0 ± 2.98^{b}**	**94.9 ± 16.77^{b}**	**5.8 ± 3.62^{c}**	ND	ND	ND	ND	ND	**1.04 ± 1.927^{c}**	ND
	(57.49 - 58.72)	(5.96 - 6.12)	(82.95 - 105.47)	(5.24 - 6.62)						(0.00 - 3.11)	
Whiteleg shrimp	**127.7 ± 70.20^{a}**	**31.7 ± 2.58^{a}**	**148.3 ± 14.52^{a}**	**90.1 ± 3.13^{a}**	**0.55 ± 0.276**	ND	ND	**0.03 ± 0.126^{a}**	**0.23 ± 0.116^{b}**	**7.48 ± 1.668^{ab}**	**0.38 ± 0.190^{a}**
	(50.68 - 347.52)	(27.54 - 33.52)	(66.53 - 191.43)	(72.05 - 97.07)	(0.00 - 2.21)			(0.00 - 0.12)	(0.00 - 0.93)	(0.17 - 13.22)	(0.00 - 1.52)
Black tiger prawn	**307.0 ± 140.41^{a}**	**23.0 ± 5.17^{a}**	**80.0 ± 29.05^{abc}**	**62.0 ± 6.26^{b}**	ND	**1.70±0.00**	ND	**0.20 ± 0.252^{a}**	**10.20 ± 0.233^{a}**	**0.70 ± 3.337^{bc}**	ND
	(307.00 - 307.00)	(23.00 - 23.00)	(80.00 - 80.00)	(62.00 - 62.00)		(1.70 - 1.70)		(0.20 - 0.20)	(10.20 - 10.20)	(0.70 - 0.70)	

Values with different letters in a column are different from each other (α =0.05)

Table 5.11 Typical feed conversion ratios (FCR) and system loads of macro-minerals from feed for production of 1 tonne (t) of five common aquaculture species.

Cultured species	FCR	System loads (kg/t)				
		S	Ca	Mg	K	Na
Channel	**2.0**	**2.43 ±0.30^{a}**	**28.76 ±6.44^{a}**	**5.49 ±0.63^{a}**	**20.19 ±1.20^{a}**	**4.28 ±0.55^{a}**
		(1.73 - 3.67)	(11.49 - 49.60)	(3.41 - 7.26)	(14.70 - 23.32)	(3.05 - 6.75)
Tilapia	**1.7**	**1.87 ±0.17^{ab}**	**25.29 ±4.65^{a}**	**5.67 ±0.52^{a}**	**17.22 ±1.31^{a}**	**6.30 ±1.00^{a}**
		(0.29 - 5.33)	(6.07 - 137.06)	(2.04 - 13.84)	(8.98 - 42.78)	(1.03 - 21.17)
Salmon	**1.1**	**0.96 ± 0.09^{c}**	**20.62 ±3.26^{a}**	**1.60 ±0.21^{c}**	**5.64 ±0.60^{c}**	**4.18 ±0.96^{ab}**
		(0.81 - 1.18)	(13.87 - 29.13)	(1.23 - 2.18)	(4.43 - 7.06)	(2.79 - 7.01)
Trout	**1.2**	**1.33 ±0.22^{bc}**	**15.89 ±2.75^{a}**	**1.54 ±0.23^{c}**	**6.54 ±0.92^{c}**	**2.65 ±0.27^{b}**
		(0.91 - 2.14)	(11.47 - 26.30)	(1.19 - 2.45)	(5.15 - 10.09)	(1.82 - 3.35)
Whiteleg shrimp	**1.5**	**1.26 ±0.21^{c}**	**15.06 ±3.28^{a}**	**3.47 ±0.23^{b}**	**13.91 ±0.45^{b}**	**5.16 ±1.58^{ab}**
		(0.38 - 2.59)	(3.37 - 31.59)	(2.60 - 4.83)	(11.48 - 16.07)	(1.16 - 12.44)

Values with different letters in a column are different from each other (α =0.05)

Table 5.12 System loads of micro-minerals from feed for production of 1 tonne (t) of five common aquaculture species.

Cultured species	System loads (g/t)										
	Fe	Mn	Zn	Cu	As	Pb	Cd	Co	Cr	Mo	Se
Channel	**571.1 ± 93.18^{ab}**	**128.3 ± 14.74^{ab}**	**239.3 ± 41.19^{a}**	**25.4 ± 1.38^{a}**	**1.59 ± 0.693^{a}**	**0.43 ± 0.204^{b}**	**0.00**	**1.52 ± 0.343^{b}**	**3.33 ± 0.321^{b}**	**4.69 ± 1.130^{a}**	**1.47 ± 0.767^{abc}**
	(295.8 - 979.2)	(75.7 - 184.0)	(171.1 - 440.3)	(20.7 - 30.6)	(0.0 - 3.5)	(0.0 - 1.2)		(0.5 - 2.4)	(2.6 - 4.4)	(2.2 - 9.5)	(0.0 - 5.1)
Tilapia	**879.2 ± 211.84^{a}**	**161.7 ± 14.17^{a}**	**227.8 ± 31.94^{a}**	**24.2 ± 2.95^{a}**	**0.84 ± 0.141^{a}**	**2.01 ± 0.366^{a}**	**1.86 ± 0.195^{ab}**	**1.33 ± 0.174^{b}**	**6.50 ± 0.854^{a}**	**2.22 ± 0.467^{ab}**	**2.68 ± 0.390^{a}**
	(228.2 - 6909.6)	(59.0 - 440.4)	(64.4 - 875.6)	(12.0 - 84.6)	(0.0 - 2.9)	(0.0 - 8.5)	(0.0 - 4.8)	(0.3 - 5.5)	(2.0 - 26.5)	(0.0 - 6.7)	(0.0 - 8.0)
Salmon	**410.6 ± 40.73^{b}**	**64.6 ± 9.54^{d}**	**169.4 ± 22.05^{a}**	**14.3 ± 0.89^{b}**	**0.04 ± 0.023^{b}**	**0.39 ± 0.255^{b}**	**2.05 ± 0.056^{a}**	**4.11 ± 0.544^{a}**	**3.64 ± 1.929^{abc}**	**0.08 ± 0.028^{c}**	**0.46 ± 0.357^{c}**
	(292.8 - 475.8)	(48.1 - 89.1)	(126.0 - 217.2)	(12.4 - 16.2)	(0.0 - 0.1)	(0.0 - 1.1)	(1.9 - 2.2)	(3.3 - 5.7)	(1.3 - 9.4)	(0.0 - 0.1)	(0.0 - 1.5)
Trout	**383.6 ± 64.16^{b}**	**77.3 ± 19.01^{cd}**	**169.1 ± 24.62^{a}**	**12.5 ± 2.10^{b}**	**0.78 ± 0.542^{ab}**	**0.36 ± 0.261^{b}**	**1.67 ± 0.175^{b}**	**0.60 ± 0.169^{c}**	**2.25 ± 0.136^{c}**	**1.31 ± 0.540^{b}**	**1.11 ± 0.321^{bc}**
	(287.8 - 633.4)	(37.1 - 142.5)	(133.0 - 262.3)	(9.2 - 20.6)	(0.0 - 2.9)	(0.0 - 1.3)	(1.0 - 2.0)	(0.4 - 1.3)	(1.8 - 2.7)	(0.4 - 3.3)	(0.2 - 2.1)
Whiteleg shrimp	**578.9 ± 41.13^{a}**	**98.5 ± 11.91^{bc}**	**175.5 ± 35.26^{a}**	**36.2 ± 6.05^{a}**	**0.97 ± 0.332^{a}**	**0.35 ± 0.203^{b}**	**1.32 ± 0.265^{b}**	**1.71 ± 0.729^{bc}**	**3.20 ± 0.656^{bc}**	**1.88 ± 1.644^{abc}**	**1.92 ± 0.617^{ab}**
	(381.3 - 857.2)	(61.6 - 198.2)	(20.2 - 361.3)	(16.5 - 81.3)	(0.0 - 2.8)	(0.0 - 1.7)	(0.0 - 2.8)	(0.3 - 6.4)	(0.4 - 6.6)	(0.1 - 5.2)	(0.7 - 5.4)

Values with different letters in a column are different from each other (α =0.05)

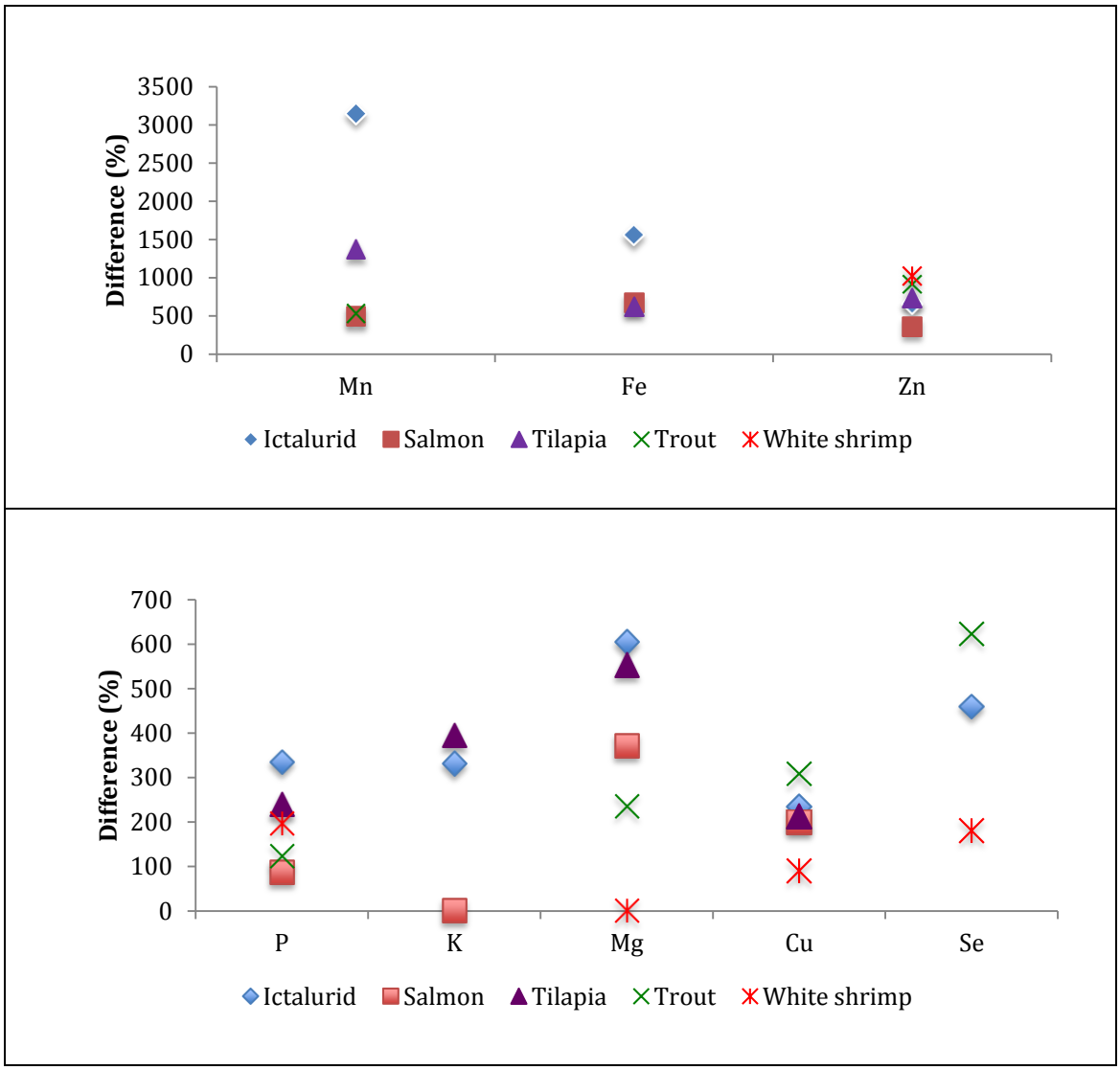

Figure 5.1 The difference between mineral concentrations in different feeds and their required levels in diets for cultured species (NRC, 2011).

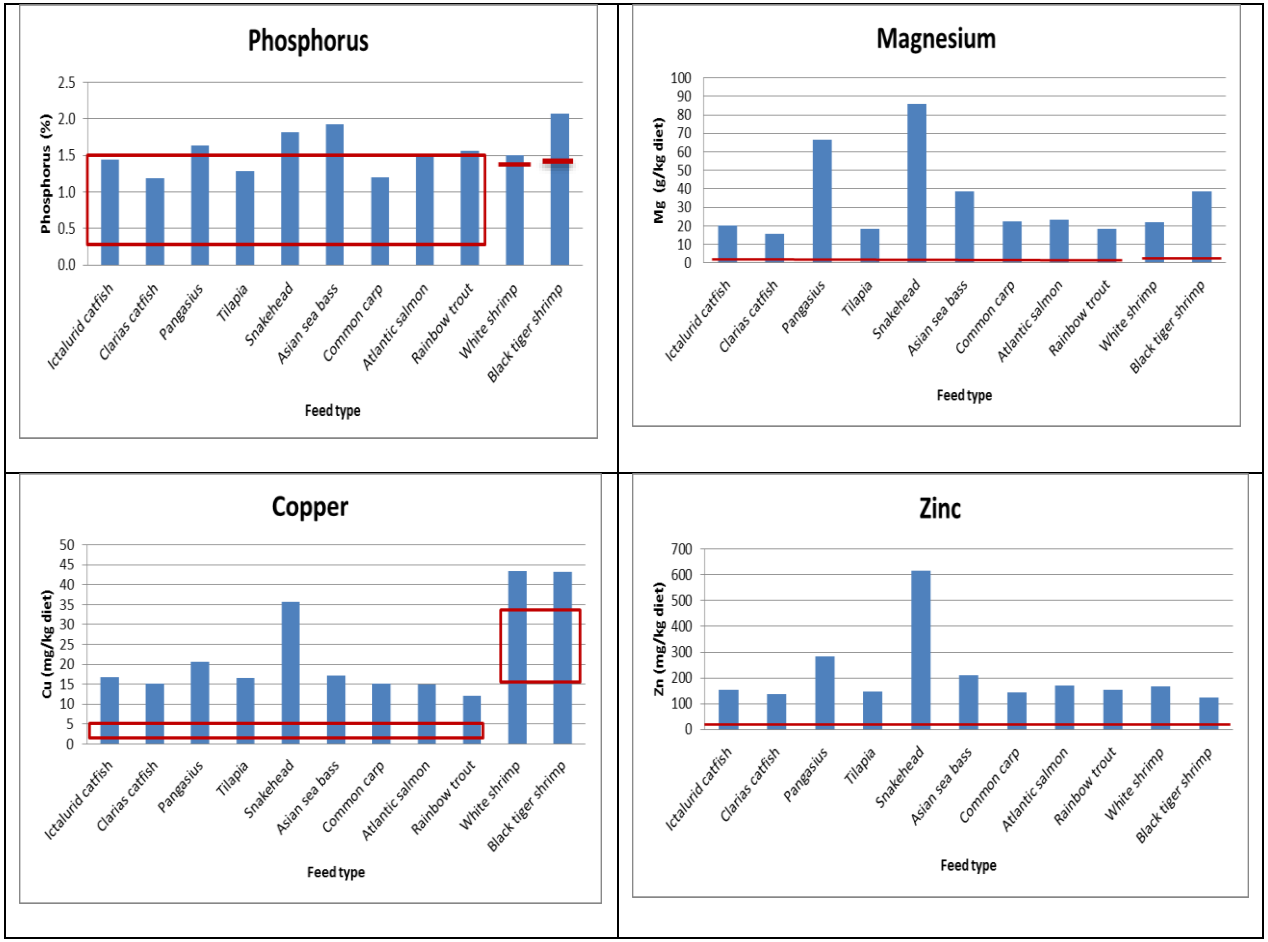

Figure 5.2 Mean phosphorus, magnesium, copper and zinc concentrations in different feeds and their required levels in diets for cultured species.

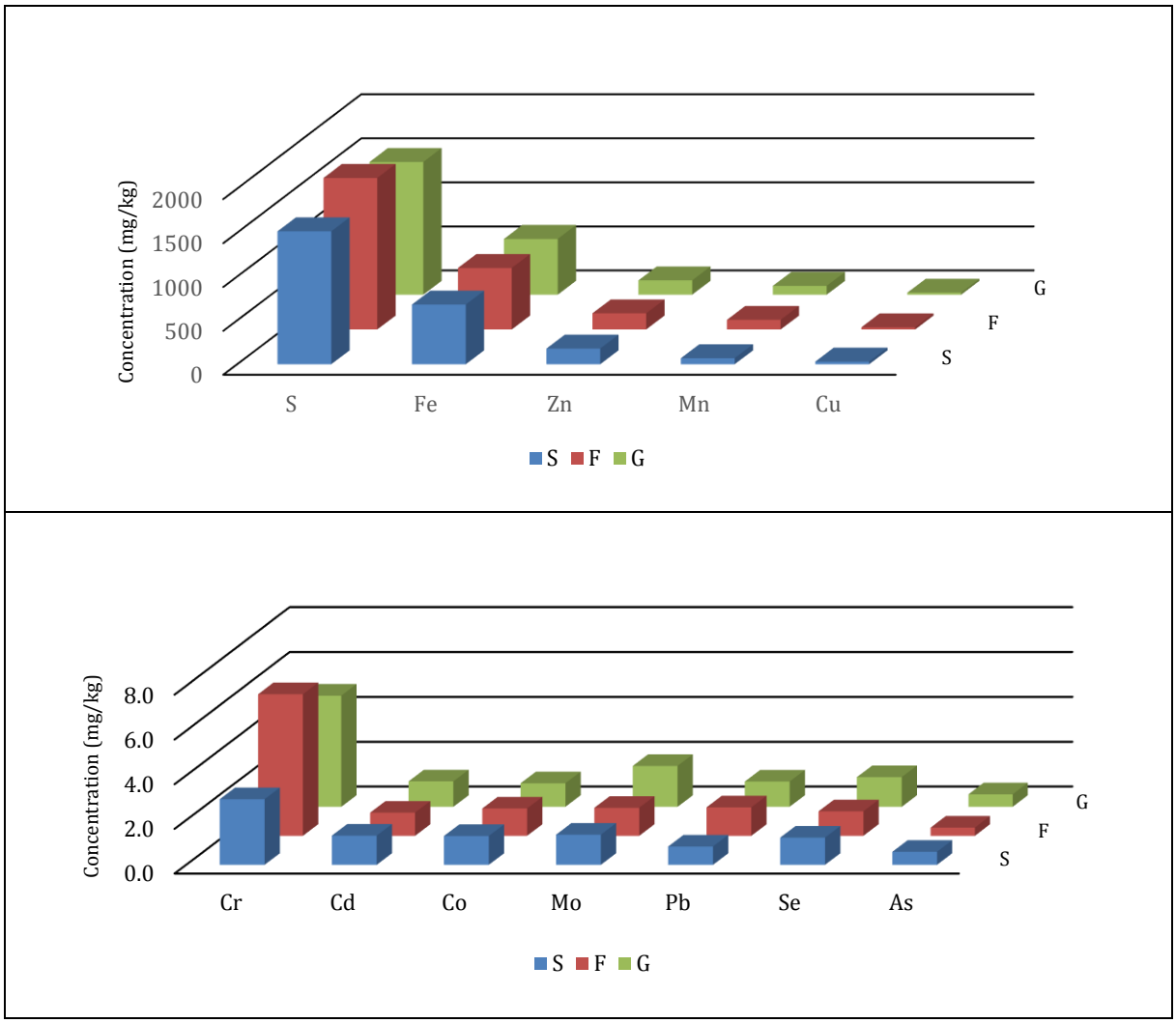

Figure 5.3 Concentrations of mineral elements for particular stage of growth in aquaculture feeds (S = starter, F = Fingerling, G = Grower).

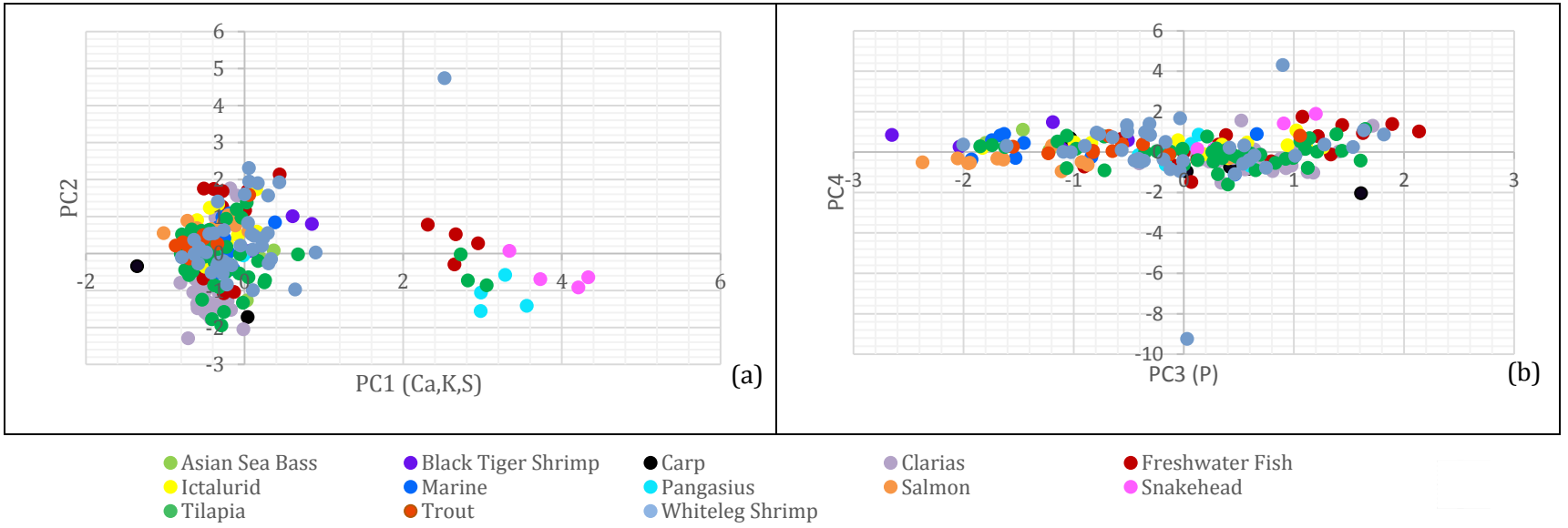

Figure 5.4 Principle component score plots for significant principal components between PC1 vs. PC2 (a), PC3 vs. PC4 (b) of grower feeds categorized by species.

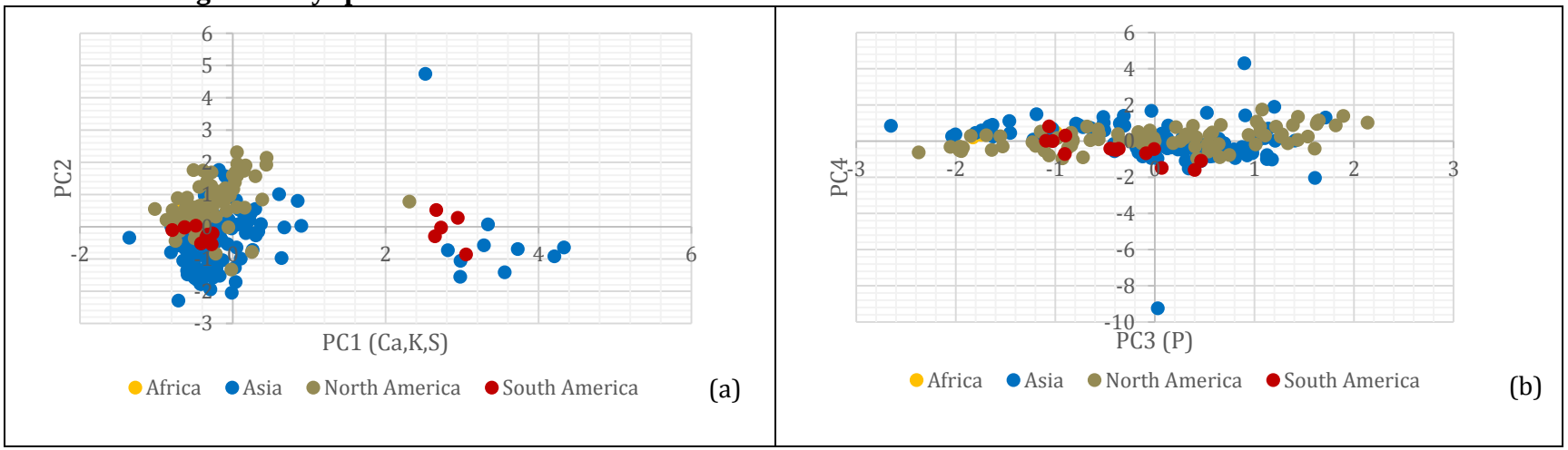

Figure 5.5 Principle component score plots for significant principal components between PC1 vs. PC2 (a), PC3 vs. PC4 (b) of grower feeds categorized by continent.

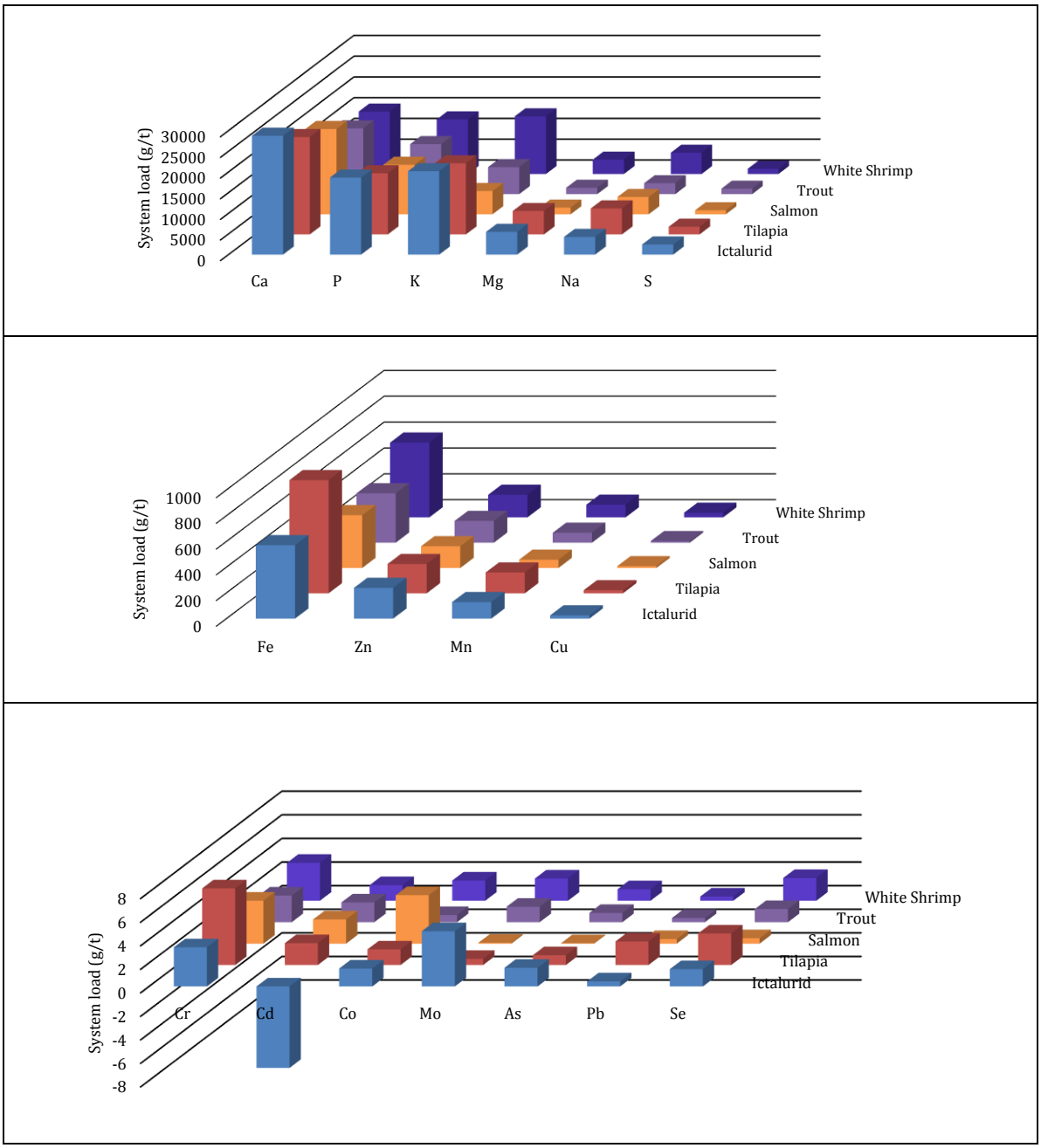

Figure 5.6 System loads of mineral elements from feed for production of 1 tonne of harvest weight.

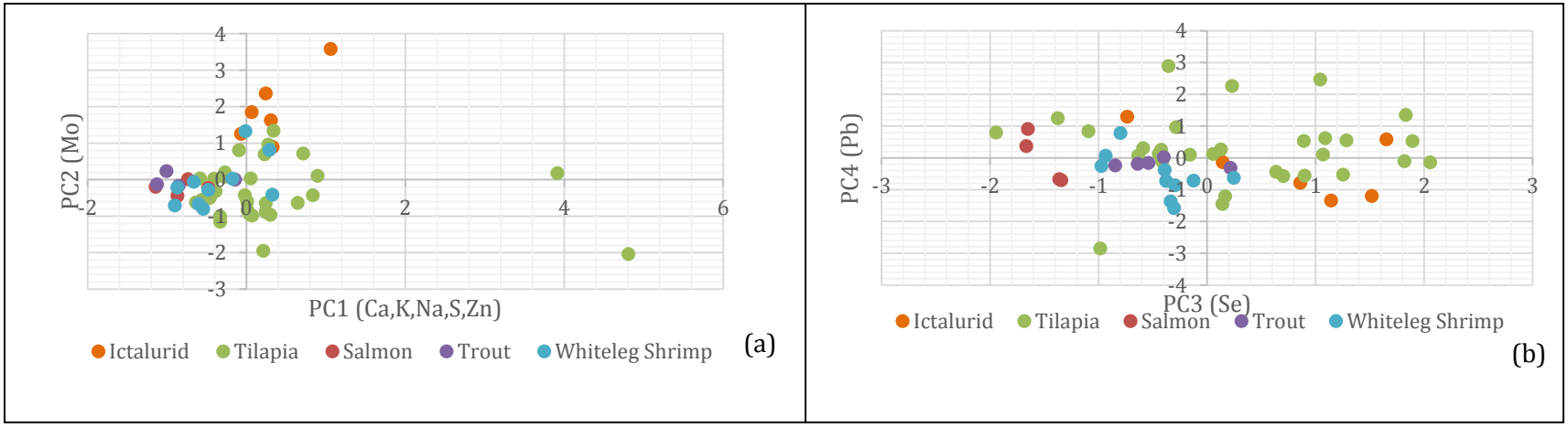

Figure 5.7 Principle component score plots for significant principal components between PC1 vs. PC2 (a), PC3 vs. PC4 (b) of element system loads categorized by species.

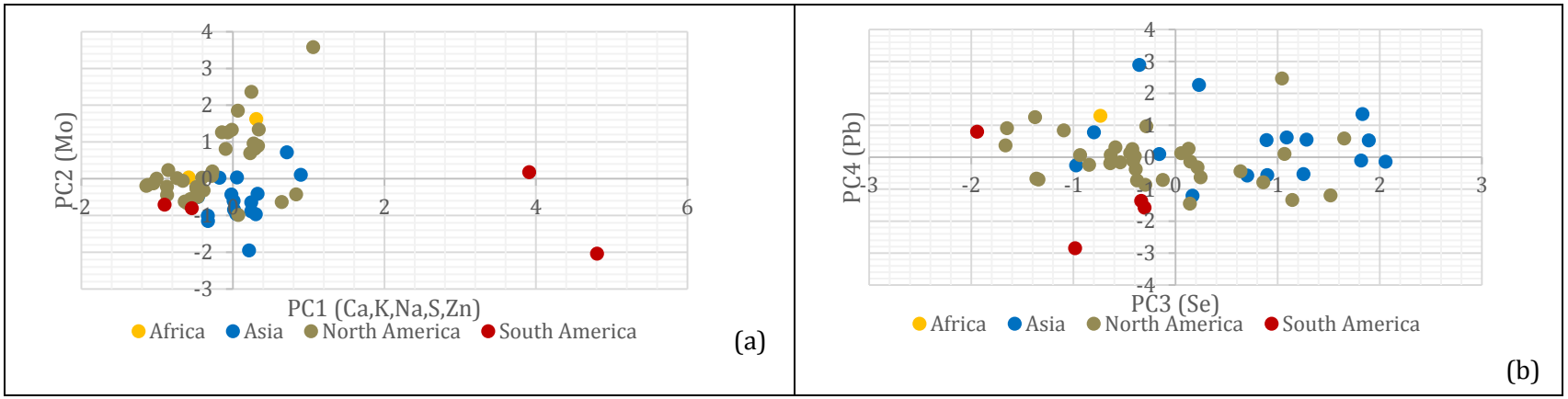

Figure 5.8 Principle component score plots for significant principal components between PC1 vs. PC2 (a), PC3 vs. PC4 (b) of element system loads categorized by continent.

References

Badsha, K., Sainsbury, M., 1978. Aspects of the Biol and heavy metal accumulation of Ciliata mustela. J. Fish Biol. 12, 213-220.

Bishop, F.J., Lall, S.P., Fisheries, C., Res., M.S., Directorate, D., 1977. Studies on Mineral and Protein Utilization by Atlantic salmon (*Salmo Salar*) Grown in Sea Water. Disease and Nutrition Section, Department of Fisheries and Environment.

Bjerregaard, P., Vislie, T., 1986. Effect of copper on ion-and osmoregulation in the shore crab Carcinus maenas. Mar. Biol. 91, 69-76.

Boyd, C.A., Boyd, C.E., Rouse, D.B., 2007a. Potassium budget for inland, saline water shrimp ponds in Alabama. Aquacult. Eng. 36, 45-50.

Boyd, C.A., Boyd, C.E., Rouse, D.B., 2007b. Potassium adsorption by bottom soils in ponds for inland culture of marine shrimp in Alabama. J. world Aquacult. Soc. 38, 85-91.

Boyd, C.E., 2000. Water Quality, an Introduction. Kluwer Academic Publishers, Boston, Massachusetts. pp.330.

Brown, M.L., Jaramillo, F., Gatlin, D.M., 1993. Dietary phosphorus requirement of juvenile sunshine bass, *Morone chrysops*♀× *M. saxatilis*♂. Aquaculture 113, 355-363.

Bryan, G., Langston, W., 1992. Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: a review. Env. Pollut. 76, 89-131.

Cardellicchio, N., Buccolieri, A., Di Leo, A., Giandomenico, S., Spada, L., 2008. Levels of metals in reared mussels from Taranto Gulf (Ionian Sea, Southern Italy). Food Chem. 107, 890-896.

Casado-Martinez, M., Smith, B., Luoma, S., Rainbow, P., 2010. Bioaccumulation of arsenic from water and sediment by a deposit-feeding polychaete (*Arenicola marina*): a biodynamic modelling approach. Aquat. Toxicol. 98, 34-43.

Cheng, Z., Man, Y.B., Nie, X.P., Wong, M.H., 2013. Trophic relationships and health risk assessments of trace metals in the aquaculture pond ecosystem of Pearl River Delta, China. Chemosphere 90, 2142-2148.

Cheng, K.M., Hu, C.Q., Liu, Y.N., Zheng, S.X., Qi, X.J., 2005. Dietary magnesium requirement and physiological responses of Mar. shrimp *Litopenaeus vannamei* reared in low salinity water. Aquacult. Nutr. 11, 385-393.

Cheng, K.M., Hu, C.Q., Liu, Y.N., Zheng, S.X., Qi, X.J., 2006. Effects of dietary calcium, phosphorus and calcium/phosphorus ratio on the growth and tissue mineralization of *Litopenaeus vannamei* reared in low-salinity water. Aquaculture 251, 472-483.

Cowey, C.B., Sargent, J.R., 1979. Nutrition. In: Hoar, W., Randell, D., (Eds.), Fish physiology, Vol. VIII. Academic Press, London, pp. 1-69.

Dall, W., Moriarty, D., 1983. Functional aspects of nutrition and digestion. Biol Crustacea. 5, 215-261.

Dalman, O., Demirak, A., Balcı, A., 2006. Determination of heavy metals (Cd, Pb) and trace elements (Cu, Zn) in sediments and fish of the Southeastern Aegean Sea (Turkey) by atomic absorption spectrometry. Food Chem. 95, 157-162.

Davis, D.A., Lawrence, A.L., Gatlin, D.M., 1993. Response of *Penaeus vannamei* to dietary calcium, phosphorus and calcium: phosphorus ratio. J. World Aquacult. Soc. 24, 504-504.

Davis, D.A., Lawrence, A.L., Gatlin III, D., 1993. Dietary copper requirement of *Penaeus vannamei*. Nippon Suisan Gakk. 59, 117-122.

Deshimaru, O., Yone, Y., 1978. Requirement of prawn for dietary minerals. B. Jpn. Soc. Sci. Fish. 44, 907-910.

Desjardins, L., Hicks, B., Hilton, J., 1987. Iron catalyzed oxidation of trout diets and its effect on the growth and physiological response of rainbow trout. Fish Physiol. Biochem. 3, 173-182.

El-Mowafi, A., Maage, A., 1998. Magnesium requirement of Atlantic salmon (*Salmo salar L.*) parr in seawater-treated fresh water. Aquacult. Nutr. 4, 31-38.

Eya, J.C., Lovell, R.T., 1997. Available phosphorus requirements of food-size channel catfish (*Ictalurus punctatus*) fed practical diets in ponds. Aquaculture 154, 283-291.

FAO, 1983. Compilation of legal limits for hazardous substance in fish and fishery products (Food and Agricultural Organization). FAO Fishery circular, No. 464, pp. 5–100.

Farmer, G., Ashfield, D., Samant, H., 1979. Effects of zinc on juvenile Atlantic salmon *Salmo*

salar: acute toxicity, food intake, growth and bioaccumulation. Env. Pollut. (1970). 19, 103-117.

Felton, S., Landolt, M., Grace, R., Palmisano, A., 1996. Effects of selenium dietary enhancement on hatchery- reared coho salmon, *Oncorhynchus kisutch* (Walbaum), when compared with wild coho: hepatic enzymes and seawater adaptation evaluated. Aquacult. Res. 27, 135-142.

Fernandes, C., Fontainhas-Fernandes, A., Peixoto, F., Salgado, M.A., 2007. Bioaccumulation of heavy metals in Liza saliens from the Esmoriz–Paramos coastal lagoon, Portugal. Ecotox. Env. Safe. 66, 426-431.

Fernandes, D., Zanuy, S., Bebianno, M.J., Porte, C., 2008. Chemical and biochemical tools to assess Pollut. exposure in cultured fish. Env. Pollut. 152, 138-146.

Gatlin, D.M., Wilson, R.P., 1984. Studies on the manganese requirement of fingerling channel catfish. Aquaculture 41, 85-92.

Gatlin, D.M., Wilson, R.P., 1986. Dietary copper requirement of fingerling channel catfish. Aquaculture 54, 277-285.

Gatlin, D.M., Wilson, R.P., 1986. Characterization of iron deficiency and the dietary iron requirement of fingerling channel catfish. Aquaculture 52, 191-198.

Gatlin, D.M., 2000. Minerals, In: Stickney, R.R. (Ed.), Encyclopedia of aquaculture. John Wiley and Sons, Inc., New York, New York, USA.

Gatlin, D.M., Phillips, H.F., 1989. Dietary calcium, phytate and zinc interactions in channel catfish. Aquaculture 79, 259-266.

Gatlin, D.M., O'Connell, J.P., Scarpa, J., 1991. Dietary zinc requirement of the red drum, *Sciaenops ocellatus*. Aquaculture 92, 259-265.

Gatlin D.M., Wilson, R.P., 1983. Dietary zinc requirement of fingerling channel catfish. J. Nutr. 113, 630-635.

Gatlin D.M., Wilson, R.P., 1984. Dietary selenium requirement of fingerling channel catfish. J. Nutr. 114, 627-633.

Hardy, R.W., Shearer, K.D., 1985. Effect of dietary calcium phosphate and zinc supplementation on whole body zinc concentration of rainbow trout (Salmo gairdneri). Canadian J. Fish.

Aquat. Sci. 42, 181-184.

Hardy, R.W., Oram, L.L., Möller, G., 2010. Effects of dietary selenomethionine on cutthroat trout (Oncorhynchus clarki bouvieri) growth and reproductive performance over a life cycle. Archives of Env. Contamination Toxicol. 58, 237-245.

Hilton, J., Hodson, P., Slinger, S., 1980. The requirement and toxicity of selenium in rainbow trout (*Salmo gairdneri*). J. Nutr. 110, 2527-2535.

Hossain, M.A., Furuichi, M., 1999. Necessity of dietary calcium supplement in black sea bream. Fish Sci. 65, 893-897.

Hossain, M., Furuichi, M., 2000. Essentiality of dietary calcium supplement in redlip mullet *Liza haematocheila*. Aquacult. Nutr. 6, 33-38.

Hossain, M.A., Furuichi, M., 2000. Necessity of calcium supplement to the diet of Japanese flounder. Fish Sci. 66, 660-664.

Hossain, M.A., Furuichi, M., 2000. Essentiality of dietary calcium supplement in fingerling scorpion fish (*Sebastiscus marmoratus*). Aquaculture 189, 155-163.

Ikem, A., Egilla, J., 2008. Trace element content of fish feed and bluegill sunfish (*Lepomis macrochirus*) from aquaculture and wild source in Missouri. Food Chem. 110, 301-309.

Ip, C., Li, X., Zhang, G., Wong, C., Zhang, W., 2005. Heavy metal and Pb isotopic compositions of Aquatic organisms in the Pearl River Estuary, South China. Env. Pollut. 138, 494-504.

Jeng, S.S., Sun, L.T., 1981. Effects of dietary zinc levels on zinc concentrations in tissues of common carp. J. Nutr. 111, 134-140.

Ketola, H., 1975. Requirement of Atlantic salmon for dietary phosphorus. Transactions of the Amer. Fish. Soc. 104, 548-551.

Khan, A., Ali, M., Biaswas, S., Hadi, D., 1987. Trace elements in Mar. fish from the Bay of Bengal. Sci. Total Environ. 61, 121-130.

Kirkpatrick, D., Coffin, D., 1974. The trace metal content of representative Canadian diets in 1970 and 1971. Can. Inst. Food Sci. Tech. J. 7, 56-58.

Lee, M.H., Shiau, S.-Y., 2002. Dietary copper requirement of juvenile grass shrimp, *Penaeus*

monodon, and effects on non-specific immune responses. Fish shellfish Immunol. 13, 259-270.

Lin, Y.H., Shie, Y.Y., Shiau, S.Y., 2008. Dietary copper requirements of juvenile grouper, *Epinephelus malabaricus*. Aquaculture 274, 161-165.

Lin, Y.H., Lin, S.M., Shiau, S.Y., 2008. Dietary manganese requirements of juvenile tilapia, *Oreochromis niloticus* × *O. aureus*. Aquaculture 284, 207-210.

Lorentzen, M., Maage, A., Julshamn, K., 1998. Supplementing copper to a fish meal based diet fed to Atlantic salmon parr affects liver copper and selenium concentrations. Aquacult. Nutr. 4, 67.

McClain, W., Gatlin, D.M., 1988. Dietary zinc requirement of *Oreochromis aureus* and effects of dietary calcium and phytate on zinc bioavailability. J. World Aquacult. Soc. 19, 103-108.

McNevin, A., Boyd, C.E., Silapajarn, O., Silaprajarn, K., 2004. Ionic supplementation of pond waters for inland culture of marine shrimp. J. World Aquacult. Soc. 35, 460-467.

Merian, E., 1991. Metals and their compounds in the environment: occurrence, analysis and biological relevance. VCH Verlagsgesellschaft mbH.

Murai, T., Andrews, J.W., Smith, R.G., 1981. Effects of dietary copper on channel catfish. Aquaculture 22, 353-357.

Murray, M.W., Andrews, J.W., 1979. Channel catfish: the absence of an effect of dietary salt on growth. Prog. Fish Cult. 41, 155-156.

Nakamura, Y., 1982. Effects of dietary phosphorus and calcium contents on the absorption of phosphorus in the digestive tract of carp. Bull. Jpn. Soc. Sci. Fish. 48, 409–413.

Ogino, C., Yang, G., 1980. Requirements of carp and rainbow trout for dietary manganese and copper. B. Jpn. Soc. of Sci Fish. 46, 455-458.

Paez-Osuna, F., Ruiz-Fernandez, C., 1995. Trace metals in the Mexican shrimp *Penaeus vannamei* from estuarine and Mar. environments. Env. Pollut. 87, 243-247.

Pan, Q., Chen, X.Y., Li, F., Bi, Y.Z., Zheng, S.X., 2005. Response of juvenile *Litopenaeus vannamei* to varying levels of calcium phosphate monobasic supplemented to a practical diet. Aquaculture 248, 97-102.

Pan, L., Zhu, X., Xie, S., Lei, W., Han, D., Yang, Y., 2008. Effects of dietary manganese on growth and tissue manganese concentrations of juvenile gibel carp, *Carassius auratus gibelio*. Aquacult. Nutr. 14, 459-463.

Pine, H.J., Boyd, C.E., 2010. Adsorption of magnesium by bottom soils in inland breackishwater shrimp ponds in Alabama. J. World Aquacult. Soc. 41, 603-609.

Robinson, E.H., LaBomascus, D., Brown, P.B., Linton, T.L., 1987. Dietary calcium and phosphorus requirements of Oreochromis aureus reared in calcium-free water. Aquaculture 64, 267-276.

Robinson, E.H., Rawles, S.D., Brown, P.B., Yette, H.E., Greene, L.W., 1986. Dietary calcium requirement of channel catfish *Ictalurus punctatus*, reared in calcium-free water. Aquaculture 53, 263-270.

Rodehutscord, M., Pfeffer, E., 1995. Requirement for phosphorus in rainbow trout (*Oncorhynchus mykiss*) growing from 50 to 200 g. Water Sci. Technol. 31, 137-141.

Roy, P.K., Lall, S.P., 2003. Dietary phosphorus requirement of juvenile haddock (*Melanogrammus aeglefinus L.*). Aquaculture 221, 451-468.

Roy, L., Davis, D., Saoud, I., Henry, R., 2007. Supplementation of potassium, magnesium and sodium chloride in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*, reared in low salinity waters. Aquacult. Nutr. 13, 104-113.

Sakamoto, S., Yone, Y., 1978. Iron deficiency symptoms of carp. B. Jpn. Soc. Sci. Fish. 44, 1157-1160.

Salman, N., Eddy, F., 1988. Effect of dietary sodium chloride on growth, food intake and conversion efficiency in rainbow trout (*Salmo gairdneri Richardson*). Aquaculture 70, 131-144.

Scarpa, J., Gatlin, D.M., 1992. Dietary zinc requirements of channel catfish, *Ictalurus punctatus*, swim-up fry in soft and hard water. Aquaculture 106, 311-322.

Shaw, H., Saunders, R., Hall, H., Henderson, E., 1975. Effect of dietary sodium chloride on growth of Atlantic salmon (*Salmo salar*). J. Fish. Board Can. 32, 1813-1819.

Shearer, K.D., 1989. Whole body magnesium concentration as an indicator of magnesium status

in rainbow trout (*Salmo gairdneri*). Aquaculture 77, 201-210.

Shiau, S.Y., Hsieh, J.F., 2001. Quantifying the dietary potassium requirement of juvenile hybrid tilapia (*Oreochromis niloticus*× *O. aureus*). Brit. J. Nutr. 85, 213-218.

Shiau, S.Y., Hsieh, J.F., 2001. Dietary potassium requirement of juvenile grass shrimp *Penaeus monodon*. Fish Sci. 67, 592-595.

Shiau, S.Y., Lu, L.S., 2004. Dietary sodium requirement determined for juvenile hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) reared in fresh water and seawater. Brit. J. Nutr. 91, 585-590.

Shiau, S.Y., Jiang, L.C., 2006. Dietary zinc requirements of grass shrimp, Penaeus monodon, and effects on immune responses. Aquaculture 254, 476-482.

Storelli, M., Barone, G., Garofalo, R., Marcotrigiano, G., 2007. Metals and organochlorine compounds in eel (*Anguilla anguilla*) from the Lesina lagoon, Adriatic Sea (Italy). Food Chem. 100, 1337-1341.

Sutton, J., Balfry, S., Higgs, D., Huang, C.H., Skura, B., 2006. Impact of iron-catalyzed dietary lipid peroxidation on growth performance, general health and flesh proximate and fatty acid composition of Atlantic salmon (*Salmo salar L.*) reared in seawater. Aquaculture 257, 534-557.

Tacon, A.G., 1992. Nutritional fish pathology: morphological signs of nutrient deficiency and toxicity in farmed fish. Food & Agriculture Org.

Tacon, A.G.J., De Silva, S., 1983. Mineral composition of some commercial fish feeds available in Europe. Aquaculture 31, 11-20.

Tashjian, D.H., Teh, S.J., Sogomonyan, A., Hung, S.S., 2006. Bioaccumulation and chronic toxicity of dietary l-selenomethionine in juvenile white sturgeon (*Acipenser transmontanus*). Aquat. Toxicol. 79, 401-409.

United Stater Environmental Protection Agency (USEPA), 1986. Quality criteria for water. Office of water regulations and standards, Washington, DC.

Vielma, J., Lall, S.P., 1998. Phosphorus utilization by Atlantic salmon (*Salmo salar*) reared in freshwater is not influenced by higher dietary calcium intake. Aquaculture 160, 117-128.

Vielma, J., Lall, S., 1998. Control of phosphorus homeostasis of Atlantic salmon (*Salmo salar*) in fresh water. Fish Physiol. BioChem. 19, 83-93.

Wang, W.N., Wang, A.-L., Zhang, Y.-J., 2006. Effect of dietary higher level of selenium and nitrite concentration on the cellular defense response of *Penaeus vannamei*. Aquaculture 256, 558-563.

Wei, T., Yang, W., Lai, Z., Zhang, Q., Liu, M., 2001. Residues of heavy metals in economic Aquatic animal muscles in Pearl River estuary, south China. J. Fish. Sci. China 9, 172-176.

Wilson, R.P., Robinson, E.H., Gatlin 3rd, D., Poe, W.E., 1982. Dietary phosphorus requirement of channel catfish. J. Nutr. 112, 1197-1202.

Yang, R., Zhang, S., Wang, Z., 2014. Bioaccumulation and regional distribution of trace metals in fish of the Tibetan Plateau. Env. Geochem. Health 36, 183-191.

Yap, C.K., Ismail, A., Tan, S.G., 2004. Heavy metal (Cd, Cu, Pb and Zn) concentrations in the green-lipped mussel *Perna viridis* (Linnaeus) collected from some wild and aquacultural sites in the west coast of Peninsular Malaysia. Food Chem. 84, 569-575.

Zhou, H., Wong, M., 2000. Mercury accumulation in freshwater fish with emphasis on the dietary influence. Water Res. 34, 4234-4242.

Chapter 6 The Effect of Carbohydrase and Phytase on Growth Performance and Nutrient Retention of Channel Catfish *Ictalurus punctatus*

6.1 Abstract

A number of plant-derived aquatic feeds contain various forms of nutrients that are not readily digested by aquatic animals due to lack of specific digestive enzymes. Hence, the addition of exogenous enzymes to diets can improve the utilization of nutrients by the animals. This study was designed to determine the effect of carbohydrase and phytase enzymes supplemented to the diet on growth performance and nutrient retention of channel catfish. Two commercially produced feeds containing 28 and 32% protein were utilized to produce six dietary treatments. Each feed was used to develop three treatments control diet, diet supplemented with carbohydrase (0.2 g/kg diet) and diet supplemented with phytase (2000 FTU/kg diet). A total of 360 fish with an average initial body weight of 100 g were randomly stocked into 24 tanks (15 fish per tank) in a recirculating system. Experiments were carried out with four replications for each of the six dietary treatments. Fish from each tank were weighed on a monthly basis and were fed to apparent satiation the assigned diet equivalent to 2.0-4.5% of their body weight, twice daily over a 12 week period. At the end of experiment, mean final weight, weight gain, survival, feed conversion ratio, protein retention, energy retention and phosphorus retentions were determined. Two-way analysis of variance indicated a significant effect of protein but not enzyme supplements ($P<0.05$). The results indicate significantly better growth performance in terms of percent weight gain and feed efficiency, as well as energy and phosphorus retention, in fish fed 32% protein diet as compared

to fish fed the 28% protein diet. However, supplementation of diets with phytase improved phosphorus retention and ash content in the fish. Greatest phosphorus retention was found in fish fed with phytase supplemented 32% protein diet treatments. Greatest ash content was found in fish fed with phytase supplemented 28% protein diet treatments. Supplementing carbohydrase or phytase into diets did not result in significant ($P>0.05$) improvement in the growth performance of fish. In conclusion, phytase 2,000 IU/ kg can be supplemented in diet in order to improve phosphorus retention in channel catfish.

6.2 Introduction

Due to rising concerns regarding the sustainability of the aquaculture industry, low pollution feeds have been designed to increase feed utilization of the cultured species and minimize nutrient loading (losses) to the environment. Presently, manufacturers have been trying to improve the nutritional value of aquatic feed by enzyme supplementations (Bedford, 2000). Fishmeal has been used as a main ingredient for protein source in aquatic feeds. However, the rising cost of fishmeal and its pollution concern have driven feed manufacturers to search for alternative protein sources (Carter, 1998; Burel et al., 2000). Plant based feeds have shown to be a good replacement for fishmeal in aquatic diets (Mbahinzireki et al., 2001; Thiessen et al., 2003; Ai and Xie, 2005). Nevertheless, plant based feedstuffs also contain some anti-nutritional compounds, which cannot be digested and/or impair the digestion process of aquatic animals (Gomes et al., 1995; Adelizi et al, 1998).

The use of exogenous enzymes in aquatic feeds is advocated as these have shown to improve nutritional value of plant-based feedstuffs. The primary purpose of the addition of these enzymes in feeds is to provide enzymes which are not normally present in the digestive system of the culture animals and improve digestibility of the feeds. These enzymes can also help to eliminate the negative impact of anti-nutritional factors and improve feed utilization, leading to an increase in the growth performance of animals (Lin et al., 2007; Soltan, 2009).

Plant feedstuff contains phosphorus in the form of phytate, which cannot be utilized by aquatic animals due to lack of phytase enzyme (Nelson, 1967). About 70% of total phosphorus in plant feedstuff is present in the form of phytate and is thus not available to aquatic species (Riche and Brown, 1996). The enzyme phytase can help make this unavailable form of phosphorus available to the aquatic animal as it can hydrolyze phytate to a more digestible form (Rodehutscord

and Pfeffer, 1995). The addition of phytase in feeds has been shown to improve the availability of phosphorus as it can increase phosphorus digestibility and phosphorus retention in cultured species, and thus also minimize phosphorus loading to the environment (Bureau, 2014). Moreover, phytate can bind with amino acid and form phytate-amino acid complex, which can reduce availability and digestibility of some amino acid, resulting in reduced growth and feed efficiency in cultured animals. It has been reported that supplementing phytase into the diets can increase the availability of proteins or amino acid in aquatic animals (Spinelli et al., 1983). In addition, phytate also has negative impact on availability of other minerals because it can chelate minerals such as calcium, magnesium and zinc in the diets (Gatlin and Phillips, 1989). Supplementation of phytase can reduce the level of phytate in feeds and consequently increase the availability of other minerals.

Another important anti-nutritional factor which can be eliminated by enzyme supplementation is non-starch polysaccharides (NSPs). NSPs are present in the plant material and are known to deteriorate the growth performance of animals once administered in the form of feeds (Bakker et al., 1998; Simon, 1998). In general, NSPs are a complex group that are comprised mainly of linked monomers by β-glycosidic bonds (Novus, 1992; van Barneveld, 1999). This has a negative effect on digestibility, since different enzymes are required to hydrolyze these bonds (Smith 1989). The anti-nutritive effects of NSPs are mainly due to an increase in viscosity of the digesta in the intestine of the animal which effectively slows down the rate of digestion. Elevated digesta viscosity in the digestive tract also causes the enclosure of nutrients making them unavailable to digestion (Choct, 1997). Since the aquatic animals lack of the intestinal enzymes for degradation of NSPs, the supplementation of exogenous enzymes in the diets is necessary. Carbohydrases have their ability to break down NSPs in carbohydrates; therefore, they can

decrease digesta viscosity and increase the amount of energy for animals to utilize (Choct, 1997; Simon, 1998).

The benefits achieved from enzyme supplementation are known to depend on a number of factors such as the raw ingredients used, the source of the enzymes, specie and age of the animals and dietary content of other nutrients in the feeds. One of the drawbacks of supplementing feedstuff with exogenous enzyme is that some enzymes are very sensitive to feed processing conditions such as temperature and pH and thus requires extra care while addition. For example, the activity of phytase decreases considerably at the improper pH environment. Phytase is considered heat labile and its activity reduces at high temperatures. Phytase cannot tolerate high temperatures associated with pre-conditioning, extruding or drying processes. Hence, phytase is usually added after pelleting by spraying onto pellets to avoid this problem. The purpose of the present study was to evaluate the effects of supplementing exogenous enzymes i.e., carbohydrase and phytase on the growth performance and nutrient retention in channel catfish.

6.3 Material and methods

6.3.1 Experimental diets

Two commercial catfish diets containing 28 and 32% protein content were used as basal diets for this experiment. Each of these diets were supplemented with carbohydrase (0.2 g/kg diet) and with phytase (2000 FTU/kg diet). Enzymes were dissolved in cold water before top coating or mixing on the pellets using mister and cement mixer. The coating and mixing was conducted over a 15 mins time period. In order to ensure stability of the feed after the addition of water, the diets were dried in an oven at a temperature below 50°C until the moisture content was < 10%. Diets

were stored in a freezer at -20°C till needed. Feed samples were collected and proximate analysis was carried out on them as per AOAC (1995) procedures to determine the composition.

6.3.2 Culture method

Catfish juveniles were reared in experimental tanks and acclimated to the control diets. The experiment consisted of six treatments with four replicates per treatment. Catfish (100 g initial weight) were stocked into a series of 650 L indoor tanks at a stocking density of 15 fish per tank in a recirculating system. The first three days after stocking, fish mortalities were observed and re-stocking was performed with reserve fish with similar weight. Tanks were supplied with dechlorinated municipal water maintained at atemperature of around 27°C. Water was continuously aerated, and photoperiod maintained at 14:10 h light:dark schedule. Water temperature and dissolved oxygen were randomly measured twice daily in the morning and afternoon (0800h, 1600h) using a oxygen meter (Yellow Springs Instrument Model 58, Yellow Springs, OH, USA). Water samples were collected weekly to determine the level of total ammonia nitrogen and nitrite. At the conclusion of the three month growth trial, fish were counted and weighed collectively. Observations such as mean final weight, weight gain, feed conversation ratio, and survival were recorded. Four fish from each tank were randomly sampled to determine the whole body composition. The indexes for assessment of fish performance including weight gain, feed conversion ratio and survival were determined using equation 6.1, 6.2 and 6.3, respectively.

$$\textit{Weight gain (g)} = \textit{Average final weight (g)} - \textit{Average initial weight (g)} \quad (6.1)$$

$$\textit{Feed conversion ratio} = \textit{Dry feed fed (g)} / \textit{Weight gain (g)} \quad (6.2)$$

$$\textit{Survival (\%)} = (\textit{Initial fish number} - \textit{Final fish number}) \times 100 \quad (6.3)$$

6.3.3 Feeding Method

Fish in four replicate tanks were randomly assigned to each of the six experimental diets. Feed input was calculated to be between 2.0 to 4.5 % of average fish weight every month. Test diets were applied twice daily at 0800 and 1600 h for a 3 month experimental period. Fish in each tank were counted and weighed collectively on a monthly basis to assess the weight gain and survival, as well as to readjust the feed input. On sampling day, fish were handled early in the morning and then fed once in the afternoon. At the conclusion of the growth trial, fish were counted and weighed collectively. Assessment factors such as mean final weight, weight gain, feed conversation ratio, and survival were determined. Four fish in each tank were euthanized and frozen for subsequent analysis.

6.3.4 Analytical Methods

Experimental diets and whole fish samples were ground using a food-processing grinder (Black and Decker, Maryland, USA). All samples were weighed and dried in an oven (105°C) to a constant weight for the determination of moisture content. The dried samples were then ground again using electric grinder (Black and Decker SmartGrind, China) and pulverized with a porcelain mortar and pestle. Samples were placed in vials, and stored in a desiccator until further analysis for ash, protein, energy, phosphorus content determination.

6.3.5 Moisture and ash contents

Moisture content of both fish and feed samples were determined using equation 6.4, by recording their original weight (wet), placing them in ceramic crucibles, drying them in isothermal oven at 105°C for 8 hours, placing them in desiccators until they were at room temperature and recording their final weight (dry). The samples were then incinerated in a furnace (62700

Barnstead Thermolyne, IOWA, USA) at 500°C for 8 hrs for ash content determination (equation 6.5).

$$Moisture\ content\ (\%) = \frac{Ww - Wd}{Ww} \times 100 \qquad 6.4$$

$$Ash\ content\ (\%) = \frac{Wa}{Wd} \times 100 \qquad 6.5$$

Where:

Ww = wet weight of the sample

Wd = weight of sample after drying

Wa = weight of sample after ashing

6.3.6 Protein Analysis by The Kjeldahl Method

The Kjeldahl method has been widely used as a standard for protein content determination by converting nitrogen to ammonia. As per the standard, all forms of nitrogen are reduced to ammonium sulfate in the presence of sulfuric acid. According to Winkler's method, after the addition of an excess amount of alkali, the ammonium ions are converted to ammonia.

Approximately, 10-15 mg of feed and fish samples were weighed on a piece of cellophane paper and were then placed in a 30 ml digestion flask. In the digestion step, 0.4 g of the catalyst (mix of $CuSO_4$ 20 g and K_2SO_4 128 g), 3.0 ml of H_2SO_4, and perforated boiling bead were added to the flask before placing it on the digestion unit for approximately 2 hours until color of the samples turned green. For distillation step, the prepared samples were poured into the inner chamber and deionized water was used to rinse the digestion flasks. About 10 ml of 40% NaOH was added into the sample cup and slowly dripped into the inner vessel unit until solution color turned brown. Samples were distilled for 5 minutes after they turned brown. Boric acid was used to capture the ammonia in distillation process. The bound boric acid was titrated with 0.02 N HCl. The total

nitrogen in the samples and apparent net protein retention in fish were calculated by using equations 6.6 and 6.7 respectively (Ma and Zuazago, 1942).

$$\%\ Nitrogen = \frac{(S - B)(14.007)(N)}{(g\ of\ sample) \times 1000} \times\ 100 \qquad 6.6$$

Where:

S = HCl to titrate sample (ml)

B = HCl to titrate blank (ml)

N = Normality of HCl

$$\text{ANPR (\%)} = \frac{(\text{P}f\ \text{in fish}\ \times\ \text{FW}\ \times\ \text{DM}f\ \text{of fish}) - (\text{P}i\ \text{in fish}\ \times\ \text{FW}\ \times\ \text{DM}i\ \text{of fish})}{\text{P in feed}\ \times \text{amount of feed consumed}} \qquad 6.7$$

Where: Pf = Protein in final fish

Pi = Protein in initial fish

DMf = Dry matter of final fish (%)

DMi = Dry matter of initial fish (%)

6.3.7 Energy Analysis

A bomb calorimeter (1425 Semimicro Calorimeter, Illinois, USA) was used to determine the energy contents of feed and fish samples. About 10 to 20 mg of sample was compressed to a pellet which was then weighed. The pellet was connected to the ignition wire and placed in a pressurized decomposition chamber/vessel filled with oxygen. The decomposition chamber was placed inside the bomb calorimeter where the samples were combusted completely and energy contents were measured by recording the change in temperature of the surrounding water bath. Apparent net energy retention in fish was calculated by using equation 6.8.

$$\text{ANER (\%)} = \frac{(\text{E}f\ \text{in fish}\ \times\ \text{FW}\ \times\ \text{DM}f\ \text{of fish}) - (\text{E}i\ \text{in fish}\ \times\ \text{FW}\ \times\ \text{DM}i\ \text{of fish})}{\text{E in feed}\ \times \text{amount of feed consumed}} \qquad 6.8$$

Where: Ef = Energy in final fish

E_i = Energy in initial fish

DM_f = Dry matter of final fish (%)

DM_i = Dry matter of initial fish (%)

6.3.8 Phosphorus Analysis

About 2 g of ash samples was digested using an acid solution (1 N HNO_3 mixed with 1 N HCl). 5 ml of acid solution was added into crucibles containing ash. Crucibles were held on a hot plate until nearly dry. The residuals were dissolved by prepared acid solution in a 100-ml volumetric flask. The resulting solutions were filtered through Whatman Number 42, acid-washed filter paper. Phosphorus concentration in cultured species and feed samples were determined by using vanadomolybdophosphoric yellow color method. Digested samples (from trace mineral analysis) were placed into a 50-ml volumetric flask, before addition of 10 ml of vanadomolybdate reagent. The solutions were diluted to a volume of 50 ml using distilled water. Samples were allowed to stand for 10 minutes so that the expected color could develop fully. A blank was prepared to be read with the samples. The colors were read in spectrophotometer at wavelength of 490 μm. Standard phosphorus solution was prepared by dissolving 0.2195 g KH_2PO_4 in 1,000 ml of distilled water. This solution was too concentrated to use directly (1 ml = 50 μg). Second solution containing 5 mg/L PO_4-P were made by diluting 50 ml of the first solution to 500 ml with distilled water. Phosphorus retentions in fish were calculated by using equation 6.9.

$$\text{ANPR (\%)} = \frac{(P_f \text{ in fish} \times \text{FW} \times DM_f \text{ of fish}) - (P_i \text{ in fish} \times \text{FW} \times DM_i \text{ of fish})}{\text{P in feed} \times \text{amount of feed consumed}} \qquad 6.9$$

Where:

P_f = Phosphorus in final fish

P_i = Phophosrus in initial fish

DM_f = Dry matter of final fish (%)

DM*i* = Dry matter of initial fish (%)

6.3.9 Ammonia Determination Method

Standard solution was prepared using ammonium chloride (NH_4Cl). In order to produce 1,000 ppm of standard solution, 1.908 g ammonium chloride was dissolved in 500 ml of distilled water. Regression analysis was employed to estimate total ammonia nitrogen in samples.

About 5 ml of the water sample was placed into a test tube, then 0.2 ml of phenol solution (20 g phenol in 200 ml of 95% v/v ethyl alcohol) was added. In sequence, 0.2 ml of sodium nitroprusside (1 g sodium nitropusside in 200 ml distilled water) and 0.5 ml of oxidizing solution (100 ml alkaline reagent mix with 25 ml chlorox) were added. The levels of total ammonia nitrogen were estimated by using a spectrophotometer (with absorbance at 640 nm) (Parsons et al. 1984).

6.3.10 Nitrite Determination Method

Standard solution was prepared by using sodium nitrite ($NaNO_2$). In order to produce 1,000 ppm of standard solution, 0.4925 g of sodium nitrite was dissolved in 1 ml of distilled water. Regression analysis was employed to estimate nitrite in samples.

About 5 ml of prepared sample was placed into a test tube, then 1 ml of diazotizing solution (5 g sulfanilamide and 50 ml concentrated HCL mixed with distilled water to get a total of 500 ml volume) was added, swirled properly and allowed to stand for 3 minutes. Then, a coupling reagent (500 mg N- (1-naphthyl)-ethylenediamine dihydrochloride in 500 ml of distilled water) was added and again the sample was swirled and allowed to stand for 10 minutes. The levels of nitrite were determined by spectrophotometer at an absorbance of 543 nm.

6.3.11 Statistical Analysis

All data was statistically analyzed using one-way and two-way analysis of variance to determine significant differences ($p < 0.05$) among treatments, which was followed by the Fisher's

Least Significant Difference test to determine significant differences among treatment means. All statistical analyses were carried out using SAS (V9.2 SAS Institute, Cary, NC, USA).

6.4 Results

6.4.1 Water Quality

Water quality parameters throughout the 8-wk period were within suitable ranges for the culture of channel catfish. The water quality parameters measured and recorded were tabulated (Table 6.1). Dissolved oxygen, temperature, salinity, total ammonia nitrogen and nitrite values were found to be 6.04±0.64 g/L, 25.05±2.18°C, 3.43±0.50 mg/L, 0.35±0.11 g/L and 0.19±0.11 g/L, respectively.

6.4.2 Fish Growth Performance

Growth performance of fish in terms of final weight, weight gain, feed conversion ratio and survival are summarized in Table 6.2. No significant differences ($P>0.05$) were observed in final weigh, feed conversion ratio and survival among different treatments. Final weight (P = 0.098) ranged from 388.78 g for 28% protein treatment to 428.00 g for 32% protein treatment. Feed conversion ratio (P = 0.037) ranged from 1.58 for 32% protein treatment to 1.75 for 28% protein supplemented with carbohydrase treatment. Weight gain of fish (P = 0.004) ranged from 180.26% for 28% protein supplemented with carbohydrase treatment to 232.02% for 32% protein treatment. Highest weight gain was observed in fish fed 32% protein treatment that received neither phytase nor carbohydrase supplementation. This weight gain was significantly greater than fish fed with 28% protein diet and 28% protein diet supplemented with carbohydrase enzyme (Figure 6.1). Supplementation of diets with enzymes (phytase and carbohydrase) had no significant effect on growth performance of fish. Result from two-way ANOVA indicated a significant effect of protein

but not enzyme supplements on final weight, percent weight gain and feed conversion ratio of fish. No significant effect was observed on the interaction of protein and enzyme on fish performance.

6.4.3 Ash content and nutrient retention

Ash content and nutrient retention values for fish are summarized in Table 6.3. No significant differences were observed in protein retention and energy retention of fish. Protein retention varied from 32.19% for 28% protein diet treatment to 36.10% for 28% protein supplemented with phytase diet treatment. Energy retention varied from 31.50% for 28% protein supplemented with carbohydrase treatment to 35.78% for 32% protein treatment. Phosphorus retention and ash content in the fish was found to differ significantly ($P<0.05$) between groups under different diet treatments. Greatest phosphorus retention (40.76%) was found in fish fed with phytase supplemented 32% protein diet treatments (Figure 6.2). This value was significantly ($P<0.05$) higher than phosphorus retention in fish under other treatments. Moreover, for fish fed with 28% protein diets (treatments), phosphorus retention was found to be significantly ($P<0.05$) higher for the group receiving phytase supplemented diet than the other 28% protein diet treatment groups. Ash content varied from 8.04% for 28% protein treatment to 10.49% for 28% protein supplemented phytase treatment (Figure 6.3).

Result from two-way ANOVA indicated a significant effect ($P<0.05$) of protein on energy and phosphorus retention of the fish, while enzyme had a significant impact ($P<0.05$) on ash content and phosphorus retention of the fish. No significant effect was observed on the interaction of protein and enzyme on nutrient retention and ash content of fish. Result from Dunnett's test showed that phytase supplementation improved ash, protein, and phosphorus retention of fish fed 28% protein diet, but only enhanced phosphorus retention of fish fed 32% protein diet (Table 6.4).

6.4.4 Body condition of fish

Fish growth performance parameters including length, weight, IP fat, liver weight and dressout were recorded in Table 6.5. No significant differences ($P>0.05$) were observed in all parameters among different treatments. Fish length ranged from 13.06 inch for 28% protein supplemented with carbohydrase treatment to 13.35 inch for 32% protein supplemented with phytase treatment. Fish weight ranged from 384.06 g for 28% protein treatment to 424.43 g for 32% protein treatment. Intraperitoneal fat of fish ranged from 6.35 g for 28% protein treatment to 8.50 g for 32% protein treatment. Liver weight ranged from 1.40 g for 32% protein supplemented with carbohydrase treatment to 1.69 g for 28% protein treatment. Dressout ranged from 273.03 g for 28% protein supplemented with carbohydrase treatment to 313.04 g for 32% protein treatment. Statistical analysis from two-way ANOVA revealed that levels of protein but not enzyme supplements in diets had significant effects on weight, intraperitoneal fat and dressout of the fish. Diet containing 32% protein was in general, found to improve fish conditions. Effect of the interaction between protein content and enzyme supplementation on fish conditions was not found to be significant.

6.5 Discussion

Protein is an important component in aquatic feeds because it significantly affects the growth of cultured animals (Carpenter, 2003). Protein is also the most expensive ingredient in aquatic feeds. Therefore, the ideal protein use is in alignment with low protein levels in feed formulations, resulting in optimal efficiency of protein utilization by animal, reduction in feed cost and minimal nitrogenous wastes (Green and Hardy, 2008). A number of studies were carried out to determine an optimal protein level in catfish feed. Page and Andrews (1973) stated that 25% protein was adequate in the diet of channel catfish of 114 to 500 g. Reis et al. (1989) conducted the experiment on catfish by feeding diets contained 26, 31, 35 and 39% protein, and found that

weigh gain of fish increased greatly, from 288 g to 407 g, as dietary protein increased from 26 to 31% ($p<0.01$), but less from 429 g to 476 g as dietary protein increased from 35 to 39%. Feed conversion ratio also was significantly ($p<0.01$) improved by an increase in level of protein from 26% (FCR=1.39) to 35% (FCR=1.15). They also found the percentage of dressing was lowest for fish fed 26% protein but not significantly different among fish fed the other diets. Fish fed 26% protein also had the greatest body fat whereas fish fed 35% protein had the lowest. Murray et al. (1977) indicated that increasing dietary protein from 25 to 35% resulted in improved weigh gain and protein conversion of channel catfish. However, Robinson and Li (1997) carried out the experiment in earthen ponds at high density, and reported that weigh gain and feed conversion ratio of channel catfish was not different between fish fed diet contained 28 and 32% protein. Despite a number of studies, there is still a debate on what protein level is the most appropriate. In the present study which is conducted under controlled conditions, we also shown that 32% protein diet significantly improved weight gain but did not have any effect on feed conversion ratio of channel catfish.

Numerous research projects have been conducted to study the effect of phytase enzyme on growth performance of different fish species. Taken together some early studies demonstrated that positive growth responses were influenced by phytase when applying diets contained entirely or almost entirely based on plant-based protein sources. Nonetheless, the improvement of growth in response to phytase is inconsistent. Zhu et al. (2015) observed that diet contained phytase 500 IU/kg did not improve yellow catfish growth response. Insignificant growth responses on phytase at the level of 500 IU/kg diet were also observed in channel catfish (*Ictalurus puntatus*) (Yan et al., 2002), Japanese seabass (*Latrobrax japonicas*) (Ai et al., 2007) and Hamilton (*Labeo rohita*) (Baruah et al., 2007). However, growth performance of rainbow trout was improved by this

supplementation level (500 IU/kg) (Vielma et al., 2002). Increasing phytase level to 1,000 IU/kg diet did resulted in positive effects on the growth of channel catfish (Jackson et al., 1996), striped bass (Papatryphon et al., 1999) and rainbow trout (Vielma et al., 2002). In this present experiment, the commercial diet contained 280 g kg^{-1} and 320 g kg^{-1} protein supplemented with phytase 2,000 IU/kg did not have significant effect on the growth performance of catfish. The inconsistent outcomes from different studies could be due to differences in feed ingredients, quality and quantity of plant-based feedstuffs in the diet formulations, fish species and experimental conditions.

Supplementation phytase to diet dramatically decreased fecal phosphorus contents in several fish species such as common carp (Schafer et al., 1995), channel catfish (Jackson et al., 1996) and rainbow trout (Sugiura et al., 2001). It indicated that phosphorus utilization and retention improved when phytase was added to diet. The increase in phosphorus retention by the fish offered diets with phytase supplementation leads to the reduction of phosphorus content in effluent water which was considered as one of the primary pollutants in water. Total phosphorus released into the water significantly decreased from 6.8 to 5.0 g/kg body weight was reported in the study of Ai et al. (2007). The result suggested that dietary phytase could improve the quality of effluents from aquaculture farming. Sugiura (2001) reported the result of feeding trail with low-ash diets on rainbow trout that the apparent absorption of phosphorus increased corresponding to the supplementation level of phytase in the diets from 27% (control diet) up to 90-93% (phytase 4,000 IU/kg diet) and the excretion of phosphorus in feces was 95-98% reduction compared with fish fed commercial trout diet. The similar findings that supplementation of phytase to fish diets reduced total phosphorus loading to the surrounding environment by 30-50% were observed in tilapia (Hardy and Shearer, 1985), salmon (Storebakken et al., 1998), carp (Schaefer et al., 1995;

Zeng et al., 2001) and rainbow trout (Rodehutscord and Pfeffer, 1995; Lanari et al., 1998). The result from present study demonstrated that supplementation of phytase in both diets containing 28% and 32% protein significantly ($P<0.05$) increased phosphorus retention in catfish.

Regarding protein retention, phytate can bind with protein and form phytate-protein complex, which can reduce availability and digestibility of the protein, resulting in reduced growth and feed efficiency in culture animals. It has been reported that supplementing phytase into diet can increase the availability of protein in aquatic animals (Usmani and Jafri, 2002).

The effect of phytase supplementation on protein retention was not observed in this study. Ai et al (2007) reported similar result in Japanese seabass, *Lateolabrax japonicas*, fed diet supplemented with phytase 500 IU/kg. However, this was in contrast to studies by Cain and Garling (1995) and Papatryphon et al. (1999), which demonstrated that the phytase supplementation level improved protein utilization in channel catfish and striped bass, respectively. In term of energy retention, a significantly improvement of energy retention was reported in Hamilton (*Pangasius pangasius*) fed with diet contained phytase 500 IU/kg (Debnath et al., 2005). Similar result was reported in rainbow trout fed diet based canola meal supplemented with phytase 1,500 IU/kg (Forster et al., 1999). However, the energy retention in the present study was not significantly affected by phytase supplementation.

Previous research has demonstrated that supplementation of phytase to diets could improve feed conversion ratio of the animals (Cain and Garling, 1995; Lanari et al., 1998; Vielma et al., 2000, 2002). In studies by Wang et al. (2009) and Hassaan et al. (2013) in tilapia revealed that phytase supplementation significantly improved feed conversion ratio in rainbow trout and tilapia. It could be due to the fact that phytase could increase the activity of digestive enzyme in fish. Li et al. (2009) conducted the experiments on tilapia, grass carp and gibel carp, and reported that

dietary phytase significantly increased amylase activities. A study carried out by Nwanna (2007) also indicated that phytase enhanced activities of amylase, protease and lipase in tilapia. Another reason in reducing feed conversion ratio from phytase may be due to the fact that phytase could enhance the release of nutrients by breaking down the bonds between phytate-protein and phytate-minerals (Vielma et al., 1998). Supplementing phytase into diet can increase the availability of these nutrients, leading to an improved growth and feed conversion ratio of animals.

Phytate has negative impacts on the availability of other minerals because phytate can also chelate with minerals such as calcium, magnesium and zinc. Supplementation of phytase can reduce the level of phytate in feeds and consequently increase the availability of other minerals. In the present study, whole-body ash content of catfish fed 28% protein treatment but not 32% protein treatment was affected by the phytase supplementation. However, Sarker et al. (2012) and Hassaan et al. (2013) could not observe the effect of phytase on whole body ash content in juvenile yellowtail (*Seriola quinqueradiata*) and tilapia, respectively. Baruah et al. (2007) also confirmed this observation in Hamilton, *Labeo rohita*, fed diet supplemented with phytase 500 IU; however, the ash content in fish was increased when adding phytase along with citic acid into the diet. This is may be due to the fact that citric acid lowers intestinal pH, which is a suitable condition for phytase to perform (Radcliffe et al., 1998; Baruah et al., 2005).

In addition to phytase there are other exogenous enzymes that may play a role in improved nutrition. The beneficial effects of carbohydrases have not been fully investigated in aquatic animals (Francis et. al, 2001; Glencross et. al, 2003). The growth response to carbohydrases supplemented to aquatic animal feeds species varies among studies. Supplementation of carbohydrases to diets helped to reduce the viscosity effect of NSP in plant-based protein ingredients (Choct, 1997; Simon, 1998). Storebakken and Austreng (1987) found that the

availability of nutrients was significantly reduced when Atlantic salmon, *Salmo salar L*., fed a diet containing NSPs compared with an NSP-free diet. It is generally believed that the positive effects of carbohydrases are based on an increase in nutrient digestibility or metabolisable energy content. Lin et al. (2007) conducted the experiment on tilapia, *Oreochromis niloticus* × *O. aureus*, and reported that supplementation of a commercial enzyme complex (neutral protease, β-glucanase and xylanase) at the level of 1.5 g kg^{-1} significantly increased feed efficiency, apparent protein retention as well as digestibility of protein, lipid and gross energy. They also demonstrated that the levels of amylase and protease activities in the intestine of hybrid tilapia were significantly higher than those fed diets without enzymes. Carter et al. (1994) reported that enzyme supplementation of diet containing 33.9% soybean meal supplemented with carbohydrases promoted fish growth as compared with those fed without enzyme. Nevertheless, the addition of a multi-carbohydrase enzyme in salmon diet containing 46.9% soybean meal did not improve the growth performance (Carter, 1998).

Feed efficiency response of culture animal corresponding with carbohydrase supplementation is also not consistent. Farhangi and Carter (2007) indicated that enzyme supplementation of a soybean-based diet with a mixture of carbohydrase and proteolytic enzymes significantly improved the FCR of rainbow trout. Nonetheless, Dalsgaard et al. (2012) conducted the experiment on rainbow trout fed diet supplemented with enzymes (β-glucanase, xylanase and protease). The result indicated that there were no differences in FCR of fish among all treatments ($P>0.05$). They also reported a significant improvement ($P<0.05$) in energy retention of fish fed soy diet supplemented with β-glucanase. This was in agreement with the result of present study which demonstrated no significant difference in feed conversion ratio of fish among the treatments. The positive effect of feed conversion ratio on carbohydrases was not observed in this study.

Carbohydrate digestibility in animals varies depending on several factors such as the type of carbohydrate, the dietary inclusion level and the processing treatments (such as raw, cooked or extruded) applied to it (Kumar et al., 2006). It has been shown in trout by Phillips et al. (1940), Pieper (1977), Bergot (1991) and Takeuchi et al. (1990), in carp by Chiou and Ogino (1975) and Mohapatra et al. (2002), in turbot by Jollivet et al. (1988), in red sea bream (*Pagrua major*) by Jeong et al. (1991) and in *Penaeus vannamei* by Davis and Arnold (1993) that digestibility of native starch was low at the level of 30 to 50 %, while that of gelatinized starch was higher at 50 to 90%. The ability of fish to digest dietary carbohydrate has been reported to be variable between species due to the different endogenous digestive carbohydrase activity in different fish (Shimeno 1982; Furuichi 1983; Smith, 1989; Wilson 1994; Shiau 1997). Carbohydrase activity in fish has been reported to be low, medium and high in the intestinal tract of carnivorous, omnivorous and herbivorous species, respectively (Smith 1989). In this study, the response of catfish on carbohydrase could not be observed. This might be due to the greater utilization of carbohydrate in omnivorous fish. Carbohydrase supplementation has been used successfully to enhance the utilization of unavailable dietary carbohydrates in mainly carnivorous species such as Atlantic salmon (*Salmo salar*), gilthead seabream (*Sparus aurata*) and tiger prawns (*Penaeus monodon*) (Kolkovski et al., 1993; Carter et al., 1994; Buchanan et al., 1997).

6.6 Conclusion

The use of exogenous enzymes as feed additive in aquatic diets currently is drawing considerable attention by the aquatic feed manufacturers. The chemical effects of these enzymes are well defined; however, data on the response, in terms of benefits to animals is still inconsistent. It was hypothesized that phytase use would increase the availability of phytate and phosphorus and would also lead to a decrease in phosphorus loading to the environment. Also, carbohydrases

would be primarily used to reduce the anti-nutritional effects of non-starch polysaccharides and increase the utilization of carbohydrates. The results from this study demonstrated that both enzyme supplementations; carbohydrase (0.2 g/kg diet) and phytase (2000 IU/kg diet), in diets did not affect the growth performance of channel catfish. However, the addition of phytase 2000 IU/kg to catfish diet improved phosphorus retention in fish fed with both 28% and 32% protein diet. Also, significant increase in the weight gain for fish fed with 28% protein diet supplemented with phytase was observed. The percentage of ash content of fish fed 28% protein could be enhanced by phytase supplementation. Results from this study indicated that the addition of phytase 2000 IU in feeds improve the availability of phosphorus as it can increase phosphorus retention in channel catfish, and also minimize the phosphorus loading to the environment.

Table 6.1 Water quality parameters. Values represent mean and standard deviation together with minimum and maximum.

Water Parameter	Mean±SD	Minimum	Maximum
Temperature (°C)	25.02±2.18	20.00	30.20
Dissolved oxygen (g/L)	6.04±0.64	4.56	7.96
Salinity (mg/L)	3.43±0.5	2.20	4.70
TAN[1] (g/L)	0.35±0.11	0.22	0.49
Nitrite (g/L)	0.19±0.11	0.06	0.36

[1]TAN: Total ammonia nitrogen

Table 6.2 Measured responses over a 12 week growth period for channel catfish fed one of six dietary treatments consisting of two levels of protein (28% and 32%) each either offered as a unsupplemented, supplemented with carbohydrase (0.2 g kg^{-1}) or supplemented with phytase (2000 FTU kg^{-1}).

Treatment	Final weight (g)	Weight gain (%)	FCR[1]	Survival (%)
28%Protein	388.78±21.37	188.67±21.61^{bc}	1.73±1.72	99.99±0.01
28%Protein+CHO	393.45±36.02	180.26±15.98^{c}	1.75±0.09	99.99±0.01
28%Protein+PHY	408.17±13.38	211.02±10.90^{abc}	1.70±0.04	100.00±0.00
32%Protein	428.00±20.80	232.02±19.62^{a}	1.58±0.10	100.00±0.00
32%Protein+CHO	420.67±19.31	226.67±21.48^{ab}	1.59±0.07	100.00±0.00
32%Protein+PHY	426.33±18.93	227.03±23.33^{ab}	1.62±0.09	100.00±0.00
P-value	0.098	0.004	0.037	0.564
PSE[2]	11.360	9.644	0.041	0.001
Two-way ANOVA				
Protein	0.007	0.0003	0.001	0.174
Enzyme	0.629	0.295	0.895	0.615
Protein*Enzyme	0.656	0.248	0.603	0.615

Values with different letters in a column are different from each other (α =0.05)

[1]FCR: Feed conversion ratio = weight gain / feed intake

[2]PSE: Pool standard error

Table 6.3 Percent ash content and nutrient retention for channel catfish fed with 28% and 32% protein diet treatments: control diet, diet supplemented with carbohydrase (0.2 g/kg) and diet supplemented with phytase (2000 FTU/kg).

Treatment	Ash Content (%)	Protein Retention (%)	Energy Retention (%)	Phosphorus Retention (%)
28%Protein	8.04±0.48[b]	32.19±2.28	32.69±3.33	27.26±1.91[d]
28%Protein+CHO	8.93±1.03[ab]	33.67±2.60	31.50±3.05	29.41±3.24[cd]
28%Protein+PHY	10.49±0.49[a]	36.10±0.62	33.18±0.86	36.02±2.86[ab]
32%Protein	9.53±1.20[ab]	34.51±0.63	35.78±2.89	34.15±3.80[bc]
32%Protein+CHO	9.97±0.56[ab]	34.25±1.83	35.67±2.04	30.33±2.43[bcd]
32%Protein+PHY	10.19±1.18[a]	34.59±1.77	35.27±1.28	40.76±2.56[a]
P-value	0.010	0.121	0.100	<0.0001
PSE[1]	0.443	0.895	1.213	1.432
Two-way ANOVA				
Protein	0.055	0.536	0.006	0.002
Enzyme	0.009	0.101	0.830	<0.0001
Protein*Enzyme	0.138	0.130	0.697	0.137

Values with different letters in a column are different from each other (α =0.05)

[1]PSE: Pool standard error

Table 6.4 Performance of channel catfish fed with 28% and 32% protein diet treatments: control diet, diet supplemented with carbohydrase (0.2 g kg^{-1}) and diet supplemented with phytase (2000 FTU kg^{-1}) performed by Dunnett's test

Treatment	Ash Content (%)	Protein Retention (%)	Energy Retention (%)	Phosphorus Retention (%)
28%Protein	8.04±0.48*	32.19±2.28*	32.69±3.33	27.26±1.91*
28%Protein+CHO	8.93±1.03	33.67±2.60	31.50±3.05	29.41±3.24
28%Protein+PHY	10.49±0.49*	36.10±0.62*	33.18±0.86	36.02±2.86*
32%Protein	9.53±1.20	34.51±0.63	35.78±2.89	34.15±3.80*
32%Protein+CHO	9.97±0.56	34.25±1.83	35.67±2.04	30.33±2.43
32%Protein+PHY	10.19±1.18	34.59±1.77	35.27±1.28	40.76±2.56*

** Values with in a column are different from each other (α =0.05)*

Table 6.5 Performance of channel catfish fed with 28% and 32% protein diet treatments: control diet, diet supplemented with carbohydrase (0.2 g kg^{-1}) and diet supplemented with phytase (2000 FTU kg^{-1}).

Treatment	Length (inch)	Weight (g)	IP fat (g)	Liver Weight (g)	Dressout (g)
28%Protein	13.10±0.26	384.06±27.08	6.35±0.59	6.52±0.86	283.86±11.65
28%Protein+CHO	13.06±0.34	390.71±38.30	6.79±1.13	6.23±1.44	273.03±38.40
28%Protein+PHY	13.14±0.18	402.80±10.18	6.66±1.54	6.33±0.25	294.78±24.86
32%Protein	13.23±0.15	424.43±18.57	8.47±0.75	6.38±0.47	313.04±21.57
32%Protein+CHO	13.22±0.17	415.43±16.46	8.50±0.86	5.81±0.52	297.80±20.94
32%Protein+PHY	13.35±0.09	416.90±21.49	8.07±1.09	6.40±0.65	306.86±16.19
P-value	0.481	0.162	0.022	0.852	0.231
PSE[1]	0.109	11.871	0.521	0.399	11.893
Two-way ANOVA					
Protein	0.076	0.014	0.001	0.622	0.036
Enzyme	0.618	0.831	0.840	0.536	0.397
Protein*Enzyme	0.929	0.549	0.778	0.826	0.760

Values with different letters in a column are different from each other (α =0.05)

[1]PSE: Pool standard error

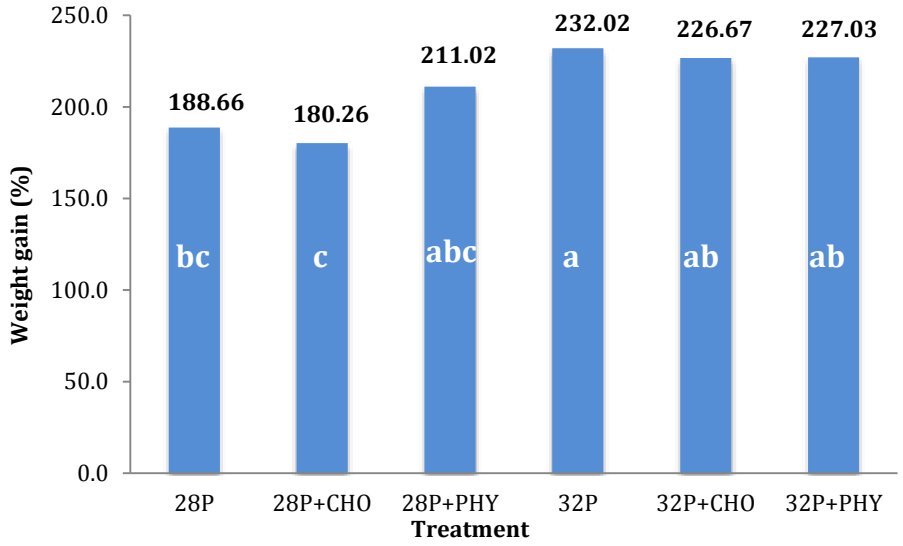

Figure 6.1 Percent weight gain in channel catfish fed with 28% and 32% protein diet treatments: control diet, diet supplemented with carbohydrase and diet supplemented with phytase.

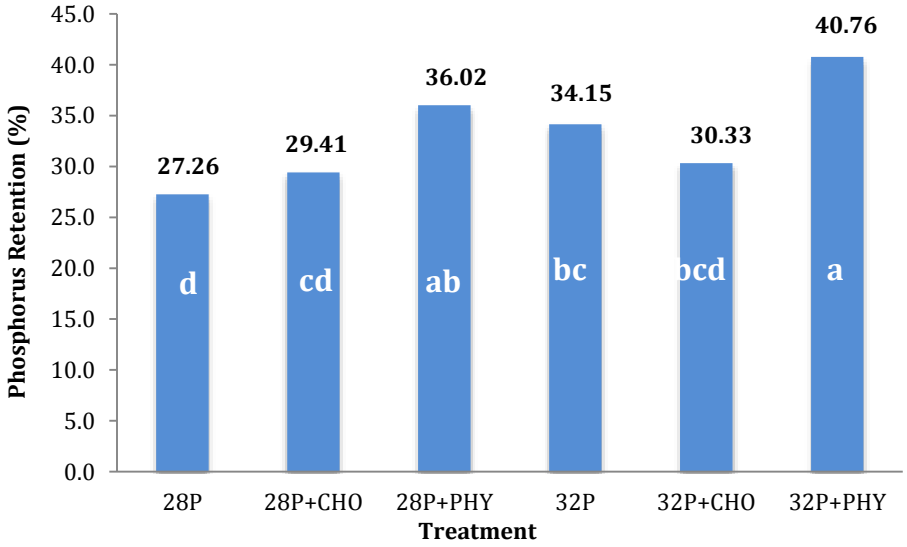

Figure 6.2 Percent phosphorus retention in channel catfish fed with 28% and 32% protein diet treatments: control diet, diet supplemented with carbohydrase and diet supplemented with phytase.

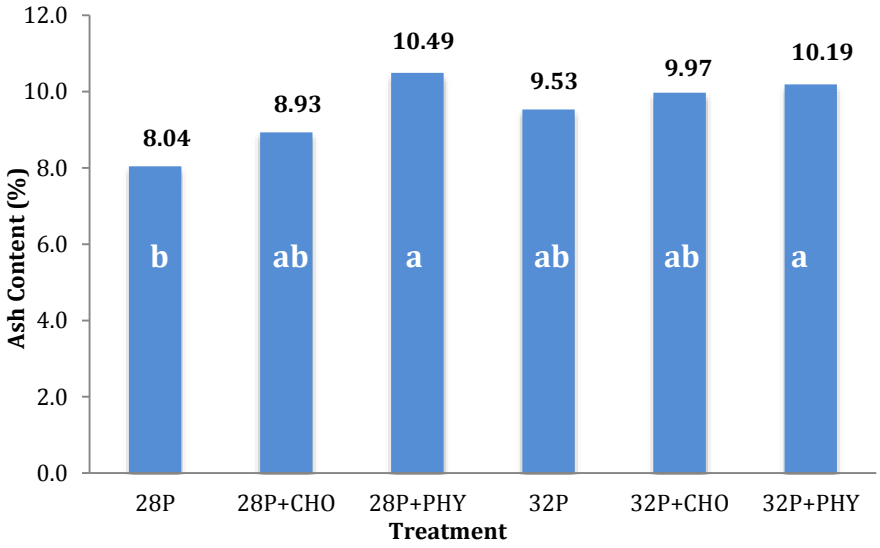

Figure 6.3 Percent ash content in channel catfish fed with 28% and 32% protein diet treatments: control diet, diet supplemented with carbohydrase and diet supplemented with phytase.

References

Adelizi, P., Rosati, R., Warner, K., Wu, Y., Muench, T., White, M., Brown, P., 1998. Evaluation of fishmeal free diets for rainbow trout, *Oncorhynchus mykiss*. Aquacult. Nutr. 4, 255-262.

Ai, Q., Xie, X., 2005. Effects of Replacement of Fish Meal by Soybean Meal and Supplementation of Methionine in Fish Meal/Soybean Meal- based Diets on Growth Performance of the Southern Catfish *Silurus meridionalis*. J. World Aquacult. Soc. 36, 498-507.

Ai, Q., Mai, K., Zhang, W., Xu, W., Tan, B., Zhang, C., Li, H., 2007. Effects of exogenous enzymes (phytase, non-starch polysaccharide enzyme) in diets on growth, feed utilization, nitrogen and phosphorus excretion of Japanese seabass, *Lateolabrax japonicus*. Comparative Biochemistry and Physiology Part A: Mol. Integrative Physiol. 147, 502-508.

Bakker, G., Dekker, R., Jongbloed, R., Jongbloed, A., 1998. Non- starch polysaccharides in pig feeding. Vet. Guart. 20, 59-64.

Baruah, K., Pal, A.K., Sahu, N.P., Jain, K.K., Mukherjee, S.C., Debnath, D., 2005. Dietary protein level, microbial phytase, citric acid and their interactions on bone mineralization of *Labeo rohita* (Hamilton) juveniles. Aquacult. Res. 36, 803-812.

Baruah, K., Sahu, N.P., Pal, A.K., Debnath, D., Yengkokpam, S., Mukherjee, S.C., 2007. Interactions of dietary microbial phytase, citric acid and crude protein level on mineral utilization by rohu, *Labeo rohita* (Hamilton), juveniles. J. World Aquacult. Soc. 38, 238-249.

Bedford, M.R., 2000. Exogenous enzymes in monogastric nutrition — their current value and future benefits. Ani. Feed Sci. Tech. 86, 1-13.

Bergot, F., 1993. Digestibility of native starches of various botanical origins by rainbow trout (*Oncorhynchus mykiss*). Colloques de l'INRA (France).

Buchanan, J., Sarac, H.Z., Poppi, D., Cowan, R.T., 1997. Effects of enzyme addition to canola meal in prawn diets. Aquaculture 151, 29-35.

Burel, C., Boujard, T., Tulli, F., Kaushik, S.J., 2000. Digestibility of extruded peas, extruded lupin, and rapeseed meal in rainbow trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). Aquaculture 188, 285-298.

Cain, K.D., Garling, D.L., 1995. Pretreatment of soybean meal with phytase for salmonid diets to

reduce phosphorus concentrations in hatchery effluents. Prog. Fish Cult. 57, 114-119.

Carter, C., 1998. Fish meal replacement in aquaculture feeds for Atlantic salmon.

Carter, C., Houlihan, D., Buchanan, B., Mitchell, A., 1994. Growth and feed utilization efficiencies of seawater Atlantic salmon, *Salmo salar L*., fed a diet containing supplementary enzymes. Aquacult. Res. 25, 37-46.

Carter, C., Houlihan, D., Buchanan, B., Mitchell, A., 1994. Growth and feed utilization efficiencies of seawater Atlantic salmon, *Salmo salar L*., fed a diet containing supplementary enzymes. Aquacult. Res. 25, 37-46.

Chiou, J., Ogino, C., 1975. Digestibility of starch in carp. B. Jnp. Soc. Sci. Fish. (Japan).

Choct, M., 1997. Feed non-starch polysaccharides: Chemical structures and nutritional significance. Feed milling international. 191, 13-26.

Davis, D.A., Arnold, C., 1993. Evaluation of five carbohydrate sources for *Penaeus vannamei*. Aquaculture 114, 285-292.

Debnath, D., Pal, A.K., Sahu, N.P., Jain, K.K., Yengkokpam, S., Mukherjee, S.C., 2005. Effect of dietary microbial phytase supplementation on growth and nutrient digestibility of *Pangasius pangasius* (Hamilton) fingerlings. Aquacult. Res. 36, 180-187.

Farhangi, M., Carter, C.G., 2007. Effect of enzyme supplementation to dehulled lupin- based diets on growth, feed efficiency, nutrient digestibility and carcass composition of rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquacult. Res. 38, 1274-1282.

Forster, I., Higgs, D.A., Dosanjh, B.S., Rowshandeli, M., Parr, J., 1999. Potential for dietary phytase to improve the nutritive value of canola protein concentrate and decrease phosphorus output in rainbow trout (*Oncorhynchus mykiss*) held in 11 C fresh water. Aquaculture 179, 109-125.

Francis, G., Makkar, H.P., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199, 197-227.

Furuichi, M., 1983. Studies on the utilization of carbohydrate by fishes. Rep. Fish. Res. Lab., Kyushu Univ. 6, 1-59.

Gatlin, D.M., Phillips, H.F., 1989. Dietary calcium, phytate and zinc interactions in channel

catfish. Aquaculture 79, 259-266.

Glencross, B.D., Boujard, T., Kaushik, S.J., 2003. Influence of oligosaccharides on the digestibility of lupin meals when fed to rainbow trout, *Oncorhynchus mykiss*. Aquaculture 219, 703-713.

Gomes, E.F., Rema, P., Kaushik, S.J., 1995. Replacement of fish meal by plant proteins in the diet of rainbow trout (*Oncorhynchus mykiss*): digestibility and growth performance. Aquaculture 130, 177-186.

Hassan, B., El-Salhia, M., Khalifa, A., Assem, H., Al Basomy, A., El-Sayed, M., 2013. Environmental isotonicity improves cold tolerance of Nile tilapia, *Oreochromis niloticus*, in Egypt. The Egyptian Journal of Aquatic Research. 39, 59-65.

Jackson, L.S., Li, M.H., Robinson, E.H., 1996. Use of Microbial Phytase in Channel Catfish *Ictalurus punctatus* Diets to Improve Utilization of Phytate Phosphorus1. J. World Aquacult. Soc. 27, 309-313.

Jackson, L.S., Li, M.H., Robinson, E.H., 1996. Use of Microbial Phytase in Channel Catfish *Ictalurus punctatus* Diets to Improve Utilization of Phytate Phosphorus1. J. World Aquacult. Soc. 27, 309-313.

Jeong, K., Takeuchi, T., Watanabe, T., 1991. Improvement of nutritional quality of carbohydrate ingredients by extrusion process in diets of red sea bream. Nippon Suisan Gakk. 57, 1543-1541.

Jollivet, D., Gabaudan, J., Metailler, R., 1988. Some effects of physical state and dietary level of starch, temperature and meal size on turbot (*Scophtalmus maximus L.*) digestive processes. ICES, CM. 25, 17.

Kolkovski, S., Tandler, A., Kissil, G.W., Gertler, A., 1993. The effect of dietary exogenous digestive enzymes on ingestion, assimilation, growth and survival of gilthead seabream (*Sparus aurata, Sparidae, Linnaeus*) larvae. Fish Physiology and Biochemistry. 12, 203-209.

Kumar, S., Sahu, N., Pal, A., Choudhury, D., Mukherjee, S., 2006. Studies on digestibility and digestive enzyme activities in *Labeo rohita* (Hamilton) juveniles: effect of microbial α-amylase supplementation in non-gelatinized or gelatinized corn-based diet at two protein levels. Fish physiology and Biochemistry. 32, 209-220.

Lanari, D., D'agaro, E., Turri, C., 1998. Use of nonlinear regression to evaluate the effects of phytase enzyme treatment of plant protein diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 161, 345-356.

Lanari, D., D'agaro, E., Turri, C., 1998. Use of nonlinear regression to evaluate the effects of phytase enzyme treatment of plant protein diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 161, 345-356.

Li, J., Li, J., Wu, T., 2009. Effects of non- starch polysaccharides enzyme, phytase and citric acid on activities of endogenous digestive enzymes of tilapia (*Oreochromis niloticus*× *Oreochromis aureus*). Aquacult. Nutr. 15, 415-420.

Lin, S., Mai, K., Tan, B., 2007. Effects of exogenous enzyme supplementation in diets on growth and feed utilization in tilapia, *Oreochromis niloticus x O. aureus*. Aquacult. Res. 38, 1645-1653.

Lin, S., Mai, K., Tan, B., 2007. Effects of exogenous enzyme supplementation in diets on growth and feed utilization in tilapia, *Oreochromis niloticus x O. aureus*. Aquacult. Res. 38, 1645-1653.

Mbahinzireki, G.B., Dabrowski, K., Lee, K.-J., El-Saidy, D., Wisner, E.R., 2001. Growth, feed utilization and body composition of tilapia (*Oreochromis sp*.) fed cottonseed meal-based diets in a recirculating system. Aquacult. Nutr. 7, 189-200.

Mohapatra, M., Sahu, N., Chaudhari, A., 2003. Utilization of gelatinized carbohydrate in diets of *Labeo rohita* fry. Aquacult. Nutr. 9, 189-196.

Murray, M.W., Andrews, J.W., DeLoach, H.L., 1977. Effects of dietary lipids, dietary protein and environmental temperatures on growth, feed conversion and body composition of channel catfish. The Journal of nutrition. 107, 272-280.

Nelson, T., 1967. The utilization of phytate phosphorus by poultry—a review. Poultry Science. 46, 862-871.

Novus, 1992. Raw material compendium: a compilation of worldwide data sources. NOVUS, St. Louis, MO (530 Maryville Centre Dr., St. Louis 63141).

NRC (National Research Council), 1993. Nutrient Requirements of Fish. The National Academies

Press, Washington, DC.

Nwanna, L., 2007. Effect of dietary phytase on growth, enzyme activities and phosphorus load of Nile tilapia (*Oreochromis niloticus*). Journal of Engineering and Applied Sciences. 2, 972-976.

Papatryphon, E., Howell, R.A., Soares, J.H., 1999. Growth and mineral absorption by striped bass *Morone saxatilis* fed a plant feedstuff based diet supplemented with phytase. J. World Aquacult. Soc. 30, 161-173.

Pieper, A., 1977. Untersuchungen über die Verwertung einiger Kohlenhydrate durch Regenbogenforellen (*Salmo gairdnerii* [i.e. gairdneri] Richardson). Georg-August-Universität.

Radcliffe, J., Zhang, Z., Kornegay, E., 1998. The effects of microbial phytase, citric acid, and their interaction in a corn-soybean meal-based diet for weanling pigs. Journal of animal science. 76, 1880-1886.

Reis, L., Reutebuch, E., Lovell, R., 1989. Protein-to-energy ratios in production diets and growth, feed conversion and body composition of channel catfish, *Ictalurus punctatus*. Aquaculture 77, 21-27.

Riche, M., Brown, P.B., 1996. Availability of phosphorus from feedstuffs fed to rainbow trout, *Oncorhynchus mykiss*. Aquaculture 142, 269-282.

Robinson, E.H., Li, M.H., 1997. Low Protein Diets for Channel Catfish *Ictalurus punctatus* Raised in Earthen Ponds at High Density1. J. World Aquacult. Soc. 28, 224-229.

Rodehutscord, M., Pfeffer, E., 1995. Effects of supplemental microbial phytase on phosphorus digestibility and utilization in rainbow trout (*Oncorhynchus mykiss*). Water Science and Technology. 31, 143-147.

Sarker, M., Alam, S., Satoh, S., Kamata, K., Haga, Y., Yamamoto, Y., 2012. Supplementation effect (s) of organic acids and/or lipid to plant protein- based diets on juvenile yellowtail, Seriola quinqueradiata Temminck et Schlegel 1845, growth and, nitrogen and phosphorus excretion. Aquacult. Res. 43, 538-545.

Schäfer, A., Koppe, W., Meyer-Burgdorff, K.-H., Günther, K., 1995. Effects of a microbial

phytase on the utilization of native phosphorus by carp in a diet based on soybean meal. Water Science and technology. 31, 149-155.

Shiau, S.Y., 1997. Utilization of carbohydrates in warmwater fish—with particular reference to tilapia, *Oreochromis niloticus x O. aureus*. Aquaculture 151, 79-96.

Shimeno, S., 1982. Effects of carbohydrate in feed on carbohydrate metabolism in yellowtail. Studies on Carbohydrate Metabolism in Fish, 91-108.

Simon, O., 1998. The mode of action of NSP hydrolysing enzymes in the gastrointestinal tract. Journal of Animal and Feed Sciences. 7, 115-123.

Smith, L.S., 1989. Digestive functions in teleost fishes. Fish Nutr. 2, 331-421.

Soltan, M., 2009. Effect of Dietary Fish Meal Replacement by Poultry By-Product Meal with Different Grain Source and Enzyme Supplementation on Performance, Feces Recovery, Body Composition and Nutrient Balance of Nile Tilapia. Pakistan Journal of Nutrition. 8, 395-407.

Spinelli, J., Houle, C.R., Wekell, J.C., 1983. The effect of phytates on the growth of rainbow trout (*Salmo gairdneri*) fed purified diets containing varying quantities of calcium and magnesium. Aquaculture 30, 71-83.

Storebakken, T., Austreng, E., 1987. Binders in fish feeds: II. Effect of different alginates on the digestibility of macronutrients in rainbow trout. Aquaculture 60, 121-131.

Storebakken, T., Shearer, K., Roem, A., 1998. Availability of protein, phosphorus and other elements in fish meal, soy-protein concentrate and phytase-treated soy-protein-concentrate-based diets to Atlantic salmon, *Salmo salar*. Aquaculture 161, 365-379.

Sugiura, S., Gabaudan, J., Dong, F., Hardy, R., 2001. Dietary microbial phytase supplementation and the utilization of phosphorus, trace minerals and protein by rainbow trout [*Oncorhynchus mykiss* (Walbaum)] fed soybean meal- based diets. Aquacult. Res. 32, 583-592.

Takeuchi, T., Jeong, K., Watanabe, T., 1990. Availability of extruded carbohydrate ingredients to rainbow trout *Oncorhynchus mykiss* and carp *Cyprinus carpio*. B. Jnp. Soc. Sci. Fish. (Japan).

Thiessen, D., Campbell, G., Tyler, R., 2003. Utilization of thin distillers' solubles as a palatability

enhancer in rainbow trout (*Oncorhynchus mykiss*) diets containing canola meal or air-classified pea protein. Aquacult. Nutr. 9, 1-10.

Usmani, N., Jafri, A.K., 2002. Influence of dietary phytic acid on the growth, conversion efficiency, and carcass composition of mrigal *Cirrhinus mrigala* (Hamilton) fry. J. World Aquacult. Soc. 33, 199-204.

Van-Barneveld, R.J., 1999. Understanding the nutritional chemistry of lupin (Lupinus spp.) seed to improve livestock production efficiency. Nutr. Res. Reviews. 12, 203-230.

Vielma, J., Ruohonen, K., Peisker, M., 2002. Dephytinization of two soy proteins increases phosphorus and protein utilization by rainbow trout, *Oncorhynchus mykiss*. Aquaculture 204, 145-156.

Vielma, J., Mäkinen, T., Ekholm, P., Koskela, J., 2000. Influence of dietary soy and phytase levels on performance and body composition of large rainbow trout (*Oncorhynchus mykiss*) and algal availability of phosphorus load. Aquaculture 183, 349-362.

Vielma, J., Lall, S.P., Koskela, J., Schöner, F.J., Mattila, P., 1998. Effects of dietary phytase and cholecalciferol on phosphorus bioavailability in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 163, 309-323.

Wang, F., Yang, Y.H., Han, Z.Z., Dong, H.W., Yang, C.H., Zou, Z.Y., 2009. Effects of phytase pretreatment of soybean meal and phytase-sprayed in diets on growth, apparent digestibility coefficient and nutrient excretion of rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquacult. Int. 17, 143-157.

Wilson, R., 1994. Utilization of dietary carbohydrate by fish. Aquaculture 124, 67-80.

Yan, W., Reigh, R.C., Xu, Z., 2002. Effects of Fungal Phytase on Utilization of Dietary Protein and Minerals, and Dephosphorylation of Phytic Acid in the Alimentary Tract of Channel Catfish *Ictalurus punctatus* Fed an All- Plant- Protein Diet. J. World Aquacult. Soc. 33, 10-22.

Zeng, H., Yao, B., Zhou, W., Fan, Z., 2001. Advance in microbial phytase research. J Aquacult Chin. 15, 87.

Zhu, Y., Ding, Q., Chan, J., Chen, P., Wang, C., 2015. The effects of concurrent supplementation

of dietary phytase, citric acid and vitamin D 3 on growth and mineral utilization in juvenile yellow catfish *Pelteobagrus fulvidraco*. Aquaculture 436, 143-150.

Chapter 7 Summary

The environmental pollution load of feed-based aquaculture is mainly a function of feed composition and the efficiency with which feed is used. Wastes generated by aquaculture systems affects water quality in culture systems and the pollution loads discharged into the surrounding environment. Waste production is an unintentional and unavoidable consequence of animal feeding and it is particular obvious in aquaculture, because of the direct discharge into water. Therefore, environmental pollution of aquaculture begins with feed application. Because manufactured feed contains more macro- and micronutrients than needed by the cultured animals, wastes generated from feed contain a certain quantity of nutrients. Although type and amount of feed waste depend on several factors including species and size of cultured animal, culture system, and management practices, nutrient loading of the receiving environment is the predominant issue.

Nutrient retention efficiency of cultured animals can be calculated by using data for final weigh of animal and dietary nutrient intake. It can be used to estimate the quantity of nutrient load released into environment. According to data presented in this study, cultured animals are able to retain only 30-35% of the ingested nitrogen and phosphorus, and they excrete the nitrogen and phosphorus which are not utilized into the environment. This excretion contributes to a significant portion to the total nutrient loading from aquaculture system. Results from the present study demonstrated that nutrient loading to the system can be approximated as 69 kg of nitrogen and 16 kg of phosphorus per ton of production.

Feed conversion ratio depends on many factors to include feed composition and principal characteristics and the metabolic characteristic of the cultured species, but it also is strongly influenced by feeding practices that determine the percentage of feed that is actually consumed by the cultured species. The amount of wasted generated per unit of production of the cultured species decreases as the FCR declines. However, a low FCR does not indicate there are no environmental concerns related to feed use for a particular specie. The most effective way of reducing the water pollution potential of feed – both within culture systems and in the water body receiving aquaculture waste – is through attention to feeds and feeding practices. Both feed quality and its management are closely interlinked and interdependent. But it should be remember that adequate water quality in cultured systems is necessary to avoid stressing the cultured species and lessening their appatite and making them more susceptible to disease.

At present, technology plays an important role in improving feed quality and making feed more friendly to the environment. Extrusion technology contributes significant impacts to the aquatic feed industry. Extruded pellets have high stability and are more durable which reduces the amount of leaching that results in less wastage of nutrients and environmental pollution. The addition of highly digestible ingredients in feeds can increase absorption and retention as well as reduce wastage by reducing nutrient excretion. In order to reduce waste nitrogen, highly digestible protein ingredients and well-balances amino acid should be used. Excess fiber should be avoided to minimize organic waste. Ingredients with a low phosphorus to nitrogen ratio can be selected to reduce the amounts of phosphorus waste. Attention increasingly is being given to greater use of enzymes which enhance nutrient utilization. The use of phytase enzyme has been shown to increase availability of phosphorus by increasing phosphorus digestibility and phosphorus retention in cultured species, and thereby minimizing loading to the environment.