

MODELING PHOSPHORUS UTILIZATION IN SALMONID FISH SPECIES

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ABSTRACT

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The development of effective nutritional strategies to manage phosphorus (P) waste output from aquaculture operations requires a detailed understanding of P utilization by fish. This thesis presents two models constructed to simulate phosphorus utilization by salmonid fish, a factorial model and a dynamic model.

Phosphorus compounds present in ingredients and feeds were classified into broad chemical categories of bone-P, phytate-P, organic P, Ca monobasic / Na / K Pi (inorganic P) supplement, and Ca dibasic Pi supplement. A fractionation protocol was modified to quantify and establish relationships between bone-P, total P, and ash in animal feed ingredients. The factorial P model was developed by integrating sub-models of P digestibility, P content, and P waste output that were constructed through statistical modeling of literature data. A subsequent validation of the digestibility sub-model by a digestibility trial suggested that there was a general good agreement between model predicted and experiment estimated values. The factorial P model provides a simple and practical tool to estimate the effects of different dietary P sources and levels on P digestibility, retention, and waste output.

A mechanistic, dynamic P model was further constructed based on biological principles of P metabolism in fish. Digestion was simulated by hydrolysis of P chemical

compounds, and absorption through passive and active uptake of Pi into the blood. Indigestible and unabsorbed P was excreted through feces. Clearance of blood phosphate was simulated by deposition into bone and soft tissues, excretion through urine, and the endogenous excretion into feces. The model was made dynamic by incorporating a growth function. The mechanistic, dynamic nature of this dynamic P model allows extrapolation of model simulations to a wide range of conditions, as well as investigations of the interactions between diet composition, nutrient deposition, and growth performance over time. The factorial P model is practice oriented. It is semi-mechanistic, direct and simple to use in practical feed formulation and aquaculture operations. The dynamic P model is research oriented. It can be further improved and used in the research of P metabolism of fish.

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LIST OF ABBREVIATIONS

ADC	Apparent digestibility coefficient
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
BW	Body weight
cAMP	cyclic adenosine monophosphate
cGMP	cyclic guanine monophosphate
CP	Crude protein
DE	Digestible energy
DM	Dry matter
DNA	Deoxyribonucleic acid
DP	Digestible protein
Dphos	Digestible phosphorus
Dphos/DE	Digestible phosphorus per unit of digestible energy
FBW	Final body weight
FM	Fish meal
FMN	Flavin mononucleotide
FTU	Phytase unit
GE	Gross energy
GTP	Guanosine triphosphate
IBW	Initial body weight

IU	International unit
kJ	kilojoule
MBM	Meat and bone meal
MJ	Megajoule
mM	Millimolar
N	Nitrogen
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
P	Phosphorus
PBM	Poultry by-product meal
Pi	Inorganic phosphate
PTH	Parathyroid hormone
PTHrP	Parathyroid hormone-related protein
RNA	Ribonucleic acid
STC	Stanniocalcin
TGC	Thermal growth coefficient

CHAPTER 1 GENERAL INTRODUCTION

Phosphorus (P) is the first limiting factor for algal growth in many freshwater ecosystems and excessive P can stimulate eutrophication. Monitoring and reducing P waste output is a key factor for environmental sustainability of aquaculture operations. The development of effective nutritional strategies to manage P waste outputs requires a detailed understanding of P utilization and accurate estimates of P digestion, retention and excretion by fish.

A variety of factors could affect digestibility of dietary P and the amount of dietary P retained and excreted by fish. Phosphorus is present as different chemical compounds in ingredients and formulated feeds, whose digestibility by fish varies significantly. In animal by-products, P exists primarily in bone as hydroxyapatite, which is fairly digestible to fish (Lall, 1991; Sugiura et al., 2000c). In plant ingredients, 60% to 80% of the total P is bound in phytate (Ravindran et al., 1995). Since fish do not possess phytase in the digestive tract, digestibility of phytate is very poor (Ogino et al., 1979; Lall, 1991). Organic P covalently linked to protein, lipid and sugar is easily hydrolyzed and presumably highly digestible. The digestibility of inorganic phosphate supplements is apparently affected by their solubility. Monobasic Ca phosphate is, for example, more digestible than dibasic Ca phosphate because of its higher solubility (Lall, 1991). Ingredient selection and quality determine the content and digestibility of P in finished feeds and this, in turn, affects P utilization. There is

evidence that high P levels negatively influence digestibility of P, however this effect is likely different amongst chemical forms.

Once digested and absorbed, P is retained in the body to support biological functions, tissue growth, and bone mineralization. Bone is the major storage site of P, whereas in soft tissues, P serves as a component of organic compounds for synthesis of cell structures and biological functions. A number of factors could affect P retention by fish, among which, the primary factors are dietary P level, ingredient/diet composition, and genetics.

Phosphorus digestibility and retention affect the quantity and forms of waste output. Similar to mammals, urinary phosphate excretion in fish is determined mostly by plasma phosphate concentration. A threshold exists below which P excretion is minimal and above which P excretion is proportional to the increase in plasma phosphate concentration (Bureau and Cho, 1999). Urinary P excretion and fecal P excretion make up total P waste output. However, urinary excreted P (soluble P waste) is more readily available to algae and can, consequently, have more immediate environmental impact than fecal excreted P (solid P waste) (Cho and Bureau, 2001). Therefore, the differentiation and accurate estimate of forms of P waste outputs would be useful in constructing nutrient management strategies to minimize the environmental impact of P waste outputs, particularly, soluble P waste outputs.

While there is a significant amount of information in the literature on P content and utilization for a number of salmonid fish species, there has been no attempt to integrate the available information through a mathematical modeling approach. Models have always been

a necessity of the scientific method (Rosenblueth and Wiener, 1945). Mathematical models can be constructed based on integration of observational data, and/or available biological principles. The vast amount of information available, including P utilization at the whole fish level, P metabolism at the tissue level, and nutritive values of a great variety of ingredients and diet formulae, provides an opportunity to simulate and estimate P utilization by salmonid fish through a quantitative modeling approach, for both practical application and research purpose.

CHAPTER 2 LITERATURE REVIEW

2.1 Phosphorus and Physiological Roles in Animals

Phosphorus (P), the element with the atomic number of 15 and atomic weight of 30.97, is the 11th most abundant element in nature (McKelvey, 1973; Berner, 1997). It was first obtained by Hennig Brand of Hamburg through distilling urine in 1669. The name ‘phosphorus’ was later given to the element, meaning ‘light bearing’ (Corbridge, 1990). In the environment, P exists in a fully oxidized state, phosphate, which due to its non-volatile nature is restricted to the lithosphere and hydrosphere (Emsley, 1980).

Phosphorus is essential for all living organisms, in which it is present in the form of phosphate, both free and combined (Frausto da Silva and Williams, 2001). Phosphates exist as inorganic phosphates (Pi) and organic phosphates. In vertebrates, most of phosphates are found as inorganic calcium salts in skeletal tissues (Corbridge, 1990). The Pi concentration is often low in intracellular and extracellular fluids, in the range of millimolar (Werner et al., 1998; Frausto da Silva and Williams, 2001). Free phosphates circulate in the body in the form of orthophosphates, H_2PO_4^- , HPO_4^{2-} , and PO_4^{3-} . These forms are interchangeable in intracellular and extracellular fluids depending on the pH. At neutral pH, the predominant form is HPO_4^{2-} (Berner, 1997; Lall, 2002). Organic phosphates are phosphate esters, in which fully oxidized P is linked to carbon through oxygen (Corbridge, 1990). They occur as mono-

di-, and triphosphoesters with protein, sugars, and lipids and their salts. Their biological functions are derived from their specific chemical properties (Berner, 1997).

Phosphate has many functions in vertebrates (Berner, 1997; Frausto da Silva and Williams, 2001; Lall, 2002). Phosphate is a major constituent of skeletal tissue of the vertebrates and is essential to its development and maintenance. In bone, P is deposited with Ca as hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$). Bone is the primary storage of P in vertebrates, accounting for 80 – 85% of total P in the body. About 14% of P is in soft tissues, especially concentrated in muscle, nerve tissues, and red blood cells. Phosphate is a component of many biologically significant compounds and an important player in intermediate metabolism. Phosphate is an essential part of nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), which carry genetic materials. P also is a constituent of phospholipid, which is a major structural component of cell membrane and intracellular organelles. Phosphate also occurs in signaling molecules such as cyclic adenosine monophosphate (cAMP), cyclic guanine monophosphate (cGMP), and inositol polyphosphate. Phosphate bond in adenosine triphosphate (ATP) upon hydrolysis releases free energy that provides the main energy source for metabolic process and muscle contraction. Other energy compounds include adenosine diphosphate (ADP), guanosine 5-triphosphate (GTP), and creatine phosphate. Phosphate is a component of many coenzymes, such as nicotinamide adenine dinucleotide (NADH), nicotinamide adenine dinucleotide phosphate (NADPH), and flavin mononucleotide (FMN). Phosphate also plays a significant role in the buffering systems of blood and body fluids.

2.2 Phosphorus Metabolism and Its Regulation in Monogastrics

2.2.1 Phosphorus Metabolism

2.2.1.1 Intestinal Absorption

In monogastric mammals and birds, the small intestine is the main site for Pi absorption (Cross et al., 1990). Phosphate is absorbed by animals in the ionic forms of inorganic phosphate (Pi), specifically, H_2PO_4^- and HPO_4^{2-} . The primary phosphate ionic form in the acidic environment of duodenum is H_2PO_4^- . At the jejunum and ileum, where the pH is around 7.5, over 80% of Pi ions are HPO_4^{2-} (Cross et al. 1990). Among the different sections of small intestine, the jejunum is the most efficient site for Pi absorption and the ileum apparently plays an insignificant role in Pi absorption (Walton and Gray, 1979). The role of the large intestine in phosphate absorption is also limited (Cross et al., 1990). Lack of difference between ileal P digestibility and fecal P digestibility has been observed in pigs (Fan et al., 2001; Fan and Sauer, 2002; and Ajakaiye et al., 2003), suggesting low P absorption capacity in the large intestine.

Pi is absorbed across the intestinal wall through the mechanisms of paracellular passive diffusion and transcellular active transport. Luminal Pi concentrations determine the routes and mechanisms of Pi absorption. Passive diffusion is predominant at high luminal Pi

concentrations, whereas active transport achieves its maximal activity at low Pi concentration (Walton and Gray, 1979; Cross et al., 1990; Schröder et al., 1996).

Active transcellular transport through the intestine consists of three possible steps: Pi enters from lumen to enterocytes across the brush border membrane; Pi migrates inside enterocytes from luminal to basolateral side; Pi exits from enterocytes to blood across the basolateral membrane (Cross et al., 1990; Schröder et al., 1996).

The active transport of Pi into enterocytes is a Na⁺ gradient driven system that follows Michaelis-Menten kinetics. The Na⁺ gradient is maintained by the ATP-dependent Na⁺/K⁺ ATPase located at the basolateral membrane (Cross et al., 1990). In fact, NaPi transporters are responsible for the active transport of Pi in various cells of monogastric animals. Three types of transporters have been identified in different cells, among which type II NaPi cotransporters are important transporters in intestine and kidney (Werner et al., 1998; Murer et al., 2003). In mammals, different sub-types of NaPi cotransporters are located in intestines and kidneys: NaPi-IIa in apical membrane of renal tubule to reabsorb Pi from glomerular filtrates, and NaPi-IIb in the brush border membrane to transport Pi from intestinal lumen. NaPi-IIa is more pH sensitive than NaPi-IIb (Murer et al., 1994). Low pH sensitivity of transporters in intestine ensures absorption over a wide range of gastrointestinal pH (Graham et al., 2003).

The migration of Pi inside the enterocyte from luminal side to the basolateral side is poorly understood. This issue is further complicated by the fact that the enterocyte itself

requires Pi for proper functioning. The intercellular compartment of Pi makes the accurate measurement a difficult task (Civitelli and Avioli, 1994; Schröder et al., 1996). Exit of Pi from enterocytes across the basolateral membrane is speculated to be actively transported by type III NaPi cotransporters (Bai et al., 2000; Werner and Kinne, 2001; Collins et al., 2004).

Fish intestine resembles mammalian small intestine, having relatively similar counterparts to duodenum, jejunum and ileum. Fish also have digestive enzymes similar to those of mammals (Guillaume and Choubert, 1999). In fish, Pi absorption also occurs through passive diffusion and active transport. Brichon (1973) first observed active transport of Pi in the intestine of freshwater eel and suggested that the transport followed Michaelis-Menten kinetics. An active co-transporter system was subsequently identified in carp. The co-transporters were Na⁺ dependent and specific for orthophosphates Pi, similar to those in mammals and birds (Nakamura, 1985). Later on, NaPi transporters were reported in flounder (Kohl et al., 1996) and rainbow trout (Avila et al., 2000; Sugiura et al., 2003). At high dietary P concentrations, passive diffusion is likely to be predominant and active transport is down-regulated (Avila et al., 2000). Higher Pi uptake was observed in the proximal intestine (Avila et al., 2000), which was in agreement with the reduction in the expression of NaPi transporters along intestinal tract (Sugiura et al., 2003). However, in fish, in contrast to the differentiation of NaPi-IIa in kidney and NaPi-IIb in intestine in mammals, NaPi-IIb is found both in intestine and kidney, specifically, NaPi-IIb1 in intestine and NaPi-IIb2 in kidney (Werner and Kinne, 2001).

It has been observed that pyloric cecum is also a site of Pi absorption in rainbow trout, where similarly to the mechanism of intestinal absorption, Pi is absorbed through passive diffusion and carrier-mediated active transport, dominated by passive diffusion (over 92%) (Sugiura and Ferraris, 2004). The active transport was also carried out by NaPi cotransporters. The nucleotide sequence of NaPi-II isomer in pyloric ceca was ~ 8% different from that of in intestine, but the Na-dependency was influenced by pH (Sugiura and Ferraris, 2004). Due to the large surface area of pyloric ceca, Sugiura and Ferraris (2004) estimated that pyloric ceca accounted for approximately 90% of Pi absorption in rainbow trout. Due to the predominant passive absorption mechanism of pyloric ceca, the authors speculated that Pi absorption in trout is largely unregulated, thus endocrinological and physiological approaches to enhance Pi absorption are limited. More research is warranted to examine this hypothesis.

2.2.1.2 Renal Handling

For monogastric animals, the major regulator of extracellular Pi homeostasis is the kidney (Tenenhouse, 1997). In mammals, renal handling of Pi is balanced by glomerular filtration and tubular reabsorption, the latter is rate-limiting and undertaken by NaPi cotransporters (Murer and Biber, 1992). Proximal tubule is the main site of phosphate reabsorption, and 70% to 80% of filtered phosphate is reabsorbed (Beck and Silve, 2001). For tubular reabsorption, the entry of Pi across apical luminal membrane into epithelial cells is undertaken by NaPi cotransporters against electrochemical gradients, the exit of Pi into peritubular interstitium has been suggested to be actively transported by type III NaPi

cotransporters (Bai et al., 2000; Werner and Kinne, 2001; Collins et al., 2004). A variety of hormonal and non-hormonal factors control Pi tubular reabsorption. These factors include parathyroid hormones, insulin, growth hormones, calcitonin, and phosphate deprivation (Murer and Biber, 1992).

It has been observed that fish are capable of net tubular secretion or net reabsorption depending on Pi load (Bijouvet and Reitsma, 1977; Kaune and Hentschel, 1987). This is a mechanism unique in fish and birds (Renfro and Gupta 1990). In the renal tubular secretive state, Pi is transported to epithelial cells across basolateral membrane from interstitium (blood) by NaPi transporters, and exits across apical membrane to the lumen (Schwab and Hammerman, 1986). In the tubular absorptive state, filtered Pi is transported by NaPi transporters across apical membrane from tubule lumen to epithelial cell and exit to interstitium, which is a mechanism apparently similar to that found in mammals (Renfro, 1997).

Recent development in molecular identification has suggested differences in mechanisms of tubular secretion and reabsorption between seawater fish and freshwater fish. Freshwater and euryhaline fish (fish capable of residing in freshwater and seawater) have typical vertebrate nephrons that are comprised of glomerulus, proximal tubule, distal tubule, and collecting duct (Beyenbach, 2004). General fish renal morphology indicated that proximal renal tubules consist of two distinct tubular segments, PI and PII. At segment PI, columnar cells have basal nuclei and bear short apical tubules, apical vacuoles and lysosomes, whereas at PII the columnar cells are taller with central nuclei and abundant

mitochondria throughout cytoplasm (Anderson and Loewen, 1975). In stenohaline seawater fish (fish residing only in seawater), glomerulus and distal tubule are reduced in size and function, or glomerulus can be entirely absent (Beyenbach, 2004). The role of glomerular filtration is diminished and that of renal tubules is promoted in seawater fish to counteract the challenge of hyperosmotic environment (Beyenbach, 2004).

In flounder, a euryhaline teleost, the NaPi cotransporters are located at the basolateral membrane of the secreting tubule PII and in the apical membrane in the collecting duct (Kohl et al., 1996; Elger et al., 1998). The location of NaPi transporters, on basolateral or apical membrane of the tubule, determines the function of transporters as secretive or reabsorptive. Therefore, the proposed mechanism of renal handling in flounder fish is that segment PII is an active secretion site of Pi and collecting duct is responsible for Pi reabsorption (Elger et al., 1998; Werner et al., 2001). In zebrafish, a freshwater fish, NaPi-IIb transporters are located at apical membrane of PII segment and collecting duct (Graham et al., 2003). This suggests that in zebrafish, renal handling of Pi is through glomerular filtration followed by NaPi-IIb reabsorption in the PII region and collecting duct (Werner et al., 2001). Similarly, in trout, it has been found that NaPi-IIb transporters are located only at apical membrane of the PI region, thus the function of the transporters is to reabsorb filtered Pi, and the renal handling of Pi in trout includes glomerular filtration and tubular reabsorption (Sugiura et al., 2003).

2.2.1.3 Bone Mineralization

Bone is the major storage site of minerals. About 98% of total body Ca and up to 85% of total body P are found in bone. Bone is made of collagen fibers, noncollagen proteins and minerals. The minerals are primarily in the form of hydroxyapatite, which is deposited in the organic matrix (Lawrence and Fowler, 1997). Comparison of chemical composition of fish bones to bovine bones indicates that they are similar in cationic composition, but fish bones are higher in P and lower in carbonate content (Weiss and Watabe, 1978).

There are three groups of bone cells in bones, including osteoblasts, osteoclasts, and osteocytes. Osteoblasts secrete the intercellular organic matrix, which calcifies and forms bone, whereas osteoclasts are responsible for the resorption of bone. Osteocytes, the trapped osteoblasts within organic matrix, have poorly-defined functions (Lawrence and Fowler, 1997). Two distinct processes apply to bone growth and mineralization: modeling and remodeling. Osteoblasts and osteoclasts are involved in both processes. In modeling process, osteoblasts and osteoclasts act independently, whereas in the remodeling process, they act cooperatively (Miller 2003). Under normal physiological conditions, the resorption and formation of bone are in equilibrium (Dorozhkin and Epple, 2002).

Fish bones of most fish species lack enclosed osteocytes. Accordingly, fish bones are commonly classified into two types: cellular bone and acellular bone (Moss, 1963). Cellular bones are only found in several groups of fish, mainly in low orders of teleost fish, such as salmonidae, cyprinadae and clupeidae. Bones of most teleost fish are acellular. Acellular bones do not have the ability to undergo extensive modeling and their apatite crystals are

smaller than those of cellular bones have (Lall, 2002). However, resorption capability of acellular bone does not seem to be affected (Lall, 2002).

2.2.2 Regulation of Phosphorus Homeostasis

In monogastric animals, phosphorus homeostasis is achieved through the cooperation between bone, kidney, and intestine: bone is a storage site of P, kidney controls P excretion, and intestine regulates dietary P absorption (Berner, 1997). Phosphorus homeostasis is subject to hormonal and non-hormonal signals. In fish, the regulation of P homeostasis appears to have distinct features.

2.2.2.1 Hormonal Regulation

Hormonal regulation of P homeostasis in mammals and birds is primarily maintained by parathyroid hormone (PTH), calcitonin and vitamin D₃, all of which are also important in the regulation of calcium homeostasis (Schröder et al., 1996). Vitamin D₃ is the most important hormone in regulating Pi intestinal absorption, whereas PTH and calcitonin are important in bone P mobilization and renal Pi handling (Schröder et al., 1996).

Hormonal regulation of P homeostasis in fish is still poorly understood. Fish do not possess parathyroid gland, thus lack PTH. Vitamin D₃ also does not seem to have a major effect. Although there are two antihypercalcemic hormones, calcitonin and stanniocalcin

(STC), which may regulate various aspects of P metabolism in fish (Lall, 2002), it has been suggested that the primary regulator of P metabolism in fish is dietary P concentration (Coloso et al., 2003b).

2.2.2.1.1 Vitamin D Metabolites

Vitamin D occurs as two natural products: ergocalciferol (vitamin D₂) from plant sources, cholecalciferol (vitamin D₃) from animal sources. Vitamin D₃, a steroid hormone, is metabolized in the liver to 25-hydroxy vitamin D₃, which is further hydrolyzed to 1, 25-(OH)₂-D₃ (calcitriol) in the kidney. Calcitriol is the most biologically active metabolite. The major biological function of vitamin D₃ is to maintain plasma Ca within a narrow range (Holick, 1996). Calcitriol stimulates Pi intestinal absorption by increasing V_{max} of the carrier system (Schröder et al., 1996), activating Pi extrusion from enterocyte across basolateral membrane (Civitelli and Avioli, 1994), and facilitating the intracellular movement of Pi inside the enterocyte (Jungluth and Binswanger, 1989). Calcitriol and other vitamin D₃ metabolites also enhance bone resorption and bone mineralization. Calcitriol responds to the decrease in plasma Ca level by stimulating the formation of osteoclasts in the bone, mobilizing Ca from bone, thus simultaneously releasing phosphate from the bone (Holick, 1996). Calcitriol can suppress PTH secretion, thus exerts indirect effect on renal handling (Berner, 1997; Jones et al., 1998). Another vitamin D₃ metabolite, 24, 25-(OH)₂-D₃ can enhance bone mineralization to lower plasma P concentration (Backström et al., 1996; Berner, 1997).

The role of vitamin D₃ metabolites in P homeostasis of fish has not been well characterized. Some fish species have storage of cholecalciferol in liver (Takeuchi et al., 1987). In fish, it was observed that 25-hydroxy vitamin D₃ was converted to 1, 25-(OH)₂-D₃ (calcitriol) primarily in the liver rather than in the kidney (Sunita Rao and Raghuramulu, 1998). In tilapia, intraperitoneal injection of vitamin D₃ and its metabolites had no effect on serum Ca and P level, or intestinal Ca absorption, or whole-body Ca and P uptake, or gill calcium binding protein activity (Sunita Rao and Raghuramulu, 1999b). Intraperitoneal injection of vitamin D₃ affected P homeostasis by increasing renal Pi reabsorption and causing hyperphosphatemia (Fenwick and Vermette, 1989). However, dietary supplemented cholecalciferol did not affect Pi intestinal absorption (Avila et al., 1999; Coloso et al., 2003b), nor did it affect P digestibility, bone mineralization and whole body P concentration (Vielma et al., 1998). Dietary supplemented cholecalciferol increased plasma phosphate concentration in some studies (Avila et al., 1999; Coloso et al., 2003b), but not in others (Vielma et al., 1998, 1999a). It did not affect renal NaPi transporter expression measured by mRNA abundance (Coloso et al., 2003b), or urinary P concentration in rainbow trout (Vielma et al., 1999a). Very high level of dietary cholecalciferol (250,000 and 2,500,000 IU/kg) resulted in reduction of growth rate and higher deposition of Ca, Mg and Zn in the kidney of rainbow trout (Vielma et al., 1998).

The role of vitamin D₃ in fish is still inconclusive and may well be different from that of mammals. It is speculated that vitamin D₃ is not required for Ca and P homeostasis in fish, because the calcium rich aquatic environment renders the regulating role of vitamin D₃

unnecessary (Sunita Rao and Raghuramulu, 1999a, b). Further research is required to determine whether vitamin D₃ regulates P metabolism of fish.

2.2.2.1.2 Parathyroid Hormone (PTH)

In mammals and birds, the NaPi transport system on proximal renal tubule is highly sensitive to parathyroid hormone (PTH) (Cross et al., 1990; Schröder et al., 1996). By inhibition of tubular Pi reabsorption, PTH increases renal excretion of Pi to reduce plasma Pi concentration (Littledike and Goff, 1987; Tenenhouse, 1997). It was long believed that the effect of PTH on intestinal absorption of mammal and birds was indirect through stimulation of calcitriol production in the kidney. However, there have been reports that there are specific PTH receptors in the intestinal cell membrane and PTH plays important direct role in enhancing Pi absorption in the intestine (Nemere, 1996; Nemere and Larsson, 2002). The effect of PTH on bone function is two-fold: homeostasis, where PTH directs osteoblasts to pump Ca and P ions from bone fluid compartment to extracellular fluid, and bone remodeling, where the effect of PTH is exerted on osteoclasts (Anderson, 1991).

There is no parathyroid gland, and consequently no PTH in fish. However, recent identification of parathyroid hormone-related protein (PTHrP) and PTH-like proteins in zebrafish (Graham et al., 2003; Gensure et al., 2004) may reveal new information on hormonal regulation of P homeostasis in fish, although their function remains unknown at present.

2.2.2.1.3 Calcitonin

In terrestrial vertebrates, calcitonin primarily works antagonistically against vitamin D₃ and PTH as an antihypercalcemic agent (Lall, 2002). The primary role of calcitonin is to regulate bone resorption (Weisbrode and Capen, 1974; Jones et al., 1998). Calcitonin can also depress renal Pi reabsorption directly or synergistically with PTH, thus enhance renal Pi excretion (Lang et al., 1981).

In fish, calcitonin inhibits Ca transport across gill (Wagner et al., 1997). However, calcitonin did not appear to affect P homeostasis (Lall, 2002). Exogenous administration of calcitonin showed inconsistent effects on P serum concentration in freshwater mud eel (Srivastav et al., 1998).

2.2.2.1.4 Stanniocalcin

Stanniocalcin (STC) is a glycoprotein hormone secreted from corpuscles of Stannius in fish (Lall, 2002). Similar to calcitonin, STC is an antihypercalcemic hormone, but its function overrides calcitonin (Ishibashi and Imai, 2002). In fish, STC reduces Ca absorption from the gill, stimulates Pi reabsorption in kidney, and probably promotes deposition of Ca and P in bones (Lu et al., 1994).

Stanniocalcin was initially believed to be unique in fish, but was subsequently discovered in humans and rats (Olsen et al., 1996). Stanniocalcin contributes to mammalian Pi homeostasis by enhancing renal Pi reabsorption (Wagner et al., 1997). Human recombinant STC increased intestinal absorption of P but decreased Ca absorption in swine and rat (Madsen et al., 1998). Human STC is expressed in many tissues, suggesting a paracrine role (Ishibashi and Imai, 2002).

2.2.2.1.5 Other Hormones

A variety of hormones in addition to those mentioned above may also affect intestinal absorption of Pi in monogastric mammals and birds. Thyroid hormones increase synthesis of the NaPi receptors thereby stimulating Pi translocation through enterocytes (Cross et al., 1990). Synergetic effect between calcitriol and thyroid hormones was suggested (Cross and Peterlik, 1988). Hormones such as insulin, glucagon, and growth hormones can increase renal Pi reabsorption in mammals (Berner, 1997; Tenenhouse, 1997).

In fish, pituitary hormones have putative roles in Pi intestinal absorption because hypophysectomy had a negative impact on P absorption (Nakamura and Hirano, 1986). However, the pituitary gland does not appear to be a significant regulator of plasma Pi concentration (Lall 2002). Bovine parathyroid hormone, ovine prolactin, and somatolactin were observed to increase net Pi reabsorption by renal proximal tubule in flounder (Lu et al., 1994, 1995).

2.2.2.2 Effects of Dietary P on Homeostasis

In addition to hormonal signals, it is particularly worth noting that dietary P levels affect Pi intestinal absorption. In mammals and birds, low P diets result in up-regulation of intestinal Pi absorption (Cross et al., 1990) by increasing number of NaPi-IIb cotransporters in the apical membrane (Hattenhauer et al., 1999). This effect is believed to be non-acute and indirect (Hattenhauer et al., 1999) and attributed to the regulation of vitamin D₃ in response to decrease in serum Pi concentration (Cross et al., 1990; Schröder et al., 1996). On the other hand, it was recently reported that intestinal NaPi transporters could adapt to low dietary P intake independent of vitamin D₃ (Segawa et al., 2004). In renal proximal tubules, low P diets enhance P reabsorption through acute adaptation of NaPi-IIb transporters (Levi et al., 1996, Lötscher et al., 1996)

In fish, dietary P level is presumably the major regulator of P metabolism (Coloso et al., 2003b). Intestinal Pi absorption was upregulated at restricted dietary P level and down regulated at high dietary P level in rainbow trout (Avila et al., 2000). Low P diet resulted in reductions in plasma Pi concentration (Coloso et al., 2003b). Expression of intestinal and renal NaPi cotransporters was enhanced by decreasing dietary P (Coloso et al., 2001; Coloso et al., 2003b). The molecular mechanism of upregulation by low dietary P is still unknown. Low dietary P increased intestinal P absorption but not in pyloric ceca (Sugiura et al., 2004).

2.3 Phosphorus Requirement and Utilization by Salmonids

2.3.1 Phosphorus Requirement by Salmonids

2.3.1.1 Deficiency and Mineral Interactions

Phosphorus is an essential dietary element for fish to maintain proper body function and adequate growth (NRC, 1993). The common P deficiency signs include depressed growth, poor feed efficiency, bone malformation, and impaired bone mineralization (Ogino and Takeda, 1976, 1978), and low hematocrit levels (Andrews et al., 1973). Phosphorus deficiency often results in increasing body fat deposition, as observed by many researchers (Sakamoto and Yone, 1978; Takeuchi and Nakazoe, 1981; Rodehutsord, 1996). In severe cases of P deficiency, mortality occurs. The exhibition of deficiency signs depends on the severity of dietary P restriction (Baeverfjord et al., 1998). Fish can mobilize body stores during subclinical deficiency without exhibition of overt deficiency signs (Hardy et al., 1993; Baeverfjord et al., 1998).

On the other hand, excessively high levels of dietary phosphorus can also be deleterious to fish health. High P dietary contents result in reduced feed efficiency, impaired growth, mortality, skeletal deformity, and cataracts (Ketola, 1979; Satoh et al. 1987; Shearer et al., 1992; Porn-Ngam et al., 1993; Satoh et al., 1993; Satoh et al., 1996). This effect could be due to the interaction between P and trace minerals, for example, Zn, consequently, the

utilization of trace mineral is impaired, and dysfunction of body metabolism occurs (Lall, 2002).

2.3.1.2 Phosphorus Requirement Values

The reported values for phosphorus requirement vary significantly. As summarized in Table 2.1, requirement values for salmonid reported in the literature range from 0.34 to 1.1% of diet.

Table 2.1 Phosphorus requirement values for salmonid fish.

Species	Requirement (% of diet)	Reference
Rainbow trout	0.37 - 0.56	Rodehutscord, 1996
Rainbow trout	0.34 - 0.54	Ketola and Richmond, 1994
Rainbow trout	0.7 - 0.8	Ogino and Takeda, 1978
Atlantic salmon	1.0 - 1.1	Asgard and Shearer, 1997
Atlantic salmon	0.6	Lall and Bishop, 1977
Atlantic salmon	0.6	Ketola, 1975

The significant variations in requirement estimates can be attributed to a variety of factors. The estimate of nutrient requirement is often based on experimental observations of ‘optimal’ response of experimental animals to graded levels of nutrient input (Mercer et al.,

1986). The 'requirement' is vague, and depends on the choice of desired response parameters and levels (Mercer et al., 1986). A number of variables have been used to estimate the requirement of P. These include live weight gain, feed efficiency, body P concentrations, bone mineralization, bone breaking strength, and enzyme activities. It has been observed that P concentrations of bone, skin and scales are more sensitive to P dietary level than whole body P levels (Skonberg et al., 1997). The dietary P level supporting maximum growth was lower than that of resulting in body saturation (Andrews et al., 1973; Rodehutscord, 1996). Therefore, requirement is not a single value; rather, it should be defined with respect to response variables.

Even within a given response variable, there are still a variety of factors that could contribute to the variability of estimate from different studies. Choice of statistical methods and fitting models to obtain the plateau of response can result in differences in requirement estimates (Baker, 1986; Remmenga et al., 1997; Rodehutscord and Pack, 1999). Differences in experimental period may also result in variations of requirement estimates. Mercer et al. (1993) illustrated that insufficient experimental period may result in underestimate of requirement, especially when growth was chosen as the response variable. Growth may not be affected in a short period in the case of subclinical P deficiency due to the contribution of body storage. Dietary history or P status at the beginning of the experiment also affects that estimate of P requirement. Requirement values obtained with fish fed P deficient diet or starved before the experiment might be higher than values obtained from fish fed P sufficient diet previously (Sugiura et al., 2000b). Furthermore, size and genetic difference could also contribute to the variability of estimates of requirement. It has been observed that smaller

fish require more dietary P (% diet) than large fish (Sugiura et al., 2000b). This is due to the fact as fish grow, muscle growth outpaces bone growth, and the concentration of P as primary bone component decreases in body, therefore large fish requires less dietary P to support body saturation (Shearer, 1994)

Experimental diets vary in nutrient composition across different requirement studies. Feed efficiency and nutrient composition of the experimental diets, especially the digestible energy density, can significantly affect estimates of P requirement. Requirement values based on total P or digestible P also contribute to variability of requirement values from different studies. Phosphorus requirement of fish has been expressed as diet content (% total P/digestible P in diet), or per unit of dietary digestible energy. Requirement expressed as a percentage of diet is not always reliable because diets can vary significantly in nutrient composition. Because the digestible energy of diets grossly determines the feed efficiency (weight gain/feed intake) and the consequent amount of P ingested per unit of weight gain, it has been recommended to express dietary requirement as digestible phosphorus per unit of digestible energy (g/MJ DE), rather than as a percentage of diet content (Rodehutscord, 1996).

The conventional method to estimate nutrient requirement through mass balance experiments limits the extrapolation of the experimental results (Baker, 1986). It would be more appropriate to use a factorial approach, which can adequately describe P requirements by accounting for major factors affecting requirement such as digestibility, utilization efficiency, and feed efficiency (Shearer, 1995). A simplified factorial model of P requirement

from Shearer (1995) can be expressed as: $P \text{ required (\%)} = (P \text{ retained (g)} / P \text{ digestibility (\%)})/ \text{feed intake (g)}$.

2.3.2 Phosphorus Contents and Chemical Compounds in Feed Ingredients

Table 2.2 presents the P contents in common feed ingredients for salmonid fish.

Table 2.2 Phosphorus content of common fish feed ingredients on dry matter basis.

Ingredient	P content (g/kg)¹
Fish meal	10.8 - 41.9
Meat and bone meal	24.9 - 70.8
Poultry by-product meal	16.5 - 34.5
Blood meal	0.8 – 17.1
Feather meal	5.4 - 12.6
Corn gluten meal	4.4 - 5.5
Soybean meal	6.4 - 8.5
Wheat middling	9.7 - 11.7

¹summarized from NRC (1993, 1994, 1998), Dale (2000), Sugiura et al. (1998b, 2000c), Sugiura and Hardy (2000).

As illustrated by Table 2.2, P contents of ingredients are highly variable. Animal protein ingredients (fish meal, poultry by-product meal, and meat and bone meal) generally have high P content and are frequently major contributors to P contents of fish feed. Animal protein ingredients are produced with a wide variety of raw materials (different fish species, whole fish versus filleting by products, various animal by-products from slaughter houses) and manufacturing techniques and equipment (Prokop, 1996; Bureau et al., 1999). The variation of P content is significant even within ingredient type. In these animal by-products ingredients, P is primarily bound with calcium in hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) deposited in the bone matrix. Hydroxyapatite has a hexagonal crystal structure (Figure 2.1), which is built from columns of calcium ions (Ca^{2+}) and oxygen atoms (O) of phosphate anions (PO_4). These ions form the walls of channels. These channels run parallel to the hexagonal axis, and contain hydroxyl ions (OH) (Corbridge, 1990; Berner, 1997). Hydroxyapatite is insoluble in water (Corbridge, 1990). In addition to the phosphates deposited in bone, phosphorus is also covalently bound to protein, lipid or carbohydrate in the animal by-products, or exists in nucleic acid. There is very little information on content and proportion of P chemical compounds in these ingredients.

In plant ingredients, a majority of P is bound in organic compounds as *myo*-inositol hexaphosphoric acid (phytic acid) and its salt, *myo*-inositol hexaphosphate (phytate-P). Phytic acid consists of inositol ring structure attached with six phosphate groups (Figure 2.2). Six of the 12 protons have pKa of 1.5, which implies very strong chelating ability to bind cations and proteins (Maenz, 2001). When it binds divalent or trivalent cations such as Ca, Mg, Zn, Fe, Cu, it may form a stable complex and is unavailable except to specific enzyme

(Figure 2.3). The calcium-magnesium salt of phytic acid is often referred to as phytin (Maenz, 2001). In plant source ingredients, the phytate-P accounts for between 60 and 80% of the total phosphorus (Ravindran et al., 1995).

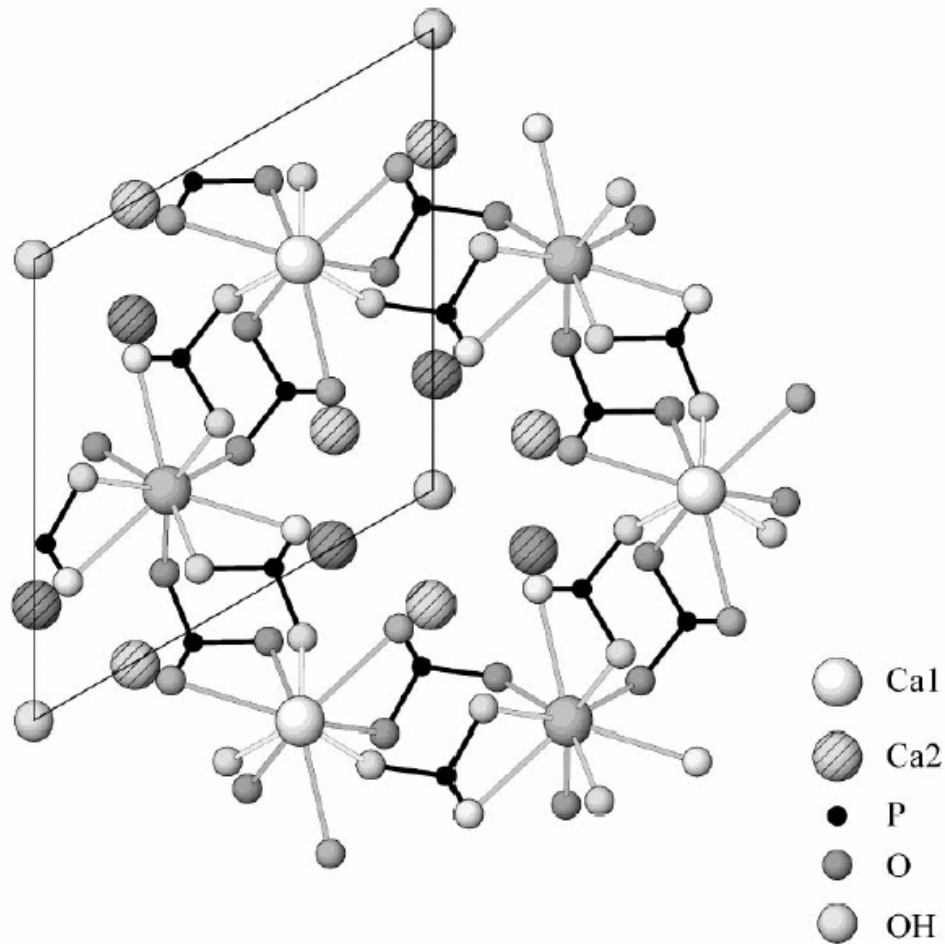


Figure 2.1 Structure of hydroxyapatite (Ivanova et al., 2001).

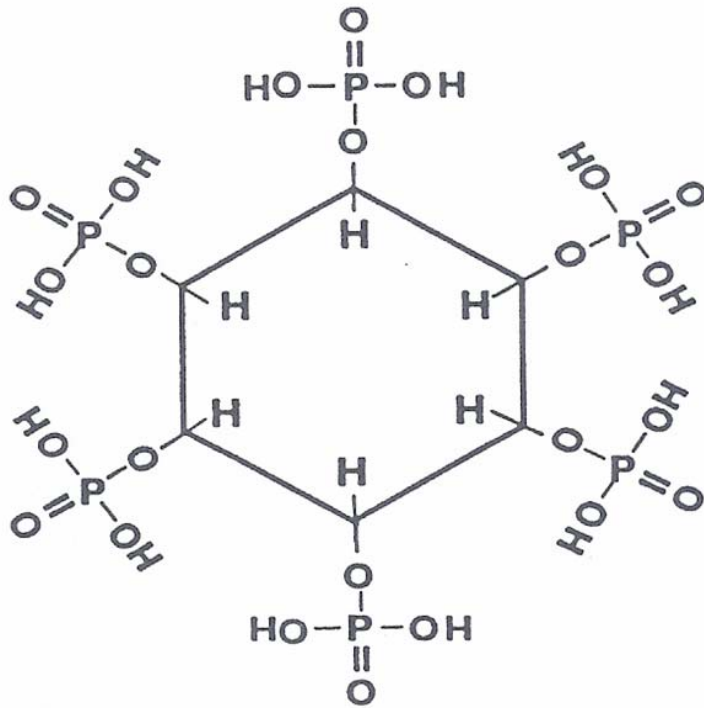


Figure 2.2 Structure of phytic acid (Anderson, 1914; Sebastian et al. 1998).

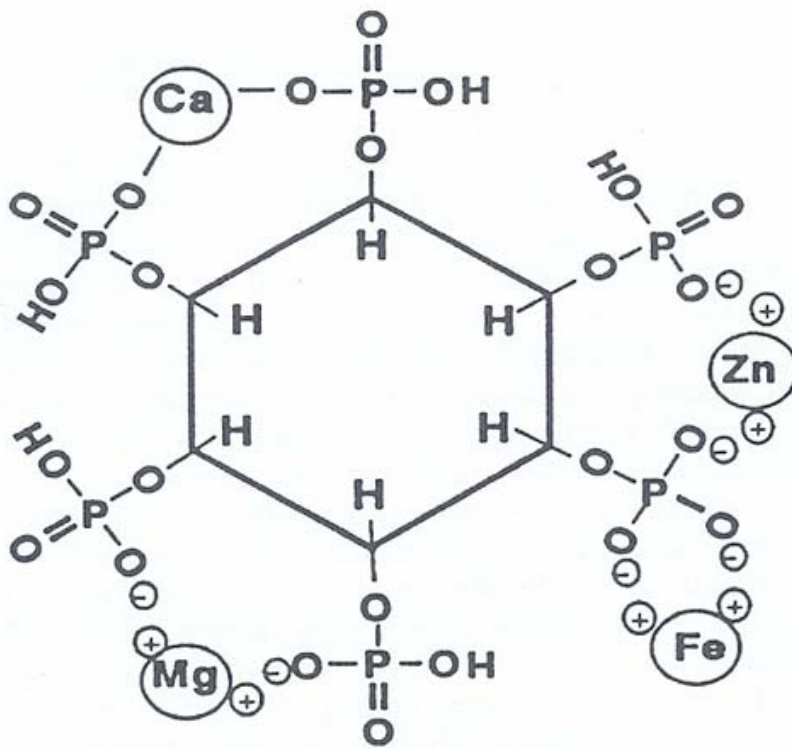


Figure 2.3 Structure of a phytic acid chelate at neutral pH (Erdman, 1979; Sebastian et al. 1998).

2.3.3 Phosphorus Digestibility by Salmonids

2.3.3.1 Apparent Digestibility as Estimate of Bioavailability

Digestibility is often used to evaluate the bioavailability of nutrients, which is defined as the amount of the nutrient that can be absorbed and utilized by animals (Ammerman, 1995). Digestibility is commonly differentiated as apparent digestibility and true digestibility (standardized digestibility by endogenous losses). The apparent digestibility of a nutrient is the proportion of the nutrient that disappears from the gastrointestinal tract, measured by the difference between nutrient intake and excretion (Ammerman, 1995). Endogenous excretion, the loss of mucosal cells and those already absorbed into body and re-secreted into the tract, is not accounted for in apparent digestibility, in contrast to true digestibility (Ammerman, 1995). Therefore, true digestibility is always higher than apparent digestibility, and is considered more precise than apparent digestibility as an indicator of bioavailability of a nutrient. The difference between true and apparent digestibility diminishes as dietary nutrient content increases, because the contribution of endogenous loss is related to dietary nutrient content negatively and exponentially, as observed for P in pigs (Fan et al., 2001; Shen et al., 2001; Ajakaiye et al., 2003).

In fish, few studies have investigated the true P digestibility of feed ingredients, and generally, apparent P digestibility is used in feed formulation. Endogenous P loss of rainbow

trout was measured using a low-P purified diet for rainbow trout, and was observed to be highly variable with large standard errors (Riche and Brown, 1996). The endogenous loss was related to fish body weight, ranging from 499.7 ± 224.8 to 76.6 ± 19.4 mg/kg body weight from day 1 to day 10, averaged at 225 mg/kg body weight per day (Riche and Brown, 1996). Apparent digestibility was similar to true digestibility of fish feed ingredients, in the same study and a subsequent study (Riche and Brown, 1996, 1999). From a practical point of view, the difference between true and apparent digestibility of nutrients in fish feed formulation is insignificant (Hardy, 1997).

2.3.3.2 Phosphorus Apparent Digestibility Values of Common Ingredients

Estimates of the apparent digestibility coefficient (ADC) of P in ingredients differ significantly. Table 2.3 presents the ADC values of P of common feed ingredients for rainbow trout.

Table 2.3 Apparent digestibility coefficients (ADC) of P in common ingredients in salmonid diets.

Ingredient	ADC (%) of P¹
Fish meal	17 - 81
Meat and bone meal	22 - 67
Poultry by-product meal	38 - 66
Blood meal	70 - 104
Feather meal	68 - 82
Corn gluten meal	< 10
Soybean meal	27 - 46
NaH ₂ PO ₄	95 - 98
Ca(H ₂ PO ₄) ₂	93 - 94
CaHPO ₄	54 - 77
Ca ₁₀ (OH) ₂ (PO ₄) ₆ or Ca ₃ (PO ₄) ₂	37 - 64

¹Apparent digestibility coefficients of phosphorus were summarized from Ogino et al. (1979), Riche and Brown (1996), Sugiura et al. (1998a), Gregus (2000), Sugiura and Hardy (2000), Sugiura et al. (2000c), Satoh et al. (2002).

2.3.3.3 Factors Affecting Phosphorus Apparent Digestibility in Ingredients

The wide variation of the ADC of P is primarily due to characteristics of P chemical compounds and their contents in feed ingredients. As mentioned, P is found in ingredients and feeds as several types of chemical compounds, namely hydroxyapatite (bone-P), *myo*-inositol hexaphosphate (phytate-P), and P compounds covalently linked to protein, lipid and sugar (organic P). Phosphorus is supplemented in fish diets in the forms of monobasic and dibasic phosphates supplements. The chemical characteristics of these P compounds affect P absorption because Pi (H_2PO_4^- , HPO_4^{2-}) has to be hydrolyzed first from these compounds to render it available for absorption across fish intestine.

Due to its complex structure, phytate can only be absorbed by animals when the phosphoester bonds are hydrolyzed. This renders major problems for monogastric animals, a majority of which do not possess phytase and cannot digest phytate-P effectively. Although it has been observed that chicks, especially older birds can utilize up to 50% of dietary phytate, there is controversy regarding whether chicks possess phytase in the intestine or the utilization is the action of non-specific phosphatases (Ravindran et al., 1995). Microbes in the intestine may also contribute to the hydrolysis of phytate in birds (Ravindran et al., 1995). Older birds were observed to be able to digest phytate better than young chicks (Underwood and Suttle, 1999). Phytate is heat stable. There have been inconsistent observations whether autoclaving at high temperature or incubation in water at moderate temperature can cause destruction of phytate and make it more available (Ravindran et al., 1995).

Fish do not possess the necessary digestive enzyme, phytase, in digestive tract, and consequently the digestibility of phytate in fish is poor. Phytate apparent digestibility in fish has been reported to be between 0 and 19% (Ogino et al., 1979; Lall, 1991). This is supported by the observations that the ADC values of P for plant ingredients are low to rainbow trout, for example, 20 - 35% for soybean meal, and less than 10% for corn gluten meal (Sato et al., 2002; Sugiura and Hardy, 2000).

Organic P covalently linked to protein, lipid and sugar is presumably highly digestible to fish. For example, ADCs of P in casein and yeast were 90% and 91%, respectively, to rainbow trout (Ogino et al., 1979).

While the hydrolysis of Pi from organic P and phytate requires enzymatic actions, the hydrolysis of bone P and dietary inorganic phosphates supplements is dependent on their solubility in gastrointestinal tract. The bioavailability of minerals is affected by gastric acidity (Wood and Serfaty-Lacroisniere, 1992). Bone particles can be completely or partially hydrolyzed (Kionka and Windell, 1972; dos Santos and Jobling, 1991). Stomachless fish such as carp cannot effectively utilize P in fish meal (Ogino et al., 1979). Dietary organic acid supplementation was observed to improve P digestibility (Sugiura et al., 1998a; Vielma and Lall, 1997). Therefore, the digestibility of bone-P may be limited by the acid output of the stomach of the fish. Sugiura et al. (2000c) suggested that ADC of bone-P is dependent on the inclusion level. It was fairly digestible (66%) at low concentration (0.47% of dry matter, DM) in the diet, whereas its digestibility decreased significantly to only 13% as the inclusion

of bone-P increased to 17% of DM. Solubility is the main influencing factor of P digestibility among inorganic phosphates supplements, with monobasic phosphates more digestible than dibasic phosphates (Lall, 1991). Digestibility of monobasic calcium phosphate is over 90%, in contrast, the digestibility of dibasic calcium phosphate is around 70% (Ogino et al., 1979; Lall, 1991).

There have been reports that high dietary P levels may depress digestibility of P in fish (Vielma and Lall, 1998a; Rodehutsord et al., 2000; Green et al., 2002). Conversely, high level of dietary P did not depress apparent digestibility of P in some studies (Nakamura, 1982; Satoh et al., 1996). This suggests that digestibility of P is not a simple function of dietary P level. Differences in the chemical characteristics and solubility of these compounds are likely to result in different digestion dynamics of P within the animal gastrointestinal tract and this, in turn, can significantly affect P digestibility.

It is well known that the Ca and P ratio in diets of terrestrial monogastric animals affect for their digestibility. Ca and P interactions can significantly affect the absorption of both minerals. Excess Ca or P results in precipitation of insoluble calcium phosphates in the intestine and subsequent reduction in absorption of both Ca and P. Therefore, in swine and poultry, an optimal dietary Ca and P ratio is recommended. For grain-soybean meal based swine diets, the recommended the Ca to total P ratio is about 1:1, and the Ca to available P ratio is between 2:1 and 3:1 (NRC, 1994). For most poultry diets, the Ca to nonphytate-P ratio is recommended to be maintained at 2:1 (NRC, 1998). Fish can absorb Ca from aquatic environment to meet their requirement for Ca (NRC, 1993). Increments of dietary Ca were

observed to depress dietary P digestibility linearly in rainbow trout (Sato et al., 1993; Encarnacao, 1997), and in carp (Nakamura, 1982). However, tissue mineralization or fish growth does not seem to be affected by Ca/P ratio as long as dietary P is sufficient and Ca is present in rearing water (Lovell, 1978; Watanabe et al., 1980; Wilson et al., 1982; Shim and Ho, 1989; Vielma and Lall 1998b, Chavez-Sanchez et al., 2000).

2.3.4 Phosphorus Deposition in Salmonids

2.3.4.1 Phosphorus Body Concentration of Salmonid

Body composition of cultured fish is affected by endogenous factors, such as genetics, size and life stage, and exogenous factors, such as environment (temperature, salinity) and diet (diet composition, feeding regime) (Shearer, 1994).

Dietary P level is perhaps the primary cause of variation of P concentration in fish. P deficiency impairs bone mineralization and results in low P body concentration (Lall, 1991). Bone is the major storage site of P. Fish can mobilize body storage in subclinical deficiency without exhibition of overt deficiency signs (Baeverfjord et al., 1998). Rainbow trout can deplete as much as 75% of normal body P concentration without affecting body function (Hardy et al., 1993).

Genetic differences may contribute to the variations of fish body P concentration. There was significant genotypic difference in body protein and ash content between strains of rainbow trout (Reinitz et al., 1979). Interspecies genetic differences in ash concentration have also been observed for lamprey, trout and largemouth bass (Niimi, 1974). It is probable that genetic difference of body composition between species and strains implies genetic difference in P body concentration. However, there has been no study on this aspect.

Fish size/body weight affect P body concentration in fish. It was observed that whole P body concentration increased with body weight in juvenile rainbow trout and decreased with body weight in post-juveniles (Shearer, 1984). The reason could be that muscle growth outpaces bone growth in juvenile to adult stage, so that concentration of P as primary bone component decreases in body (Shearer, 1994).

In vertebrates, bone is the major storage site of P. The majority of P (80- 85%) exists in skeletal tissues as hydroxyapatite, less than 14% is organic P in various soft tissues and organs, and only very small amount is presented as free ions or soluble inorganic P (Pi) in blood and body fluids (Lall, 1991; Berner, 1997). Therefore, a change of the proportion of bone mass and soft tissue mass would result in change in body P concentration. Numerous factors could potentially affect the proportion of bone mass and soft tissue mass. These factors remain to be elucidated.

2.3.4.2 Phosphorus Deposition

Literature reports of percent of dietary P retained of rainbow trout diets varies from 15 to 75%, whereas percent of digestible P retained varies from 48 to 92%, when dietary P concentration ranged from 0.4 to 3.0% of diet in these studies (Wiesmann et al., 1988; Heinen et al., 1993; Ketola and Harland, 1993; Lanari et al., 1995; Satoh et al., 1996; Kim et al., 1998; Medale et al., 1998; Ruohonen et al., 1999; Barrias and Oliva-Teles, 2000; Johnson and Summerfelt, 2000; Green et al., 2002; Lanari and D'Agaro, 2002; Vielma et al., 2002; Coloso et al., 2003a).

A significant number of factors affect P retention efficiency, including dietary P level, digestibility of P, and requirement of fish. Because P retention in fish is commonly calculated as the difference of body P content at initial and final body weight, the above mentioned various factors affecting body P concentration eventually contribute to the variability of P retention efficiency as percent of digestible P intake. The varying digestibility of P of experimental diets further amplifies the difference in P retention efficiency expressed as percent of dietary P intake.

2.3.5 Phosphorus Excretion and Waste Output

Dietary P not retained by fish is released into the environment. The undigested fraction of the P of the diet is egested in the feces by fish, whereas digestible P supplied over maintenance and growth is excreted mostly as phosphates via the urine. Similar to the excretion mechanism in mammals, urinary phosphate excretion in fish is determined

primarily by plasma phosphate concentration. In trout, the excretion threshold has been determined to be approximately 86 mg/L, below which phosphate excretion is minimal and above which phosphate excretion is proportional to the increase in plasma phosphate (Bureau and Cho, 1999).

Fecal P and urinary P represent the bulk of solid waste and soluble waste, respectively. Reports of total P waste, solid P waste, and soluble P waste are highly variable. The variation could be due to factors such as dietary P inclusion levels and their digestibility, P requirement and utilization efficiency by fish. For example, for rainbow trout only, total P wastes reportedly vary from 1.4 to 25.5 g/kg fish weight gain (Wiesmann et al., 1988; Heinen and Hankins, 1993; Gomes et al., 1995; Lanari et al., 1995; Kim et al., 1998; Ruohonen et al., 1999; Vielma et al., 2000; Green et al., 2002; Lanari and D'Agaro, 2002; Vielma et al., 2002). Solid waste outputs vary from 0.8 to 10.2 g/kg fish weight gain (Wiesmann et al., 1988; Gomes et al., 1995; Green et al., 2002). Soluble waste outputs vary from 0.3 to 13.8 g/kg fish weight gain (Wiesmann et al., 1988; Cain and Garling, 1995; Gomes et al., 1995; Green et al., 2002). This illustrates the high variability of quantity and forms of P waste outputs, subjecting to factors such as diet formulation, fish requirement of P, and rearing conditions. Soluble P waste is more readily available to algal growth and thus has potentially immediate and greater impact to environment than solid waste (Cho and Bureau, 2001). Therefore, it is useful to estimate not only the quantity of total P waste, but also the quantity of different forms of P waste output.

The biological method based on mass balance of feed intake and nutrient utilization to estimate P soluble and solid waste output is more accurate, flexible and economical than direct measurement of P concentration in aquaculture effluent (Cho et al., 1991, 1994). This method of estimating P soluble and solid waste output requires accurate estimates of P digestibility of the diet, and P retention in fish.

2.4 Phosphorus in Freshwater System

Phosphorus is the most limiting factor for algae growth and eutrophication in many freshwater ecosystems. Eutrophication refers to the enrichment of the water ecosystem by nutrients, causing accelerated algal growth and associated undesirable disturbance to the water ecosystem (Wetzel, 2001). The corresponding phenomenon in the marine system is more often referred to as hypernutrification (Pillay, 1992). Redfield (1958) identified that the composition of aquatic biomass in terms of carbon, nitrogen and phosphorus is relatively constant, with the relationship of C: N: P being 105:15:1. The Redfield ratio suggests that any element below the ratio in nature system is likely to be the limiting factor. Phosphorus is limiting in freshwater ecosystem, whereas nitrogen is usually limiting in marine system (Dugdale, 1967).

In freshwater ecosystems, the concentration and availability of P has direct effect on the biomass of algae, since algal growth is limited by available phosphorus (Gibson, 1997). Boström et al. (1988) defined bioavailable P as the sum of immediately available P and those

forms that can yield available P under the conditions of physical, chemical and biological processes. The most available P form is orthophosphates (H_2PO_4^- , HPO_4^{2-} , and PO_4^{3-}), although organic P can be taken up in variable amount by algae (Wetzel, 2001). However, the uptake of organic P has to be preceded by external phosphatase hydrolysis to release orthophosphates (Jansson, 1988). Therefore, the input of Pi into aquatic systems is an important factor that determines the primary productivity.

Anthropogenic activities are the major sources of P input into the aquatic system. This includes urban and industrial activities, agricultural fertilization and animal manure (Carpenter et al., 1998). In intensive agricultural production systems, a great amount of dietary P supplied to farmed animals is released to the environment. In addition, a proportion of the fertilizer P applied to the soil unutilized by crops can become fixed in the soil in an unavailable form to plant. Accumulated soil P can be released to runoff and end up in water bodies as either dissolved form or particulate form (Sharpley and Rekolainen, 1997). In fish farming, especially cage farming, P is discharged directly into the water as feces, metabolic waste products, and feed wastage (Pillay, 1992).

2.5 Nutritional Approach to Reduce Phosphorus Waste Output

In fish culture, feed is the ultimate source of P waste outputs. Nutrient management through feed formulation is believed to be the most effective and most feasible approach to reduce phosphorus output to the environment. The most important factors affecting the forms

and quantify of P waste output are the supplied total dietary P and digestible P levels, and the partitioning of digestible P between body deposition and urinary excretion. Therefore, it is necessary to improve dietary P digestibility by selection of highly available ingredients to minimize solid P waste outputs. Furthermore, it is desirable to just meet but not exceed fish requirement for digestible P in order to minimize soluble P waste output.

Apart from selection of highly digestible ingredients, there are a number of strategies to enhance digestibility of P in formulated diets. Acidification can improve the digestibility of phosphorus. Organic acids have been used in swine and poultry diets to improve P utilization. The early study of Pileggi et al. (1956) showed that citric acid improved the P utilization in phytate-containing diets to rats. Citric acid also increased phytate-P utilization in chicks and pigs (Boling et al., 2000). Similarly, dietary supplementation of citric acid, Na citrate, and EDTA was able to improve P digestibility of fish meal to rainbow trout (Sugiura et al., 1998a). The effect was probably due to the solubilization of bone minerals in fish meal, as well as a chelating effect that reduces the antagonistic interaction between Ca and P that could precipitate Ca and P at the intestinal brush border (Sugiura et al., 1998a). Formic acid at supplement level of 4 and 10 ml/kg diet significantly improve P digestibility from 69.5% at zero supplement to 73.6% and 75.0% for fish meal based diets (Vielma and Lall, 1997). Supplementing citric acid at levels of 4, 8, 16 g/kg diet to 28% herring bone meal based diet linearly increased rainbow trout body ash concentration indicating higher bone mineralization and a better P utilization by fish, despite the fact that the effect on body P concentration was not statistically significant (Vielma et al., 1999b). These authors cautioned

the use of acidified diets because of the possible disturbance of acid-base balance and mineral homeostasis. More research is warranted in this aspect.

Exogenous phytase (microbial or fungal phytase) can be supplemented in diets to improve the P digestibility of plant ingredients. Phytate present in plant ingredients has to be dephosphorylated, primarily by phytase, to be available for intestinal absorption. Phytase (*myo*-inositol hexaphosphate phosphohydrolase) catalyzes the stepwise removal of Pi from phytic acid (Ravindran et al., 1995). Phytase is differentiated into 3-phytase and 6-phytase based on their initial dephosphorylation position of ester bond of the phytate. In general, microbial phytase is 3-phytase, whereas phytase in plants and fungi is 6-phytase (Ravindran et al., 1995). Certain plant ingredients have high endogenous phytase activities, such as rye, wheat, and barley (Weremko et al., 1997). In monogastric mammals, such as pig and poultry, endogenous phytases present in plant ingredients contribute to the hydrolysis of phytate. However, phytase is heat labile, and a temperature of 70-80°C can cause partial or total inactivation of endogenous phytase (Ravindran et al., 1995). Extrusion, the major processing method for fish diets, is likely to destroy the endogenous phytase activity (Hughes and Soares, 1998; Forster et al. 1999; Vandenberg, 2001). Therefore, the research of effect of phytase on fish diets has been focused on exogenous phytase. The incorporation of exogenous phytase in diets has proved to improve the digestibility of P in salmonid (Rodehutscord and Pfeffer, 1995; Lanari et al., 1998; Vielma et al., 1998; Sugiura et al., 2001; Vandenberg, 2001) and other fish species, such as carp (Schäfer et al., 1995), channel catfish (Jackson et al., 1996; Yan et al., 2002), African catfish (Van Weerd et al., 1999), striped bass (Hughes and Soares 1998), Japanese flounder (Masumoto et al., 2001), and

seabass (Oliva-Teles et al., 1998). There is some indication that in efficacy of microbial phytase is more prominent in warm water species (Forster et al., 1999). However, for carnivorous fish such as salmonid, the value of exogenous phytase has been questioned since the diets are formulated with animal products rather than plant products as major ingredients. The beneficial effect of exogenous phytase was not evident to rainbow trout fed high fish meal diets (Sugiura et al., 2001). Similarly, Oliva-Teles et al. (1998) also observed that phytase significantly improves soybean meal-based diet P digestibility, but not fish meal-based diet for seabass.

In addition to incorporation of exogenous microbial phytase, other methods such as pre-treatment of plant ingredients also can improve P digestibility and utilization. Dephytinization of plant ingredient improved P utilization in Atlantic salmon (Storebakken et al., 1998) and rainbow trout (Vielma et al., 2002). Low phytate cultivar grains, in which single gene mutation resulting in blockage of phytic acid accumulation, have been developed to improve phytate P digestibility. P digestibility in low phytate barley, corn, and barley was significantly higher than regular grains for rainbow trout (Sugiura et al., 1999; Overturf et al., 2003).

2.6 Modeling Nutrient Utilization of Farm Animals

Models are description of systems and have always been a necessity of the scientific method (Rosenblueth and Wiener, 1945). A system can be defined as “any collection of

interacting elements for which there are cause-and-effect relationships among the variables” (Close et al., 2002). Live organisms can be considered as systems at a steady state maintained through nutritional processes (Sauvant, 1992).

There are numerous of types of models depending on classification schemes and field of application (DiStefano and Landaw, 1984; Massoud et al., 1998). Mathematic models are particularly of interest as a quantitative approach in the investigation of nutrient utilization of farm animals. Mathematical models are description of systems through one or a set of mathematical equations (France and Thornley, 1984; Gill et al., 1989; Close et al., 2002). As information accumulates, mathematical models are increasingly utilized as a tool to simulate the behavior of biological systems by integration of available experimental data and biological concepts (Gill et al., 1989; Sauvant 1992). Mathematical models have become important and even critical to the progress of agricultural science, in that, variance could be explained and evaluated by mathematically articulated theories instead of by conducting the same type of descriptive experiments in slightly different situations (Baldwin, 1995). The extension of current knowledge can be achieved by improvement of observational data, and/or by integration of data and concepts into representations of the system – mathematical models (France and Thornley, 1984; Baldwin, 1995; McNamara, 2004).

Mathematical models in nutrient utilization of farm animals are commonly classified as empirical models versus mechanistic models, static models versus dynamic models or deterministic models versus stochastic models (France and Thornley, 1984).

The differentiation between empirical and mechanistic models is based on the model targeted hierarchy organization of a biological system, where several levels can be identified: cells, tissues, organs, animal, and herd of animals. Empirical models rely on the statistical interpretation of relationships based on experimental data, and they target one level of hierarchy organization, usually at the animal level (France and Thornley, 1984; Forbes and France, 1993). Empirical models do not seek to understand the biological system; rather, they are solely based on system input and system output (Mercer et al., 1986). The curve fitting model is the best fit of the experimental data, thus for a certain experimental condition, it is an accurate description of the observations. However, empirical models can be faithfully descriptive under one specific condition, but lack of accuracy for extrapolation if the condition changes dramatically. In contrast, mechanistic models utilize a reductionism approach and investigate the fundamental underlying principles based on lower levels of hierarchy organization, often at lower levels such as cells, tissues and organs, to describe the behavior of higher levels such as the whole animal level (France and Thornley, 1984; Forbes and France, 1993, Baldwin, 1995). Mechanistic models predict a response based on cause and effect relationships; therefore, they are useful in understanding biological interactions, and can be applied to a wider range of conditions. Construction of mechanistic models also requires adequate knowledge of the system (Baldwin, 1995). The application of mechanistic models may be potentially hindered by the fact that they tend to be comparatively complicated and often require more computational capacity, thus may be more of a research tool than of practical usage. This is becoming less an issue because of ever-increasing computer power and speed.

Dynamic models are generally based on differential equations and are integrated over time, whereas static models are based on algebraic equations and solved for specific conditions at a time point (France and Thornley, 1984; Baldwin, 1995). Stochastic models incorporate probability distribution functions to variables so that the solutions are not exact, in contrast to deterministic models, which often imply average and exact solutions to equations (France and Thornley, 1984; Baldwin, 1995). Deterministic models represent the mean behavior of a group of animals and account for a majority of animal models that are current available (Black, 1995).

In biological systems, models are often built on multiple factors as independent variables (Mercer, 1980). These models are called factorial models. Factorial models can be classified as empirical, deterministic, and static. It is commonly used to estimate nutrient requirements, through a sum of factors, such as requirements of maintenance, gain, activity, etc (Baldwin, 1995). One of the greatest advantages of the factorial method is that the results of many different experiments, most of them conducted for completely different purposes, can be combined (Kienzle, 1998), and this approach has proven to be very practical and realistic if used within the boundaries of which the data are collected (McNamara, 2004).

Classification of models are not absolute and sometimes the distinction is somewhat blurred in the case of empirical models and mechanistic models. Some empirical models can be given physical interpretations (DiStefano and Landaw, 1984), or they may contain mechanistic elements, whereas mechanistic models can incorporate some empirical equations (Baldwin, 1995). Different types of models are constructed based on differing objectives and

should not be judged on the degree of empirical or mechanistic elements (Baldwin, 1995). Mathematical models are tools. Models can be integration of current data based on statistical resolution of experimental findings. More importantly, models can be built to focus on individual components of the system or to present a coherent view of the whole system. Consequently, instead of being merely a summary of experimental data, they can be used to generate and evaluate hypothesis, estimate parameter values not directly measurable, demonstrate the gaps in our knowledge, and present opportunities for further research (Gill et al., 1989; France and Thornley, 1984; Forbes and France, 1993; Baldwin, 1995).

For farm animals, models have been constructed to simulate growth, nutrient utilization and metabolism of animals, to estimate nutrient requirement of animals, and to evaluate nutritive values of feedstuffs. A limited number of models have been devoted to P utilization and metabolism. A few kinetic and dynamic models of P utilization have been developed for sheep (Grace, 1981; Schneider et al., 1987), pigs (Fernández, 1995), goats (Vitti et al., 2000), and dairy cows (Kebreab et al., 2004). However, there has been no systematic modeling of P utilization in fish, either through empirical factorial integration of factors affecting P digestibility, retention, and waste output, or in a mechanistic, dynamic manner.

Over the past decades, many studies have focused on P nutrition in fish, notably with salmonid fish species. A large number of observations on P digestibility, retention, and waste output at whole fish level is available in the literature. Details of P metabolism at tissue levels, such as mechanisms of P intestinal absorption, renal handling and deposition have

been characterized. In addition, nutritive values of a great variety of ingredients and diet formulae have been evaluated. This vast amount of information provides an opportunity to simulate and estimate P utilization (P digestion, retention, and waste output) by salmonid fish through a quantitative modeling approach, for both practical application and research purpose.

2.7 Objectives

The objectives of this thesis were to:

- (1) Identify some of the factors that affect P utilization in salmonid fish and develop a factorial P model by integrating these factors through a statistical modeling approach,
- (2) Identify and generate information on certain poorly documented aspects for the development of the factorial P model,
- (3) Conduct a feeding trial to validate certain components of the factorial P model,
- (4) Develop a mechanistic, dynamic model of P utilization by integrating current available biological concepts.

CHAPTER 3 DETERMINATION OF PHOSPHORUS FRACTIONS IN ANIMAL PROTEIN INGREDIENTS*

3.1 Abstract

Phosphorus (P) is present in different chemical compounds in animal feeds, and the solubility and digestibility of these different compounds are known to differ significantly. Animal protein ingredients generally have a high P content and are major contributors to total P of feeds for fish and other domestic animals. Estimation of different P compounds in these ingredients could help to improve the accuracy of estimates of digestible P contents of feeds. Bone-P and organic P contents were quantified in 32 animal protein ingredients, including 10 fish meals, 14 meat and bone meals, and 8 poultry by-products meals, using a fractionation protocol. The total P contents of the ingredients ranged from 2.1% to 8.3% on a dry matter (DM) basis. Organic P contents varied between 0.3% and 1.3% of DM. Highly significant ($p < 0.001$) linear relationships were observed between total P and ash and between bone-P and ash for all ingredients combined: total P (%) = $0.185 * \text{ash} (\%)$ ($r^2 = 0.88$), and bone P (%) = $0.188 * \text{ash} (\%) - 0.852$ ($r^2 = 0.94$). These results suggest that bone-P can be easily and reliably estimated on the basis of ash content in animal protein ingredients.

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KEY WORDS: Phosphorus, Feed, Digestibility, Composition, Diet, Animal protein ingredients

3.2 Introduction

Managing phosphorus (P) waste outputs is a key factor for environmental sustainability of animal production operations. The development of effective nutritional strategies to manage P waste outputs requires a detailed understanding of P nutrition (supply, digestion, accretion, excretion) of animals.

Phosphorus is a component of several different types of chemical compounds found in ingredients and feeds. These compounds include hydroxyapatite (bone-P), *myo*-inositol hexaphosphate (phytate-P), P compounds covalently linked to protein, lipid, and sugar (organic P), and various inorganic phosphate supplements. These compounds are present in various amounts in animal feeds depending on feed formulation and the compositional variability of the ingredients used. Differences in the chemical characteristics and solubility of these compounds are likely to result in different digestion dynamics of P within the animal gastrointestinal tract, and this, in turn, can significantly affect P digestibility. It is consequently necessary to quantify the different P forms in ingredients to better understand and/or predict the digestibility of P in feeds.

Animal protein ingredients (fish meal, poultry by-product meal, and meat and bone meal) generally have high P contents and often contribute a significant proportion of the total P of feeds for fish and, occasionally, other domestic animals. Animal protein ingredients are produced from a wide variety of raw materials, and manufacturing techniques and equipment (Prokop, 1996; Bureau et al., 1998). Consequently, P content and the proportion of chemical compounds in these ingredients may be highly variable, even for a given type of ingredient. A survey of the literature indicates that there are between 16 and 42 g/kg of P in fish meal, from 25 to 56 g/kg of P in meat and bone meal, and from 17 to 35 g/kg of P in poultry by-products meal (NRC, 1993, 1994, 1998; Sugiura et al., 1998b, 2000c; Sugiura and Hardy, 2000). Very little information on the proportion of P chemical compounds in these ingredients is available in the literature, although it is well-known that in the body of vertebrates, the majority of P (85 - 88%) exists as bone-P, about 10-15% is organic P, and only a small amount is present as free ions or soluble inorganic P phosphates (P_i) (Lall, 1991; Berner, 1997).

Estimates of the digestibility of P for animal protein ingredients are highly variable even for similar ingredients. For example, estimates of apparent digestibility of P in fish meal vary between 17% and 81% for rainbow trout (Ogino et al., 1979; Riche and Brown, 1996; Sugiura et al., 1998b, 2000c; Sugiura and Hardy, 2000). Differences in the levels of different P chemical forms could explain part of the variability in the estimates of apparent digestibility of P. Information on the contents of various chemical forms of P in animal protein ingredients would enable better prediction of digestibility of P in feed and/or P waste output by animal production operations (Cho and Bureau, 2001). There have been attempts to

estimate bioavailability of P in ingredients and feeds based on chemical extractions (Pettersen, 1988; Satoh et al., 1992b, 1997; Buyukates et al., 2000). A fractionation method was also used for estimates of composition of animal manures (Pettersen, 1988; Garcia-Ruiz and Hall, 1996; Dou et al., 2002; Wienhold and Miller, 2004). However, limited work has been carried out to quantify specific chemical compounds in animal protein ingredients. There is also a need for simple methods of estimating total P and bone-P contents of feed ingredients based on routine chemical analyses (e.g. proximate analysis).

The objectives of the study were to (1) quantify bone-P and non bone-P in animal ingredients and (2) determine the relationship among bone-P, total P and proximate analysis parameters.

3.3 Materials and Methods

3.3.1 Sources of Samples

Thirty-two animal ingredients, including 10 fish meals, 8 poultry by-products meals, and 14 meat and bone meals, were obtained from various suppliers in North America. These ingredients were selected to cover a wide range of raw materials and finished products for each ingredient type.

3.3.2 Chemical Analyses

Duplicate samples of ingredients were analyzed for proximate composition. Dry matter (DM) was analyzed by heating samples at 105°C for 24 h. Ash was analyzed according to AOAC gravimetric method 942.05 (AOAC, 1995). Crude protein (%N x 6.25) was analyzed according to the Kjeldahl method using a Kjeltech 1030 autoanalyzer (Tecator, Höganäs, Sweden). Lipid was analyzed according to AOAC acid hydrolysis method 954.02 (AOAC, 1995) by a commercial laboratory (AgriFood, Guelph, ON, Canada). A coefficient of variation (CV) of replicates below 5% was considered to be acceptable.

The P fractionation protocol was carried out as detailed in Ruban et al. (2001 a; b) but with slight modifications (Figure 3.1). Triplicate ingredient samples (0.4 g) were incubated in 1 N NaOH overnight with shaking, and then centrifuged. An aliquot of supernatant was incubated in 3.5 N HCl overnight, whereas pellets were incubated in 1 N HCl overnight with shaking, and then centrifuged. The supernatants and pellets were evaporated to dryness on a hot plate. The resulting P fractions included bone-P, organic P, and residual P (P resistant to acid and alkaline extraction, and thus unaccounted for in analysis). Phosphorus contents in animal protein ingredients and fractioned samples were ashed at 900°F (482°C) for 10 h and then analyzed according to the colorimetric method of Heinonen and Lahti (1981).

3.3.3 Calculations and Statistical Analyses

The total P content of each ingredient analyzed was compared to the sum of bone-P, organic P and residual P by *t* test. Relationships between all analyzed variables were subjected to linear regression using SAS software (SAS Institute, 1999). Probability (*p*) of < 0.05 was considered to be significant.

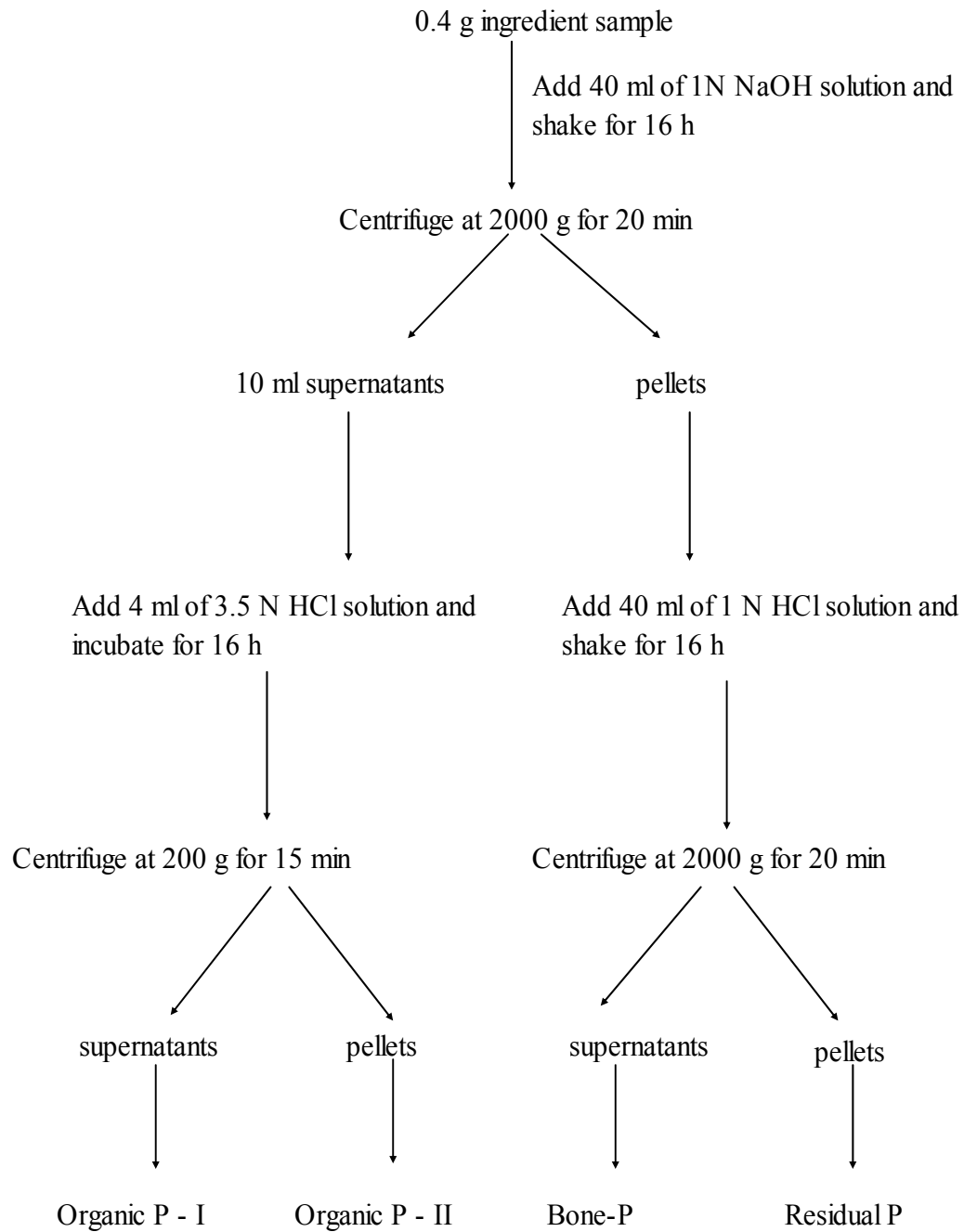


Figure 3.1 P fractionation protocol.

3.4 Results

Table 3.1 summarizes the results of crude protein, lipid, ash, total P, bone-P, organic P, and residual P on a DM basis in fish meals, poultry by-products meals, and meat and bone meals. Overall, the total P contents of all ingredients samples varied from 2.1% to 8.3%, and ash contents varied from 10% to 37% on a DM basis. The total P contents of fish meals ranged from 2.5% to 4.7% on a DM basis, whereas bone-P contents were between 1.4% and 3.5%. Bone-P accounted for 53% to 79% of total P in fish meal. In poultry by-products meals, total P contents and bone-P contents ranged from 2.1% to 3.6% and from 1.2% to 3.1% on a DM basis, respectively. This translated into 60% to 91% of the total P being present as bone-P in poultry by-products meals. In meat and bone meals, total P content varies from 2.2% to 8.3% of DM, of which between 71% and 93% was bone-P. On a DM basis, bone-P contents of the 14 meat and bone meals varied between 1.6% and 7.0%. Organic P varied between 0.3% and 1.3% in all ingredients. Residual P represented < 2.5% of total P in all ingredients. The difference between total P and the sum of bone-P, organic P and residual P did not exceed 10% in all ingredients and was not significantly different ($p > 0.05$).

Figure 3.2 illustrates the relationship between the analyzed variables. Highly linear relationships ($p < 0.0001$) were observed among bone-P (%), total P (%), ash (%), and protein (%) as follows:

$$\text{bone-P} = 0.980 * \text{total P} - 0.711 \quad (r^2 = 0.97, p < 0.0001)$$

$$\text{total P} = 0.185 * \text{ash} \quad (r^2 = 0.88, p < 0.0001)$$

$$\text{bone-P} = 0.188 * \text{ash} - 0.852 \quad (r^2 = 0.94, p < 0.0001)$$

Relationship between proportion of bone-P in total P (%) and ash (%) appeared to be asymptotic and could be in practice described by the following quadratic equation:

$$\text{bone-P/total P} = -0.057 * \text{ash}^2 + 3.749 * \text{ash} + 26.839 \quad (R^2 = 0.76, p < 0.0001)$$

A significant linear equation was obtained to describe the relationship between bone-P (%), protein (%) and lipid (%) content as illustrated by Figure 3.3 and the following equation:

$$\text{bone-P} = 13.520 - 0.139 * \text{protein} - 0.150 * \text{lipid} \quad (R^2 = 0.82, p < 0.0001)$$

Table 3.1 Contents of dry matter (DM), crude protein (CP), lipid, ash, total P and P fractions¹ in fish meals, poultry by-products meals, and meat and bone meals.

	DM	CP	Lipid	Ash	Total P	Bone-P	Organic P - I	Organic P - II	Residual P
	%								
	%DM								
Fish meals (FM)									
FM – 1	90.1	78.3	12.4	10.7	2.6	1.4	0.7	0.3	0.0
FM – 2	85.4	74.0	11.1	12.0	2.5	1.5	0.8	0.2	0.0
FM – 3	75.6	71.9	10.2	14.5	2.6	1.7	0.8	0.1	0.0
FM – 4	93.4	66.7	13.6	17.6	3.7	2.7	0.7	0.1	0.0
FM – 5	92.9	68.3	10.5	19.8	4.7	3.5	1.1	0.2	0.1
FM – 6	91.1	68.0	9.2	20.8	3.8	2.8	0.7	0.1	0.1
FM – 7	90.6	68.6	6.1	20.6	3.8	3.0	0.7	0.1	0.1
FM – 8	92.0	68.1	14.0	17.8	3.4	2.5	0.8	0.1	0.0
FM – 9	94.2	73.6	10.0	16.2	2.5	1.7	0.6	0.2	0.0
FM – 10	92.0	73.1	8.8	15.8	2.7	1.8	0.9	0.1	0.0
Poultry by-products meals (PBM)									
PBM – 1	96.2	67.6	13.4	14.4	2.7	1.8	0.6	0.1	0.0
PBM – 2	93.7	68.2	14.7	12.7	2.5	1.8	0.6	0.1	0.0

PBM – 3	94.1	70.1	16.8	9.8	2.1	1.2	0.7	0.1	0.0
PBM – 4	98.5	61.4	15.0	18.9	3.4	3.1	0.6	0.1	0.1
PBM – 5	94.2	68.3	14.9	13.6	2.6	2.0	0.6	0.1	0.0
PBM – 6	93.6	64.6	10.9	19.7	3.6	3.1	0.5	0.1	0.1
PBM – 7	96.3	72.0	14.9	13.1	2.6	1.7	0.7	0.1	0.0
PBM – 8	93.9	69.8	9.4	14.4	2.7	1.9	0.6	0.1	0.0

Meat and bone meals (MBM)

MBM – 1	95.0	54.8	13.6	22.3	4.2	3.5	0.5	0.1	0.1
MBM – 2	96.3	61.8	10.0	22.5	3.5	3.0	0.4	0.0	0.1
MBM – 3	96.1	54.0	12.8	27.7	4.7	3.9	0.4	0.0	0.1
MBM – 4	95.1	49.0	11.8	35.5	6.3	5.9	0.3	0.0	0.1
MBM – 5	96.5	57.0	12.7	23.5	3.5	3.2	0.4	0.1	0.1
MBM – 6	90.5	57.0	14.3	23.1	4.0	3.3	0.4	0.1	0.1
MBM – 7	94.5	50.9	12.8	27.8	5.0	4.3	0.5	0.1	0.1
MBM – 8	95.2	55.2	12.5	24.9	4.0	3.2	0.5	0.1	0.1
MBM – 9	96.0	45.7	12.1	37.3	8.3	7.0	0.8	0.2	0.2
MBM – 10	95.6	49.6	11.8	26.9	5.5	4.3	0.9	0.2	0.1
MBM – 11	95.0	59.8	19.7	13.2	2.2	1.6	0.6	0.1	0.0
MBM – 12	94.3	50.5	12.0	30.8	5.4	5.0	0.4	0.1	0.1
MBM – 13	92.2	55.6	10.7	23.8	3.8	3.2	0.4	0.1	0.1
MBM – 14	95.2	63.7	12.3	21.4	4.0	3.3	0.4	0.0	0.1

¹Refer to Figure 3.1 and text for P fractions.

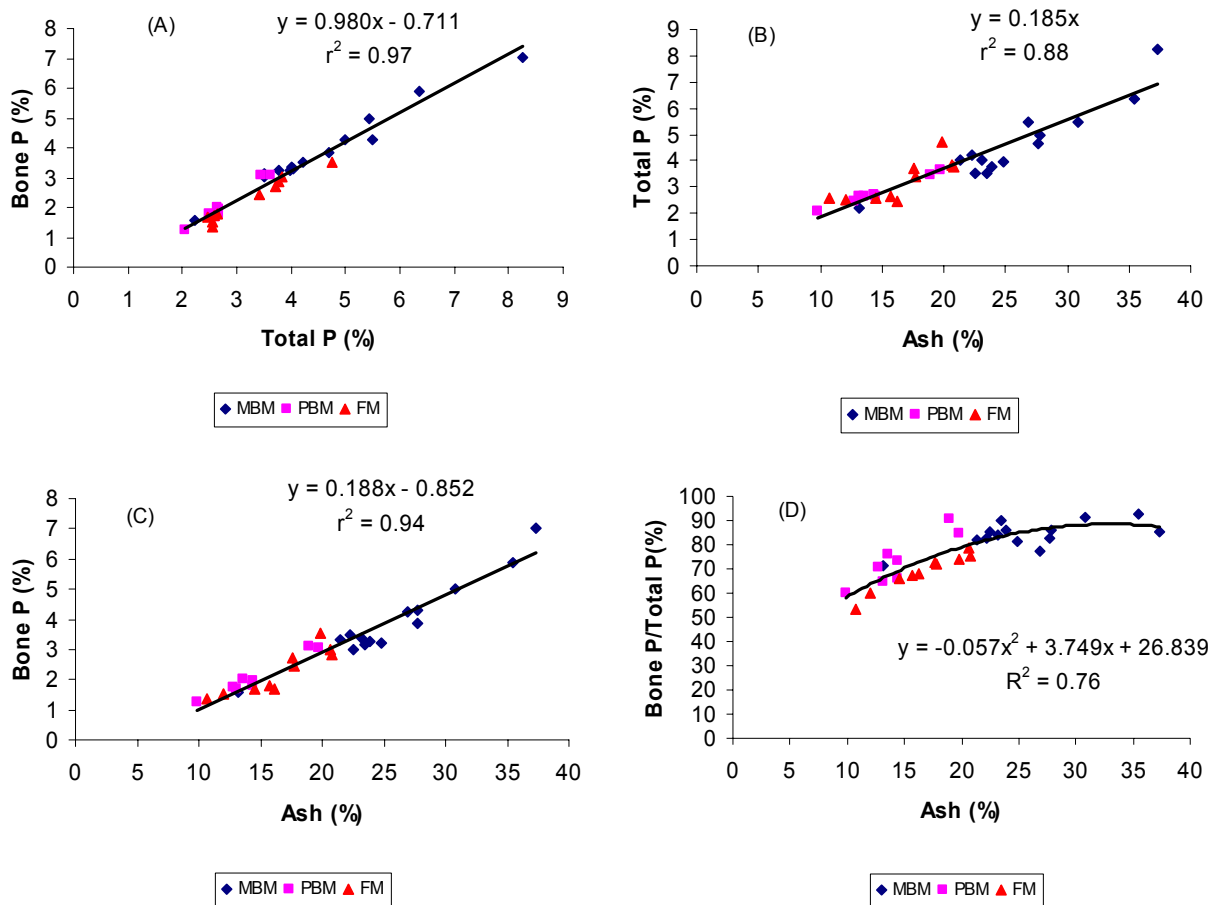


Figure 3.2 Relationship among bone-P, total P, ash, and bone-P/total P in meat and bone meal (MBM), poultry by-product meal (PBM), fish meal (FM).

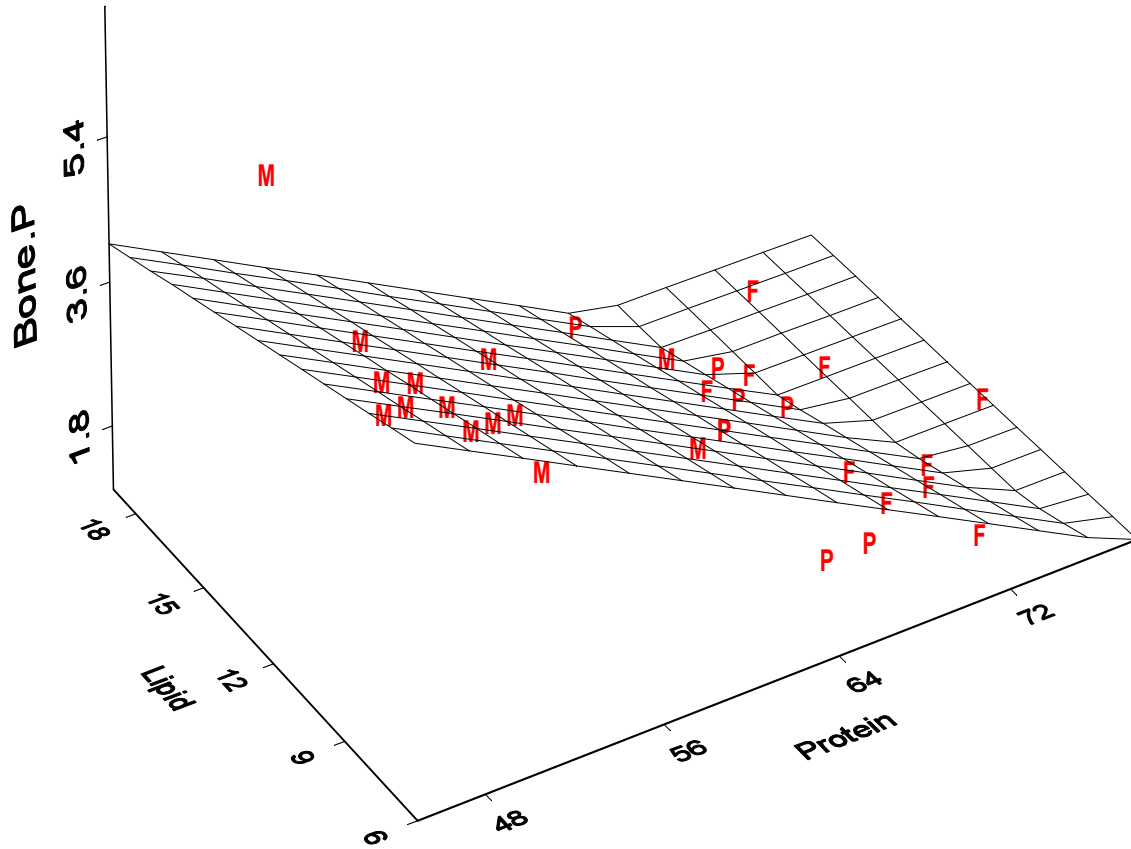


Figure 3.3 Relationship among bone-P (%), protein (%), and lipid (%) in meat and bone meal (M), poultry by-product meal (P), fish meal (F). The linear relationship was described as $\text{bone-P} = 13.520 - 0.139 * \text{protein} - 0.150 * \text{lipid}$ ($R^2 = 0.82$).

3.5 Discussion

In the present study, bone P accounted for 53% to 93% of total P in the animal protein ingredients analyzed, reflecting the variability of the types and proportion of raw materials used in the manufacturing of these ingredients. Bone is a prominent raw material component in high-ash animal protein ingredients. Bone P content was negatively correlated with protein and lipid contents (Figure 3.3) and positively correlated with ash content (Figure 3.2). Bone-P/total P ratio approached an asymptote at high ash levels (Figure 3.2). Organic P content represented a minor proportion of total P content, especially at high ash levels. Residual P represented < 2.5% of total P in all ingredients. Contents of organic P were analyzed as organic P – I and organic P – II, with organic P – I representing the portion more easily hydrolyzed and/or dissolved, and thus probably more digestible to fish. The results suggest that organic P – I accounted for a major portion of total organic P. However, organic P represents several organic phosphate compounds such as phosphoproteins, phospholipids, phosphosugars and nucleic acids. The employed fractionation scheme could not further differentiate organic phosphate compounds within organic P – I and II. The subsequent analysis of relationships between total P, bone-P, and organic P suggested that there was no specific advantage in separating out organic P – I and II, or measuring organic P directly instead of estimating it as the difference between total P and bone P. Within each ingredient type, the relationships between analyzed variables were also explored separately; it appeared that the relationships with all ingredients combined are representative of each ingredient type.

Relationship between proportion of bone-P in total P (%) and ash (%) was described by a quadratic equation in this study (Figure 3.2). True asymptotic relationship was also explored by fitting a Michaelis-Menten equation and was described as follows:

$$\text{bone-P/total P} = 115.8/(1+9.6/\text{ash}) \quad (R^2 = 0.75, p < 0.0001)$$

The Michaelis-Menten equation did not improve the goodness of fit of the data, as there was no improvement in R^2 (0.75 for the Michaelis-Menten equation versus 0.76 for the quadratic equation) or residual standard error (5.15 for the Michaelis-Menten equation versus 5.12 for the quadratic equation). Therefore, the presented quadratic function is an adequate description of the asymptotic relationship. Although a quadratic equation is only asymptotic within a certain range of independent variable, ash contents of the animal ingredients tested in this study were from 9.9 to 37.3 g/kg, which fall in a wide range and are representative of ingredients used in practical feed formulation. A quadratic equation is also more readily applicable than a true asymptotic equation such as the Michaelis-Menten equation in practice.

Very little information on the proportion of P chemical compounds in these ingredients is available in the literature. The information of P content and the proportion of bone-P to organic P in whole animal body cannot provide reliable estimates of bone-P and organic P contents in animal protein ingredients because the compositions of the raw material vary significantly. Whole fish or filleting by-products from a variety of species can be used to produce fish meal. Meat and bone meal, and poultry by-products meal are often composed of offals, fat, feet, legs, bones, etc, from slaughterhouses and butcher shops (Prokop, 1996;

Bureau et al., 1999). The heterogeneity of ingredient compositions is reflected in this study by the wide variations in contents of total P, bone-P and organic P in these animal protein ingredients.

The wide variation of bone P content appears to explain the variation of P digestibility of animal by-products reported in the literature. For salmonid fish, P digestibility ranges from 17% to 81% for fish meal, from 22% to 45% for meat and bone meal, and from 15% to 64% for poultry by-product meal (Ogino et al., 1979; Riche and Brown, 1996; Sugiura et al., 1998b, 2000c; Sugiura and Hardy, 2000; Cheng and Hardy, 2002). For swine, P digestibility was in the range of 66%-85% for meat and bone meal and 85%-90% for fish meal (Jongbloed and Kemme, 1990; Rodehutscord et al., 1997). In poultry, P digestibility was reported to be 74% for fish meal and 66% for meat and bone meal for 3-week-old broilers (Van der Klis and Versteegh, 1996). Because bone-P is generally believed to be less digestible than organic P to fish (Lall, 1991) and that its digestibility is not additive (Sugiura et al., 2000c), the content of bone P in ingredients and the inclusion level of ingredients in experiment diets will greatly affect P digestibility of an ingredient. The depressing effect of dietary P level on P apparent digestibility in fish (Vielma and Lall, 1998a; Rodehutscord et al., 2000; Sugiura et al., 2000c) may be primarily due to the limited capacity of the fish gastrointestinal tract to solubilize hydroxyapatite, when diets were formulated with high level of animal ingredients, rather than through down-regulation of intestinal active transport by high P_i concentration (Avila et al., 2000). Therefore, quantification of different dietary P forms in feeds is needed to better understand and predict apparent digestibility of P.

Analysis of bone P and total P contents of different batches of animal protein ingredients is an expensive and tedious process. The heterogeneous nature of animal protein ingredients, in particular, high-ash meat and bone meal, further complicates analysis. Given the very good relationships between contents of bone P, total P and ash, our study suggests that bone P content in animal protein ingredients can be easily and reliably estimated on the basis of total P content or ash content of the ingredients. Our study also suggests that there is no advantage in measuring organic P directly instead of estimating it as the difference between total P and bone P.

CHAPTER 4 DEVELOPMENT OF A MODEL TO ESTIMATE DIGESTIBLE PHOSPHORUS CONTENT OF SALMONID FISH FEEDS

4.1 Abstract

Accurate estimation of digestible phosphorus (P) content of fish feeds is essential to formulating feed that meet nutritional requirements but minimizing dissolved P waste outputs from fish culture operations. Phosphorus is found as different chemical compounds in feeds and on the basis of their chemical characteristics, the digestibility of these different forms should differ significantly. In addition, at high P levels, a depression of P digestibility has been reported in some studies.

A digestibility model was constructed by integrating data from 22 studies with rainbow trout. Phosphorus-containing compounds present in ingredients and feeds were classified into broad chemical categories of bone-P, phytate-P, organic P, Ca monobasic / Na / K Pi supplement, and Ca dibasic Pi supplement. The relationship between digestible P content and various P chemical compounds contents was modeled through a multiple regression approach. The model was described as follows:

$$\text{Digestible P} = 0.68 \text{ bone-P} + 0 \text{ phytate-P} + 0.84 \text{ organic P} + 0.89 \text{ Ca monobasic / Na / K Pi supplement} + 0.64 \text{ Ca dibasic Pi supplement} + 0.51 \text{ phytase/phytate} - 0.02 (\text{phytase/phytate})^2 - 0.03 (\text{bone-P})^2 - 0.14 \text{ bone-P} * \text{Ca monobasic / Na / K Pi supplement} (p$$

< 0.0001, $R^2 = 0.96$. The units for all variables are g/kg diet, except for phytase/phytate ratio the unit is 100 FTU phytase/g phytate).

The results suggest that the digestibility of different P chemical compounds differ significantly. Digestibility of bone-P is not additive, as indicated by the significant quadratic function and its negative interaction with Ca monobasic / Na / K Pi supplement. This model provides a simple means of predicting digestible P content of salmonid fish feeds.

KEY WORDS: Phosphorus, Feed, Digestibility, Model, Trout

4.2 Introduction

Phosphorus (P) waste is of great concern in freshwater aquaculture since P is generally the most limiting factor for algal growth and excess P results in eutrophication. The use of feeds containing digestible P levels closely meeting, but not exceeding, the requirement of the fish has been shown to be an effective way of minimizing P waste outputs (Sugiura and Hardy, 2000; Cho and Bureau, 2001). This necessitates accurate estimate of digestible P content in fish feed.

Apparent digestibility coefficients (ADC) of P in common fish ingredients vary greatly in the literature. For example, for rainbow trout, ADC of P of fish meal has been reported to vary from 17 to 81%, meat and bone meal from 22 to 45%, poultry by-product

meal from 15 to 64%, soybean meal from 20 to 35% (Ogino et al., 1979; Riche and Brown, 1996; Sugiura et al., 1998b, 2000c; Sugiura and Hardy, 2000; Cheng and Hardy, 2002). P is present in different chemical compounds in ingredients and feeds. In animal by-products, P exists primarily in bone as hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), which is fairly digestible to fish (Lall, 1991; Sugiura et al., 2000c). In plant ingredients, 60 to 80% of the total P is bound in phytate (*myo*-inositol hexaphosphate) (Ravindran et al., 1995). Since fish do not possess phytase in the digestive tract, digestibility of phytate is very poor (Ogino et al., 1979; Lall, 1991). Organic phosphates covalently linked to protein, lipid and sugar are easily hydrolyzed by alkaline phosphatase and presumably highly digestible. The digestibility of inorganic phosphate supplements is affected by their solubility. For example, monobasic Ca phosphate ($\text{Ca}(\text{H}_2\text{PO}_4)_2$) is apparently more digestible than dibasic Ca phosphate (CaHPO_4) because of its higher solubility (Lall, 1991). It has been observed that high dietary P levels negatively affect digestibility of dietary P (Vielma and Lall, 1998a; Rodehutsord et al., 2000; Sugiura et al., 2000c). Conversely, high levels of dietary P did not depress apparent digestibility of P in other studies (Nakamura, 1982; Satoh et al., 1996). This suggests that digestibility of P is not a simple function of dietary P level. The dynamics of P digestive process (solubilization of different P chemical compounds and subsequent absorption of P in available forms) in fish gastrointestinal tract imply that chemical characteristics of P compounds may greatly affect dietary P digestibility. Accurate estimates of P digestibility of the diets can only be achieved based on differentiation of P compounds rather than aggregates of total dietary P. There is a large amount of information in the literature; however, no attempt has been made to integrate the available information through a quantitative modeling approach to estimate digestible P content of salmonid fish feeds.

Indigestible dietary P is excreted through feces by fish and represents most of the solid P waste output of fish culture operations. Digestible P intake exceeding the fish requirement is mostly excreted through the urine, and represents the bulk of the soluble/dissolved P waste output of fish culture operations. Soluble P waste is more readily available to algae and can, consequently, have potentially more immediate environmental impact than solid P waste (Cho and Bureau, 2001).

Accurate estimation of digestible P content in fish feed and characterization of the P waste outputs could be achieved through a modeling approach taking into account the digestibility of the different P chemical compounds and the effect of their inclusion levels.

The objective of this study was therefore to develop a model that estimates digestible P content of fish feed based on P chemical forms and their inclusion levels by integrating currently available information from the literature.

4.3 Materials and Methods

4.3.1 Modeling Dataset

The modeling dataset included 137 dietary treatments from 22 studies conducted with rainbow trout (Figure 4.1). Feces collection methods used in these digestibility studies fell into four major methods/collection systems: the Guelph system (Cho et al., 1982), the St-Pée system (Choubert et al., 1982), the Tokyo University of Fisheries (TUF) system (Ogino et al., 1973), and the stripping method (Austreng, 1978).

Phosphorus compounds in ingredients and diets were characterized into five broad chemical categories: bone-P, phytate-P, organic P, Ca monobasic / Na / K Pi supplement, and Ca dibasic Pi supplement (Figure 4.2). Organic P in this scheme represents non-bone P from animal ingredients and non-phytate P from plant ingredients, which include phosphoproteins, phosphosugars, phospholipids, and nucleic acids. The contents of total P and various P chemical compounds of the diets of modeling datasets were estimated from diet formulae reported in the original papers. P contents of individual ingredients, including contents of total P of animal and plant ingredients, contents of phytate-P and non-phytate P from plant ingredients, were either tabulated from reported values in the original papers or estimated from various sources in the literature (Wisman et al., 1958; Nelson et al., 1968; Bielorai et al., 1983; Hopkins, 1988; Kirby and Nelson, 1988; Jongbloed and Kemme, 1990; March, 1991; Anderson et al., 1993; NRC, 1993; Dungelhoeft et al., 1994; Eeckhout and De Paepe, 1994; NRC, 1994; Ravindran et al., 1994; Ravindran et al., 1995; Riche and Brown, 1996; Anderson et al., 1997; Johnson and Parsons, 1997; Parsons et al., 1997; Mendez and Dale, 1998; NRC, 1998; Dale, 2000; Hertrampf and Piedad-Pascual, 2000; Kasim and Edwards, 2000; Shirley and Parsons, 2001; Vandenberg, 2001). Contents of bone-P and non bone-P

from animal ingredients were estimated by the relationship between bone-P and total P:
bone-P content (%) = 0.980 * total P content (%) – 0.711 ($r^2 = 0.97$) (Chapter 3).

The modeling dataset included a wide range of semi-purified and practical diets. The dietary total P contents ranged from 0.6 to 30.5 g/kg on dry matter basis. Estimated contents of P compounds on dry matter basis in these diets varied from 0 to 17.1 g/kg for bone-P, from 0 to 7.5 g/kg for phytate-P, from 0.2 to 6.8 g/kg for organic-P, from 0 to 25.4 g/kg for Ca monobasic / Na / K Pi supplement, and from 0 to 3.2 g/kg for Ca dibasic Pi supplement. Exogenous phytase inclusion levels were from 500 to 6000 FTU when phytate-P content were 1.6 to 7.5 g/kg, the phytase to phytate ratio (100 FTU/ g phytate) in these studies were between 0.7 to 17.4.

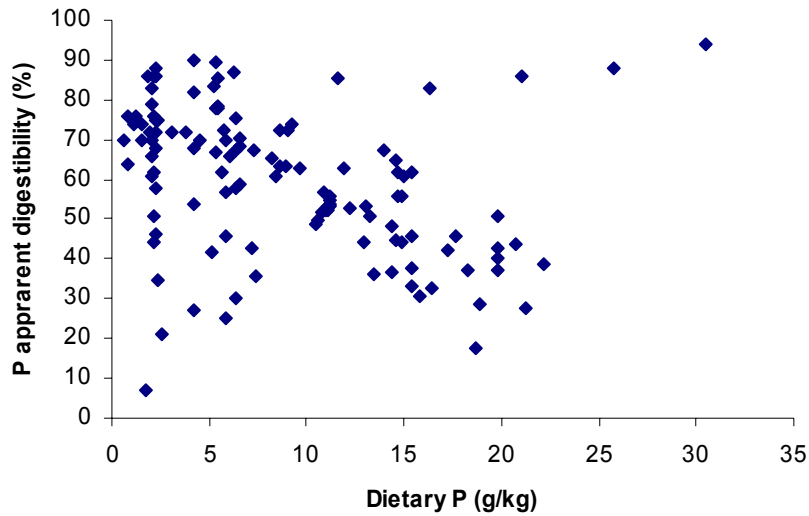


Figure 4.1 Modeling dataset consisting of data from 22 studies¹.

¹These studies included Gomes et al. (1995), Rodehutsord and Pfeffer (1995), Satoh et al. (1996), Encarnacao (1997), Yamamoto et al. (1997), Azevedo et al. (1998), Lanari et al. (1998), Sugiura et al (1998a), Vielma et al. (1998), Forster et al. (1999), Sugiura et al. (1999), Barrias and Oliva-Teles (2000), Gregus (2000), Johnson and Summerfelt (2000), Rodehutsord et al. (2000), Sugiura et al. (2000c), Sugiura et al. (2001), Vandenberg (2001), Green et al. (2002), Satoh et al. (2002), Cheng et al. (2004), Ogunkoya (2004).

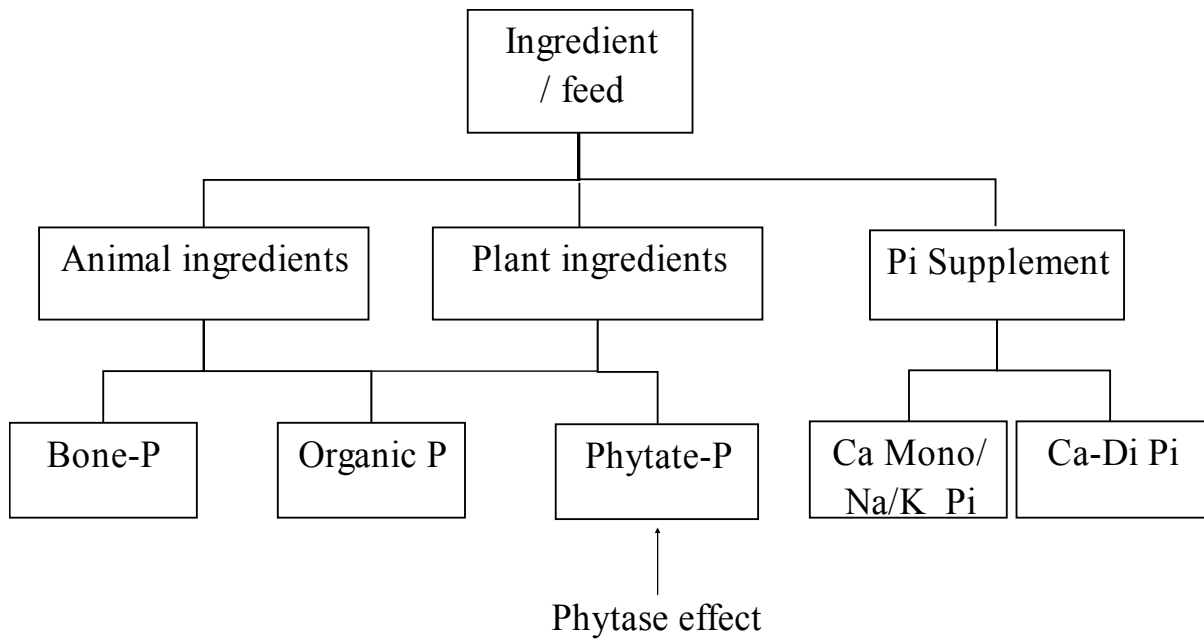


Figure 4.2 Classification scheme for P compounds in ingredients and feeds.

4.3.2 Modeling Digestible Phosphorus Content by Multiple Regression

Multiple regression approach was used to examine the relationship between the content of digestible P and inclusion levels of P chemical compounds in diets. Digestible P was considered as dependent variable, whereas bone-P, phytate-P, organic P, Ca monobasic / Na / K Pi supplement, Ca dibasic Pi supplement, and exogenous phytase were all independent variables. The independent variables were first tested for absence of collinearity (intercorrelation) using SAS (SAS Institute, 1999) before the following second order polynomial linear model was used:

$$Y_i = \beta_0 + \beta_{1i} X_i + \beta_{2i} X_i^2 + \beta_{3i} X_i * X_{i-1} + \varepsilon_i$$

Where Y_i = i th observation of the digestible P (g/kg of diet)

X_i = inclusion level of i th chemical form of P in the diet (g/kg of diet)

β_0 = intercept

β_{1i} = partial regression coefficient of 1st order response of dependent variable to the i th independent variable

β_{2i} = partial regression coefficient of 2nd order response of the dependent variable to the i th independent variable

β_{3i} = partial regression coefficient of the interaction between the independent variables

ε_i = random error

Confidence intervals of the regression coefficients were estimated by bootstrapping, a random resampling technique to obtain statistical inference (Efron and Tibshirani, 1993), using the S-Plus software (Insightful Corporation, Seattle, WA). A total of 1,000 new datasets were randomly sampled from the original data set, multiple regression analysis was performed on these 1,000 datasets, and a bootstrap distribution of regression coefficients was obtained. The resulting replicates are used to calculate the bootstrap estimates of confidence intervals of the regression coefficients.

4.4 Results

Figure 4.1 presented the data used in modeling exercise. Multiple regression analysis yielded the following model:

$$\text{Digestible P} = 0.68 \text{ bone-P} + 0 \text{ phytate-P} + 0.84 \text{ organic P} + 0.89 \text{ Ca monobasic / Na} \\ / \text{ K Pi supplement} + 0.64 \text{ Ca dibasic Pi supplement} + 0.51 \text{ phytase/phytate} - 0.02 \\ (\text{phytase/phytate})^2 - 0.03 (\text{bone-P})^2 - 0.14 \text{ bone-P} * \text{Ca monobasic / Na / K Pi supplement}.$$

The units for all variables are g/kg diet, except for phytase/phytate ratio the unit is 100 FTU phytase/g phytate. The model was highly significant ($p < 0.0001$), and $R^2 = 0.96$. The standard errors for regression coefficients were 0.07 for bone-P, 0.05 for organic P, 0.02 for Ca monobasic / Na / K Pi supplement, 0.10 for Ca dibasic Pi supplement, 0.06 for phytase effect, 0.00 for quadratic effects of phytase and bone-P, and 0.01 for bone P and Ca

monobasic / Na / K Pi supplement interaction. Bootstrapping 95% confidence intervals of regression coefficients were 0.55 to 0.83 for bone-P, 0.70 to 0.94 for organic P, 0.80 to 0.96 for Ca monobasic / Na / K Pi supplement, 0.46 to 0.86 for Ca dibasic Pi supplement, 0.40 to 0.60 for phytase effect, -0.03 to -0.01 for phytase quadratic effect, -0.04 to -0.02 for bone P quadratic effect, and -0.19 to -0.11 for bone P and Ca monobasic / Na / K Pi supplement interaction.

4.5 Discussion

The modeling dataset included a wide range of total dietary P and ADC values of P. Figure 4.1 suggested that there was no evident relationship between ADC of P and total P. It illustrates that apparent digestibility of P is not a simple function of total dietary P level.

Phosphorus compounds in ingredients and diets were characterized into broad chemical categories. Organic P in the modeling scheme represents non-bone P from animal ingredients and non-phytate P from plant ingredients, which include phosphoprotein, phosphosugar, phospholipids, and nucleic acid. Therefore, organic P represents a group of heterogeneous P compounds. It could be further differentiated into specific organic phosphate compounds if sufficient data regarding their contents in animal and plant ingredients are available. Organic phosphates are easily hydrolyzed by alkaline phosphatase and presumably highly digestible. Phytate-P is essentially an organic compound as well. It was separated from other organic P compounds in the scheme, because monogastric animals

including fish do not possess phytase in the digestive tract and consequently digestibility of phytate is very poor (Ogino et al., 1979; Lall, 1991).

The model explained 96% of the variance of the data and well described the observations of the dataset (Figure 4.3). Apparent digestibility coefficients of specific P chemical compounds can be estimated from the multiple regression coefficients as follows: 68% for bone-P, 0% for phytate-P, 84% for organic P, 89% for Ca monobasic /Na /K Pi supplement, 64% for Ca dibasic Pi supplement. It has been reported that for rainbow trout, apparent digestibility values of inorganic phosphate were 54 to 77% for dicalcium phosphate (CaHPO_4), 93 to 98% for monobasic calcium phosphate ($\text{Ca}(\text{H}_2\text{PO}_4)_2$) and potassium and sodium phosphate (KH_2PO_4 , NaH_2PO_4) (Ogino et al., 1979; Gregus, 2000). Organic P has been assumed to be highly digestible since P in yeast and casein, in which organic P was the primary P form, is approximately 90% digestible to rainbow trout (Ogino et al., 1979).

The model suggests that phytate-P is not digestible to salmonid fish. This is in agreement with the literature where apparent digestibility of phytate-P for fish was documented to be between 0 to 19%. Our results are consistent with the physiology of fish, which did not appear to possess phytase within their gastrointestinal tract (Ogino et al., 1979; Lall, 1991). Phytase is heat labile and the high temperature associated with the extrusion and pelleting in the manufacturing of fish diets is likely to destroy phytase activity, and consequently endogenous phytase activity in plant ingredients is probably minimal or absent in fish diets (Forster et al. 1999; Vandenberg, 2001). Therefore, only exogenous phytase supplementation was considered in this model. Phytase effect was described by a quadratic

function, an asymptotic relationship to phytate content of the diet, which is agreeable with the dose-response effect of phytase supplementation observed in fish diets (Jackson et al., 1996; Sugiura et al., 2001; Cheng et al., 2004).

The model suggested that digestibility of bone-P is not additive, since a significant quadratic function and negative interaction with Ca monobasic / Na / K Pi supplement were observed. Hydroxyapatite is the most stable and least soluble calcium phosphate (Dorozhkin and Epple, 2002). The quadratic function of bone-P digestibility is probably a reflection of solubility of hydroxyapatite in fish gastrointestinal tract, which is likely to be limited by gastric acid output. Solubility of P chemical compounds is the important factor for P digestibility, because the available forms of P for intestinal absorption are orthophosphates (HPO_4^{2-} and H_2PO_4^-) in fish, similar to mammals and birds (Nakamura, 1985). ADC of bone-P was observed to decrease with increasing inclusion levels (Sugiura et al., 2000c).

The negative interaction of bone-P and Ca monobasic / Na / K Pi supplement is probably due to the formation of insoluble calcium phosphates in the intestine. It is known that calcium can bind phosphate in the intestine (NRC, 1993). The factors that enhance insoluble calcium phosphates formation include increase in Ca/P ratio and pH (Song et al., 2002). The Ca/P ratios of monobasic Ca phosphate, dibasic Ca phosphate, and hydroxyapatite are 0.5, 1, and 1.67 respectively (Fernández et al., 1999; Dorozhkin and Epple, 2002). It can be hypothesized that when pH changes from acidic to alkaline along the intestine, Ca released from bone particles will interact with Pi from Ca monobasic / Na / K Pi supplement to insoluble calcium phosphates precipitate. Since at neutral pH, the predominant

ion is divalent HPO_4^{2-} , the resulting insoluble calcium phosphates in the intestine could be dibasic calcium phosphates. This is probably the reason that there was interaction between bone-P and Ca monobasic / Na / K Pi supplement observed in this model, but not between bone-P and Ca dibasic Pi supplement. Interaction of Ca and P was observed *in vivo* where increment of dietary Ca linearly depressed dietary P digestibility in rainbow trout (Sato et al., 1993; Encarnacao, 1997), and in carp (Nakamura, 1982). Therefore, although monobasic Pi supplement is readily digestible to fish, but when combined in feed with bone-P, its digestibility is significantly reduced.

The effect of high dietary P inclusion level on P digestibility may well be two-fold: 1) Pi concentration at intestinal absorption site results in regulatory response of Pi absorption, because intestinal uptake of Pi is through mechanisms of passive diffusion and active transport, the latter is saturable and down-regulated by high Pi concentration in the intestine (Avila et al., 2000), and 2) The chemical characteristics of P compounds result in different solubility of P compounds in fish gastrointestinal tract and consequently different available Pi concentration at intestinal absorption site, and formation of insoluble Ca phosphates along the intestine. Being empirical in nature, the present model was based on experimental observations and did not attempt to specifically represent the regulatory mechanism at the intestinal absorption level. This aspect is included in a mechanistic, dynamic model of P utilization for salmonid fish (Chapter 8).

The differentiation of P compounds offered a semi-mechanistic feature to this model and enabled it to be more physiological sound and biological relevant. However, this model

is essentially an empirical model. One of the inherent characteristics of an empirical model is its dependence on data property, range and the conditions under which the data generated. It is the best fit of experimental data and a faithful description of the observations within the boundary of the modeling dataset. Therefore, this model can only be applied with confidence to conditions similar to those used to develop the model; extrapolation beyond the data range should be exercised with caution. Although the model was constructed based on literature data containing a wide range of feed ingredients and diets, and is expected to be applicable to a majority of feed formulation encountered in practice, the limitations of the model should always be recognized.

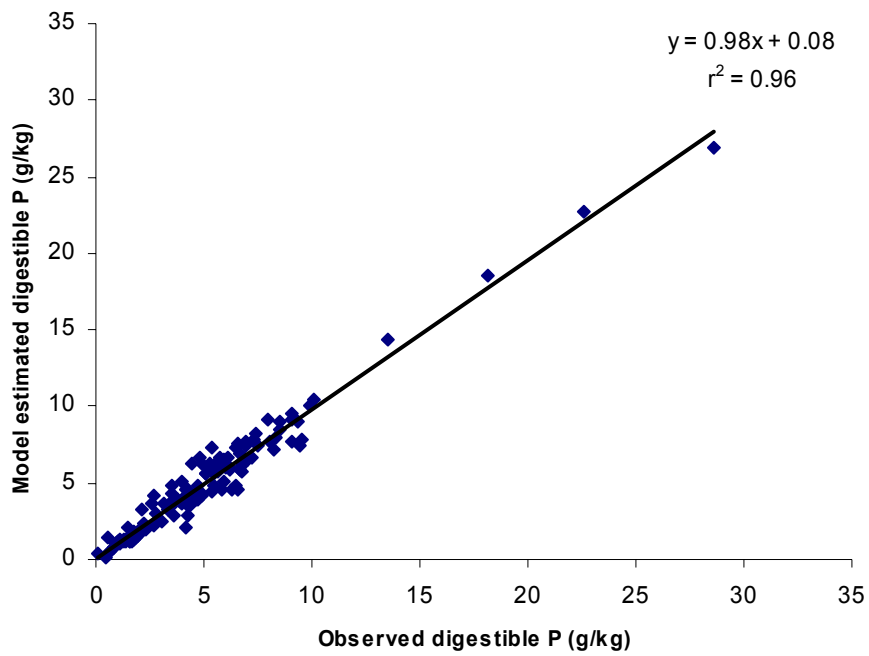


Figure 4.3 Comparison of observed and model estimated digestible P content (g/kg) of the diets from dataset.

4.6 Conclusion

In summary, a model was constructed to estimate digestible P content of salmonid fish feed based on the levels of different P chemical compounds: bone-P, phytate-P, cellular P, Ca monobasic / Na / K Pi supplement, and Ca dibasic Pi supplement. The model suggests that digestibility of bone-P was best described by a quadratic equation and there was significant negative interaction between bone-P and Ca monobasic / Na / K Pi supplement. The present model requires validation by independent experimental data.

CHAPTER 5 VALIDATION OF A PHOSPHORUS DIGESTIBILITY MODEL FOR SALMONID FISH

5.1 Abstract

A digestibility trial was conducted to validate a mathematical model that estimates the digestible phosphorus (P) content of salmonid feeds. The P digestibility model estimates digestible P contents of fish feed based on the dietary inclusion level of different P chemical compounds, which were characterized into five categories: bone-P, phytate-P, organic P, Ca monobasic / Na / K Pi supplement, Ca dibasic Pi supplement. The model suggests that the digestibility of these P compounds differs significantly, and the digestibility of bone-P is not additive. Test diets were formulated with two types of fish meal, two types of poultry by-products meal, two types of soy protein concentrates, and one type of meat and bone meal. The digestibility trial was carried out with rainbow trout (initial body weight ~ 48 g/fish) using the Guelph feces collection system. Comparison between experimental observed and model predicted values suggested that the model well predicted digestible P content of diets formulated with a wide variety of ingredients used in practical feed formulation. The model can be a useful tool in practical feed formulation.

KEY WORDS: Phosphorus, Chemical compounds, Digestibility, Model, Validation, Salmonid

5.2 Introduction

Phosphorus (P) released from freshwater aquaculture operations is of great concern since P is generally the most limiting factor for algal growth and excess P results in eutrophication. Nutritional management is believed to be the most effective approach to reduce P waste outputs, but this approach requires feed formulators to very accurately estimate digestible P content of the feed (Sugiura and Hardy, 2000; Cho and Bureau, 2001). This is often a challenge due to the great variability in estimates of apparent digestibility coefficient of P of common feed ingredients reported in the literature.

A mathematical model was constructed to predict digestible P content of salmonid fish feeds by integrating available literature information (Chapter 4). The digestibility model classified P chemical compounds into bone-P, phytate-P, organic P, Ca monobasic / Na / K Pi supplement, Ca dibasic Pi supplement. Digestible P content of diets was estimated based on contents of P chemical compounds. The model was described as follows:

$$\text{Digestible P} = 0.68 \text{ bone-P} + 0 \text{ phytate-P} + 0.84 \text{ organic P} + 0.89 \text{ Ca monobasic / Na / K Pi supplement} + 0.64 \text{ Ca dibasic Pi supplement} + 0.51 \text{ phytase/phytate} - 0.02 (\text{phytase/phytate})^2 - 0.03 (\text{bone-P})^2 - 0.14 \text{ bone-P} * \text{Ca monobasic / Na / K Pi supplement}.$$

The units for all variables are g/kg diet, except for phytase/phytate ratio the unit is 100 FTU phytase/g phytate. The model suggests that the digestibility of different P chemical compounds differs significantly and digestibility of bone-P is not additive, as indicated by the

significant quadratic function and its negative interaction with Ca monobasic / Na / K Pi supplement.

The mathematical model was constructed through integration of data from unrelated studies focusing on different ingredients or chemical compounds and using different experimental approaches. There is a need to validate the model *in vivo* using diets formulated with ingredients containing different levels of P chemical compounds fed to fish reared under the same experimental conditions. Therefore, a digestibility experiment was conducted to evaluate/validate the P digestibility model.

5.3 Materials and Methods

5.3.1 Ingredients and Diets

A low digestible P reference diet meeting the nutrient requirements recommended by NRC (1993) was formulated with herring meal, wheat middlings, corn gluten meal, and fish oil (Table 5.1). A series of test diets was formulated with 20% of two fish meals, two soybean protein concentrates, one meat and bone meal, and two poultry by-products meals that substituted 20% of corn gluten meal in reference diet (Table 5.1). Acid-washed diatomaceous silica (Celite AW521, Celite Corp., Lompoc, California) was included in diets to serve as a digestion indicator. The diets were mixed using a Hobart mixer (Hobart Ltd,

Don Mills, Ontario) and pelleted using a laboratory steam pellet mill (California Pellet Mill Co., San Francisco, California). The feed pellets were dried in a forced air drier at room temperature for 24h, and were kept at 4°C until used. Amount of diets required was measured out weekly and then kept at room temperature.

Table 5.1 Formulation and composition of diets used in the digestibility trial.

Ingredients	1	2	3	4	5	6	7	8
				%				
Fish meal, herring	15	35	15	15	15	15	15	15
Fish meal, menhaden			20					
Meat-bone meal 56% CP				20				
Poultry by-product meal (low ash)					20			
Poultry by-product meal (regular)						20		
Soy protein concentrate							20	
Soy protein concentrate, dephytinized								20
Wheat middlings	20	20	20	20	20	20	20	20
Corn gluten meal	45	25	25	25	25	25	25	25
Vitamin premix ¹	1	1	1	1	1	1	1	1
Mineral premix ¹	1	1	1	1	1	1	1	1
Lysine	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Celite	1	1	1	1	1	1	1	1
Fish oil	15.5	15.5	15.5	15.5	15.5	15.5	15.5	15.5
Total	100	100	100	100	100	100	100	100
Analyzed Composition (DM basis)								
				%				
Dry matter	95.9	96.3	96.3	96.2	96.1	96.3	96.3	96.2
Crude protein (N x 6.25)	40.5	43.4	42.7	41.4	44.7	41.2	40.1	40.6
Ash	4.5	6.7	7.8	9.0	7.1	8.1	5.4	5.4

¹Composition of vitamin and mineral premixes were as reported in Bureau et al. (1998).

5.3.2 Fish, Feeding, Sample Collection

Rainbow trout were obtained from a commercial hatchery (Rainbow Springs Trout Hatchery, Ontario, Canada). Initial fish body weight averaged 48 g. The fish were stocked in an aquatic system equipped with feces settling columns (the Guelph System) described by Cho et al. (1982). Maximum loading was kept below 3.5 kg of fish for each tank during the experiment. The velocity of the water flow was adjusted to minimize settling of the feces in the drainpipe and maximize recovery of the feces in the settling column. The fish were treated in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 1984) and the University of Guelph Animal Care Committee.

The experimental diets were randomly allocated to collection units. The fish were acclimated to both the tanks and the dietary regime for six days before collection of feces began. A total of four fecal samples were collected. Two fecal samples per diet were collected over a 3-week period. The experimental diets were randomly re-allocated to new tank collection units for a second period and two additional fecal samples per diet were collected in the following 2 weeks.

The fish were hand fed to apparent satiation three times daily between 0930 and 1600 hours. One hour after the last meal, the drainpipe and the settling column were brushed out to remove feed residues and feces from the system. One-third of the water in the tanks was drained to ensure that the cleaning procedure was complete. At 0900 hours the following day,

the settled feces and surrounding water were gently withdrawn from the base of the settling column into a large centrifuge bottle. These feces were free of uneaten feed particles and considered a representative sample of the feces produced throughout the 24-hour period. The feces were centrifuged at 5,000 g for 15 min and the supernatant discarded. The feces were then freeze-dried, ground, and stored at -20°C until analysis.

5.3.3 Chemical Analyses

Samples of ingredients, diets and feces were analyzed for dry matter and ash according to AOAC (1995). Crude protein (N x 6.25) was analyzed by the Kjeldahl method using a Kjeltech 1030 autoanalyzer (Tecator, Höganäs, Sweden). Acid insoluble ash was analyzed according to Atkinson et al. (1984).

Bone-P content of animal ingredients and diet samples was analyzed following a P fractionation protocol (Chapter 3). Phosphorus content was determined according to the spectrophotometric method of Heinonen and Lahti (1981).

5.3.4 Calculations and Statistical Analyses

The apparent digestibility coefficients (ADC) for the nutrients and energy of the test diets were calculated as follows (Cho et al., 1982):

$$\text{ADC} = 1 - (\text{F}/\text{D} \times \text{Di}/\text{Fi})$$

where D = % nutrient (or kJ/g gross energy) of diet

F = % nutrient (or kJ/g gross energy) of feces

Di = % digestion indicator (AIA) of diet

Fi = % digestion indicator (AIA) of feces

Validation of P fractionation protocol was carried out by regressing predicted bone-P content (based on bone-P contents of individual ingredients) on analyzed bone-P content of diet and by subsequently examining the slope and intercept of the regression. Similarly, validation of the model was carried out by examining the slope and intercept of the regression of model predicted digestible P contents on experimental observed values. The statistical analysis was performed by software GraphPad Prism (version 3.0, GraphPad Software, San Diego, CA) and S-Plus (Insightful Corporation, Seattle, WA).

5.4 Results

The chemical composition of the test ingredients are presented in Table 5.2. All the experimental diets were well accepted by the fish. Growth rates, expressed as thermal-unit growth coefficients (Cho, 1992), ranged between 0.19 and 0.25, whereas feed efficiency (gain/feed) ranged between 0.9 and 1.2.

Regression of predicted bone-P content (based on bone-P content of ingredients) on analyzed bone-P content gave a highly significant ($p < 0.0001$) linear relationship (Figure 5.1):

$$\text{Predicted bone-P content (g/kg)} = 0.95 * \text{analyzed bone-P content (g/kg)} + 0.53 \quad (r^2 = 0.99)$$

The slope and intercept were not significantly different from 1 and 0, suggesting good agreement and free of bias between predicted bone-P content and analyzed bone-P content.

Contents of P fractions in the experimental diets are presented in Table 5.3. ADC values of dry matter, crude protein and ash of the diets are reported in Table 5.4. Comparisons of ADC of P and digestible P content (g/kg) from experimental observations and model predictions are reported in Table 5.5 and Table 5.6, respectively. There was no statistical difference ($p > 0.05$) between model prediction and experimental observations of ADCs of P and digestible P contents of the diets, except for Diet 1, and 7 (Table 5.5, 5.6).

A highly significant ($p < 0.0001$) linear relationship was observed between predicted digestible P content and observed values (Figure 5.2):

Predicted digestible P content (g/kg) = $1.04 * \text{observed digestible P content (g/kg)} - 0.73$ ($r^2 = 0.99$).

The intercept was not significantly different from 0, and the slope was not significantly different from 1, suggesting good agreement between model predicted and experimental estimated values.

Table 5.2 Chemical composition of the test ingredients used in the digestibility trial.

Ingredients	Composition (Dry matter basis) ¹			
	DM	CP	Ash	Total P
	%	%	%	%
Fish meal, herring	93.5	71.0	13.4	2.5
Fish meal, menhaden	93.4	66.7	17.6	3.7
Meat-bone meal 56% CP	96.5	57.0	23.5	3.5
Poultry by-product meal (low ash)	97.8	59.5	14.3	2.6
Poultry by-product meal (regular)	98.5	61.4	18.9	3.4
Soy protein concentrate	92.4	59.9	6.9	1.0
Soy protein concentrate, dephytinized	91.0	59.3	6.5	0.8

¹DM, dry matter; CP, crude protein (N x 6.25)

Table 5.3 P chemical compounds in experimental diets (g/kg dry matter)¹.

Diet No.	Diet	Total P	Bone-P	Phytate-P	Organic P
1	Reference	7.3	2.9	2.6	1.8
2	Fish meal, herring	12.0	6.3	1.9	3.8
3	Fish meal, menhaden	13.7	8.0	2.0	3.8
4	Meat-bone meal 56% CP	14.6	8.7	1.9	3.9
5	Poultry by-product meal (low ash)	12.6	6.7	1.9	4.0
6	Poultry by-product meal (regular)	14.5	8.4	1.9	4.2
7	Soy protein concentrate	8.7	3.1	3.1	2.6
8	Soy protein concentrate, dephytinized	8.5	3.0	2.2	3.3

¹Total P and bone-P contents were analyzed, phytate-P contents were estimated from diet formulae, and organic P contents were calculated by difference.

Table 5.4 Apparent digestibility coefficients (%) of dry matter (DM), crude protein (CP), and ash of the experiment diets (Mean \pm SE)^{1,2}.

Diet No.	Diets	DM	CP	Ash
		%	%	%
1	Reference	75 \pm 0.7	92 \pm 0.4	42 \pm 1.2
2	Fish meal, herring	74 \pm 0.5	91 \pm 0.3	38 \pm 1.0
3	Fish meal, menhaden	71 \pm 0.2	90 \pm 0.2	35 \pm 0.8
4	Meat-bone meal 56% CP	68 \pm 0.9	88 \pm 0.4	38 \pm 0.9
5	Poultry by-product meal (low ash)	75 \pm 0.4	92 \pm 0.2	43 \pm 0.9
6	Poultry by-product meal (regular)	70 \pm 1.0	89 \pm 0.4	37 \pm 0.9
7	Soy protein concentrate	74 \pm 0.8	93 \pm 0.2	43 \pm 1.3
8	Soy protein concentrate, dephytinized	72 \pm 0.6	93 \pm 0.3	45 \pm 0.2

¹Mean (n= 4 replicates)

²CP, crude protein (N x 6.25)

Table 5.5 Comparison of apparent digestibility (%) of P from experimental observation (n = 4) with model prediction (Mean \pm SE)¹.

Diet No.	Diet	Observation	Prediction
1	Reference	53.4 \pm 1.4 ^a	45.1 \pm 1.6 ^b
2	Fish meal, herring	53.4 \pm 0.8	51.6 \pm 1.1
3	Fish meal, menhaden	50.8 \pm 0.5	48.1 \pm 1.0
4	Meat-bone meal 56% CP	48.2 \pm 1.0	46.8 \pm 1.0
5	Poultry by-product meal (low ash)	56.8 \pm 1.2	51.5 \pm 1.0
6	Poultry by-product meal (regular)	52.1 \pm 1.3	48.2 \pm 0.9
7	Soy protein concentrate	53.3 \pm 1.3 ^a	46.1 \pm 1.2 ^b
8	Soy protein concentrate, dephytinized	59.3 \pm 1.3	53.9 \pm 1.3

¹Values within row with different subscript letters are significantly different ($p < 0.05$).

Table 5.6 Comparison of digestible P (g/kg DM) from experimental observation (n = 4) with model prediction (Mean \pm SE)¹.

Diet No.	Diet	Observed	Model Prediction
1	Reference	3.9 \pm 0.1 ^a	3.3 \pm 0.1 ^b
2	Fish meal, herring	6.4 \pm 0.1	6.2 \pm 0.1
3	Fish meal, menhaden	7.0 \pm 0.1	6.6 \pm 0.1
4	Meat-bone meal 56% CP	7.0 \pm 0.2	6.8 \pm 0.1
5	Poultry by-product meal (low ash)	7.2 \pm 0.2	6.5 \pm 0.1
6	Poultry by-product meal (regular)	7.6 \pm 0.2	7.0 \pm 0.1
7	Soy protein concentrate	4.6 \pm 0.1 ^a	4.0 \pm 0.1 ^b
8	Soy protein concentrate, dephytinized	5.1 \pm 0.1	4.6 \pm 0.1

¹Values within row with different subscript letters are significantly different ($p < 0.05$).

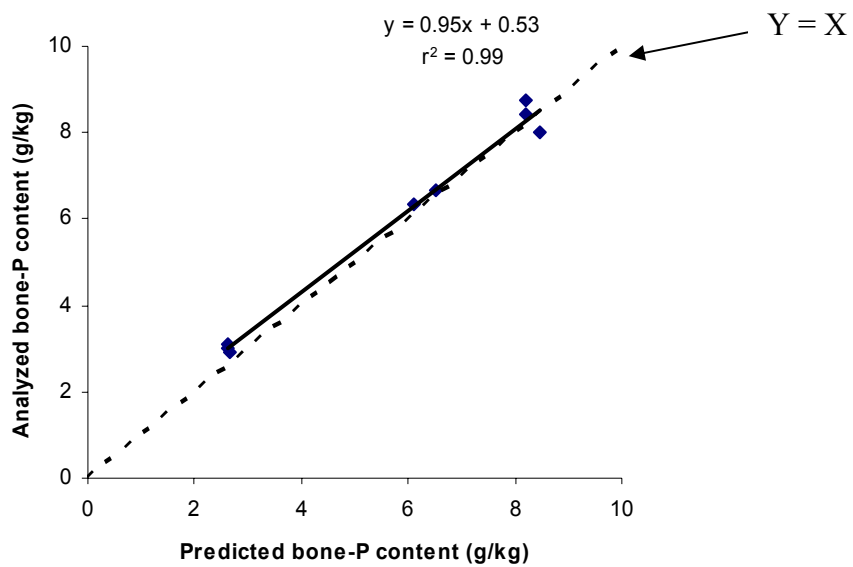


Figure 5.1 Predicted and analyzed bone-P content of the experiment diets (g/kg DM).

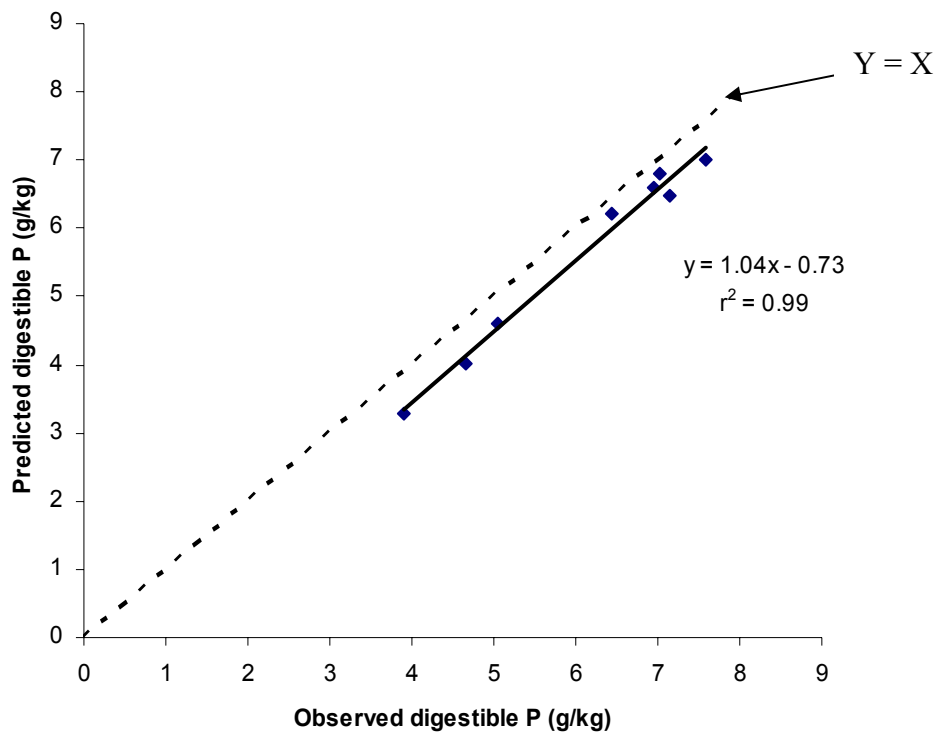


Figure 5.2 Predicted P and observed digestible P content of the experiment diets (g/kg DM).

5.5 Discussion

Good agreement was observed between predicted bone-P content of the diets based on bone-P contents of ingredients and analyzed bone-P content directly from diets (Figure 5.1), suggesting that the P fractionation protocol is not only suitable for analysis of animal ingredients, but also suitable for compounds diets. The slight underestimate of P digestible contents by the model could be due to leaching of P from feces or a result of an overestimation of phytate-P contents in these diets. Nevertheless, the overall model prediction was accurate and non-biased across all experimental diets, suggested by the slope and intercept of the linear regression of the model predicted values on experimental observed values (Figure 5.2).

Phosphorus contents and digestibility values of individual ingredients are highly variable, depending on quality of raw materials and processing method. For rainbow trout, apparent digestibility of P of fish meal varies from 17 to 81%, meat and bone meal from 22 to 45%, poultry by-product meal from 15 to 64%, soybean meal from 20 to 35% (Ogino et al., 1979; Riche and Brown, 1996; Sugiura et al., 1998a, 2000c; Sugiura and Hardy, 2000; Cheng and Hardy, 2002). Dietary P levels have variable effects on digestibility of P (Sato et al., 1996; Vielma and Lall, 1998a; Rodehutschord et al., 2000; Sugiura et al., 2000c). This large amount of information has not been integrated through a quantitative modeling approach.

The P digestibility model is the first mathematical model to estimate P digestibility and digestible P content of fish feeds, based on inclusion levels of different P chemical compounds. Although empirical in nature, the model incorporated mechanistic elements and its prediction of P digestibility is based on differentiation of P compounds rather than aggregates of total dietary P. The feature of an empirical model with mechanistic elements enables this model to be more physiologically relevant and biologically meaningful than a pure empirical model, yet it is more readily applicable in practical feed formulation than a pure mechanistic model. The experiment results suggested that P digestibility model well predicted P digestible P contents of the experimental diets formulated with a variety of ingredients used in practical feed formulation. The model can be a useful tool to estimate P digestibility of practical feeds containing bone-P, phytate-P and organic P. Future validation of model with respect of various types of inorganic P supplement is necessary.

The characteristics of static empirical models imply that the model can only be applied with confidence to conditions similar to those used to develop the model; extrapolation beyond the data range should be exercised with caution. The model was constructed based on literature data covering a broad variety of feed ingredients and diets. The contents of dietary P and P fractions of the experimental diets in this digestibility trial fall within the range of data and represent typical practical feed formulation. Specifically, the model was built on digestibility data from fish fed diets with total P contents between 0.6 and 30.5 g/kg, bone-P between 0 to 17.1 g/kg, phytate-P between 0 to 7.5 g/kg, and organic-P between 0.2 to 6.8 g/kg. Extrapolation of the model beyond the data range is not recommended. Nevertheless, the model is based on a broad range of contents of P fractions,

and is expected to be applicable to the majority of situations encountered in practical feed formulation.

5.6 Conclusion

The experiment results suggest that P digestibility model accurately estimated P digestibility and digestible P contents of the experimental diets formulated with a wide variety of ingredients used in practice. The P digestibility model is the first mathematical model developed to estimate digestible P content of fish feeds and it can be a useful tool in practical feed formulation.

**CHAPTER 6 DELINEATION OF FACTORS AFFECTING PHOSPHORUS
BODY CONCENTRATION AND DEVELOPMENT OF A P CONTENT MODEL
FOR RAINBOW TROUT**

6.1 Abstract

A biological method utilizing mass balance approach to estimate phosphorus (P) retention and subsequent waste outputs for fish culture operations requires accurate estimates of body P content and concentration of fish. Phosphorus concentration in rainbow trout is reported to vary from 0.24 to 0.62% of wet body weight in the literature. Various factors appear to affect P body concentration and this study represents a preliminary attempt to delineate some of these factors.

A model to estimate phosphorus content of fish was constructed by integrating various data from the literature. The effects of body weight and dietary digestible P on fish P body content were first examined. The effect of body weight (BW, g) on P content (g) was modeled through allometric analysis, which suggested a slight decrease in P concentration with increasing body weight. The effect of dietary digestible P (DPhos, per unit of digestible energy, g/MJ DE) was modeled by a kinetic saturation model, which described an asymptotic effect of digestible P on fish body P concentration. The resulted P content model was represented by the following equation: $P = (0.005 - 0.0015 / (1 + (Dphos/0.11)^{8.80})) * BW^{0.96}$

The P content model appears to be a practical model to estimate P content for rainbow trout. However, significant variations in P concentration remain to be better explained. The further improvement of the model requires better understanding and quantification of other factors that affect P body concentration, such as genetic factors and the effect of diet composition. The dynamic nature of the P deposition indicates that better characterization of fish body P concentration perhaps should be achieved through construction of a dynamic model or through a more comprehensive model predicting nutrient depositions in fish.

KEY WORDS: Phosphorus, nutritional model, carcass, waste, feed

6.2 Introduction

Phosphorus (P) is the most limiting factor for algal growth in freshwater and excess P can stimulate eutrophication of water bodies. Minimizing P environmental impact is of primary concern in freshwater aquaculture operations. Biological method has been shown to be an accurate, economical, and flexible approach to estimate P retention and subsequent waste outputs for fish culture operations (Cho et al., 1991, 1994). This method is a mass balance approach, which estimates solid waste output based on apparent digestibility of nutrients and estimates soluble waste output based on digestible nutrient retention. In order to

employ this approach to partitioning P waste output, accurate estimates of body P content of fish are required.

Estimates of P concentration of rainbow trout reported in the literature are highly variable, ranging between 0.24 and 0.62% of wet body weight (Figure 6.1). Even at similar body weights, estimates of P body concentration of rainbow trout varies significantly. For example, P concentration of rainbow trout weighing approximately 100g has been reported to vary between 0.29 and 0.52% (Wiesmann et al., 1988; Rodehutscord, 1996; Azevedo et al., 1998; Lanari and D'Agaro, 2002; Bureau et al., 2003). Fish P body concentration appears to be affected by numerous endogenous (biological) and exogenous (dietary, environmental) factors (Shearer, 1994). Digestible P level is perhaps one of the factors that have the most significant effect on body P concentration. Phosphorus deficiency results in poor bone mineralization and low body P concentration (Ogino and Takeda, 1976, 1978). In vertebrates, bone is the major storage site of P. The majority of P (80% to 85%) exists in skeletal tissues as hydroxyapatite, less than 14% of P is covalently bound as organic P in various soft tissues and organs, and only very small amount is presented as free ions or soluble inorganic P (Pi) in blood and body fluids (Lall, 1991; Berner, 1997). Therefore, in addition to digestible P supply, body P concentration may also be affected by a variety of factors that result in change in carcass composition, such as lifestage, genetic variations, as well as dietary and environmental factors. These factors remain to be delineated and quantified.

Therefore, the objective of this study was to delineate some of the factors that affect P concentration of rainbow trout and use this information to develop a practical model to estimate P content of rainbow trout.

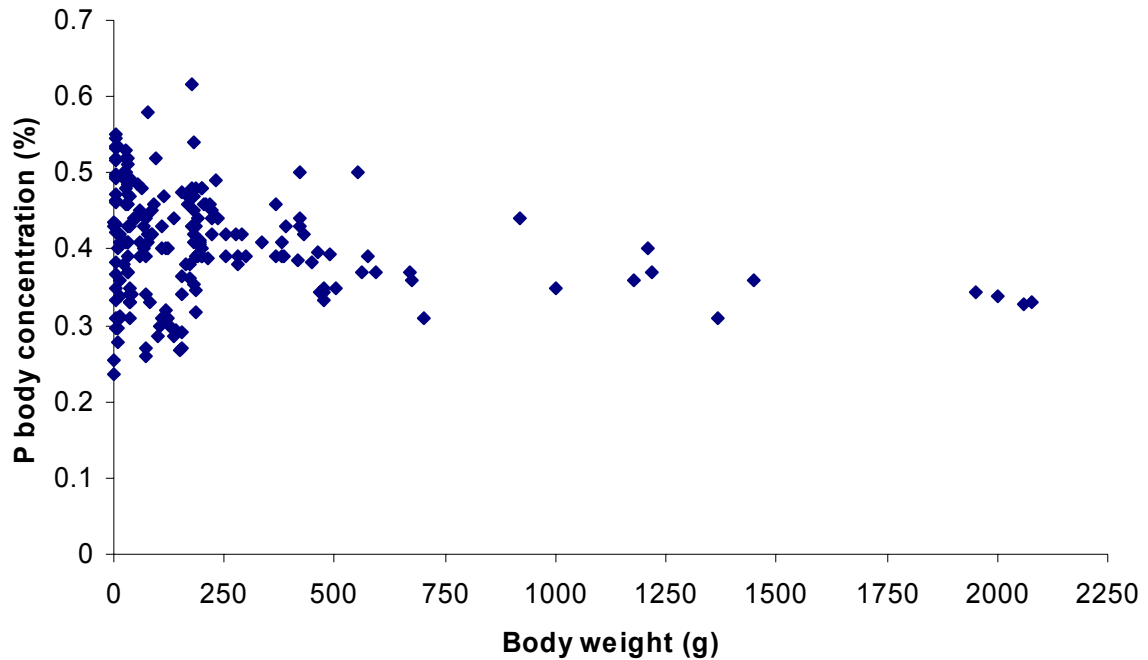


Figure 6.1 Body P concentration of rainbow trout¹.

¹data from Ogino and Takeda (1978), Shearer and Hardy (1987), Wiesmann et al. (1988), Heinen et al. (1993), Ketola and Richmond (1994), Rodehutschord (1996), Satoh et al. (1996), Encarnacao (1997), Skonberg et al. (1997), Azevedo et al.(1998), Vielma et al. (1998), Bureau and Cho (1999), Vielma et al. (1999b), Johnson and Summerfelt (2000), Barrias and Oliva-Teles (2000), Vielma et al.(2000), Coloso et al. (2001), Green et al. (2002), Lanari and D'Agaro (2002), Bureau et al. (2003), Coloso et al. (2003a), Ogunkoya (2004).

6.3 Materials and Methods

6.3.1 Effect of Body Weight

A database was constructed based on a survey of 22 studies (see footnote of Figure 6.1). A total of 142 estimates of P body concentrations from fish of different weights fed diets deemed adequate in digestible P were included in the modeling dataset. The body weight of fish included in the data ranged from 2.5 g to 2080 g.

The effect of body weight on P concentration was examined by allometric analysis (Huxley, 1932; Shearer, 1995):

$$P (g) = a * BW (g)^b$$

The allometric equation was log transformed and then solved as a linear regression equation:

$$Y = \alpha + \beta X_i + e_i$$

where $Y = \log P$,

$$X = \log BW,$$

$$\alpha = \log a,$$

$$\beta = \log b,$$

$$e_i = \text{residual error.}$$

6.3.2 Effect of Dietary Digestible P Level

The effect of dietary digestible P level on P body concentration was examined by a saturation kinetics model (Mercer, 1982; Rodehutscord and Pack, 1999) using raw data (12 data points) from Rodehutscord (1996). In Rodehutscord (1996), rainbow trout were fed with 12 graded levels of dietary P for 53 day growing from 50 g to 200 g. The diets were semipurified diets containing 0.1 to 1.1% of dietary P.

$$Y = (d (K_{0.5})^n + Y_{\max} (x)^n) / ((K_{0.5})^n + (x)^n)$$

Where Y = body weight standardized P concentration (%)

X = digestible P (Dphos) per unit of digestible energy (DE) (g/MJ DE)

Y_{\max} = plateau of curve

d = intercept on y axis

$K_{0.5}$ = concentration for $\frac{1}{2}$ of ($Y_{\max} + d$)

6.3.3 Model Response

The model response was theoretically simulated by using two diets: a low digestible P diet (digestible P = 0.10 g /MJ DE) versus a high digestible P diet (digestible P = 0.25 g /MJ DE), for fish of body weight ranging 1 g to 1000 g.

The statistical analyses in this study were performed with software GraphPad Prism version 3.0 (GraphPad Software, San Diego, CA, USA).

6.4 Results and Discussion

6.4.1 Effect of Body Weight

The allometric equation obtained from allometric analysis was $P \text{ content} = 0.0047 * BW^{0.96}$ ($p < 0.0001$, $R^2 = 0.99$). Exponent b was estimated to be 0.96. It was significantly different from 1. The allometric analysis suggested that P concentration decreases slightly by a factor of 0.96 when body weight increases. This is in agreement with Shearer (1995) who estimated an exponent of 0.965 through allometric analysis. Therefore, in this study, exponent 0.96 were used to standardize P across body weight / fish size, which was used in further analyses.

6.4.2 Effect of Dietary P Level

Because digestible energy of the diets grossly determine the feed efficiency (weight gain/feed intake) and the consequent amount of P ingested per unit in weight gain, it has been recommended to express dietary requirement as digestible phosphorus per unit of digestible energy (g/MJ DE), rather than as a percentage of diet content (Rodehutscord, 1996). The

efficiency of DE utilization was not affected by P deficiency in rainbow trout (Rodehutsord et al., 2000). It was also observed that P requirement increased with increasing dietary energy content in carp (Sato, 2000). Therefore, digestible P contents of the diets were represented here as digestible P per unit of digestible energy.

The effect of dietary P level on size standardized body P concentration was established by the following equation:

$$\text{Standardized P (\%)} = (0.35 * 0.11^{8.80} + 0.50 * \text{Dphos}^{8.80}) / (0.11^{8.80} + \text{Dphos}^{8.80})$$

$$R^2 = 0.97, p < 0.01.$$

where Standardized P (%) = P content / BW^{0.96} * 100

Dphos = digestible P per unit of digestible energy (DE) (g/MJ DE)

Therefore, P content was described by the following equation after mathematical simplification:

$$P \text{ (g)} = (0.005 - 0.0015 / (1 + (\text{Dphos}/0.11)^{8.80})) * \text{BW}^{0.96}$$

Low dietary P level is known to affect fish P body concentration. P deficiency results in poor bone mineralization and low body P concentration (Ogino and Takeda, 1976; 1978). Bone is the major storage site of P. Fish can mobilize body storage when P is deficient in diet, but the exhibition of deficiency signs depends on the severity of dietary P restriction (Baeverfjord et al., 1998). On the other hand, at body P saturation, increase in digestible P results in no additional P retention but linearly excretion of P through urine (Bureau and Cho, 1999; Sugiura et al. 2000a, b). The effect dietary P was well reflected in the highly

significant asymptotic relationship to body P concentration ($p < 0.01$) and good degree of fitness ($R^2 = 0.97$) of the saturation kinetic model (Figure 6.2).

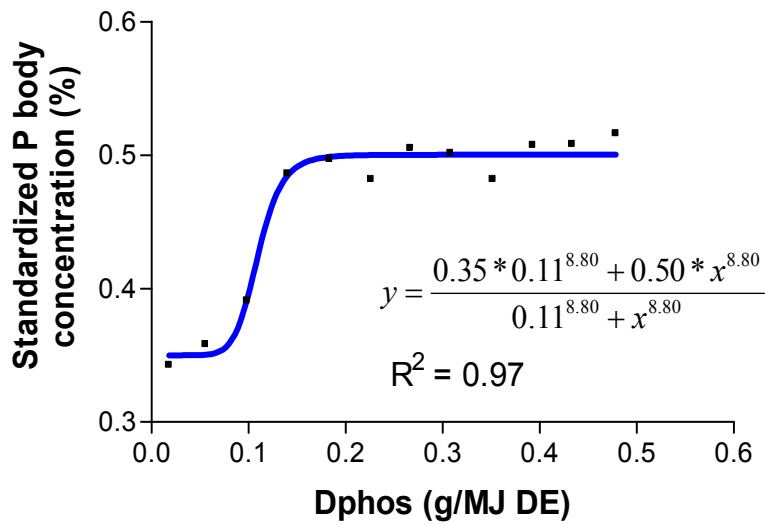


Figure 6.2 Effect of dietary digestible P level¹ on standardized body P concentration^{2,3}.

¹digestible P, Dphos, per unit of dietary energy concentration, g/MJ DE

²standardized body P concentration (%) = P content / BW^{0.96} * 100

³raw data from Rodehutscord (1996).

6.4.3 Model Response

The P content model is an integration of effects of body weight and digestible P level. The first effect was modeled through allometric analysis, which explained 99% of the variance of the P dataset. The second effect was modeled through saturation kinetic equation, which explained 97% of the variance of the raw data from Rodehutsord (1996). The integrated P content model was further partially evaluated by model simulation in comparison to the dataset. This was a partial evaluation because the dataset did not permit evaluation of varying digestible P level. Regression of model simulated P content on observation resulted in a highly significant linear relationship (Figure 6.3):

$$\text{Simulated P content} = 1.06 * \text{observed P content} - 0.03 \quad (p < 0.0001, r^2 = 0.99).$$

The slope was significantly different from 1; the intercept was not significantly different from 0. The result suggested that model simulation was slightly biased and had the tendency to overestimate observation at high P content from large fish, by about 5%. The slight model bias towards large body weight is likely due to the fact that the data for large fish were scarce; the majority of data were for fish size lower than 1000 g. Nevertheless, there is overall a good agreement between model simulation and dataset observation of P content.

Model simulated P concentration (%) was compared to observed P concentration across body weight (Figure 6.4). Since the sufficient P dataset did not permit evaluation of model response to different dietary P levels, two diets, low digestible P diet (digestible P = 0.10 g /MJ DE) versus high digestible P diet (digestible P = 0.25 g /MJ DE), were used to

theoretically simulate the model response to digestible P levels across body weight from 1g to 1000g (Figure 6.5). According to the model, the digestible P level sufficient to result in body saturation was 0.22 g/MJ DE. The simulation illustrated that a low P diet (insufficient to support body saturation) resulted in lower body P concentration than a high P diet.

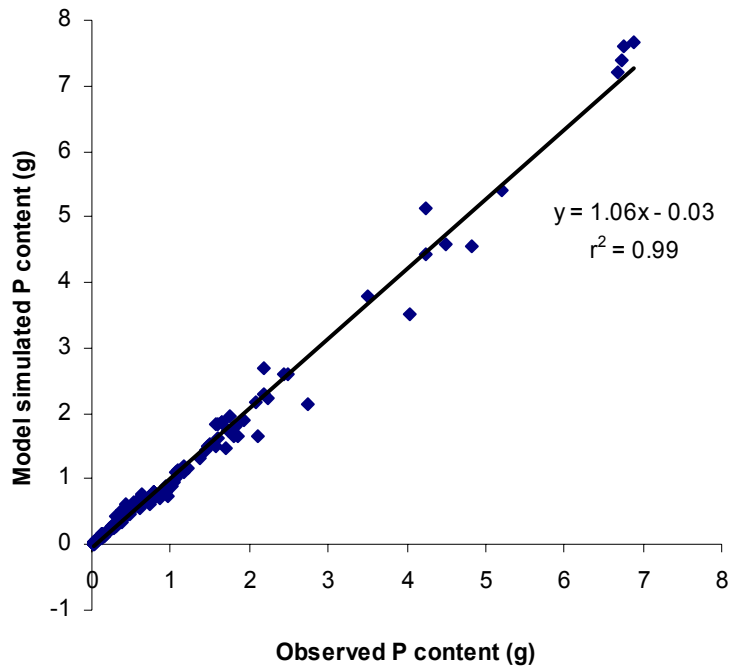


Figure 6.3 Comparison of model simulated and observed P body content (g).

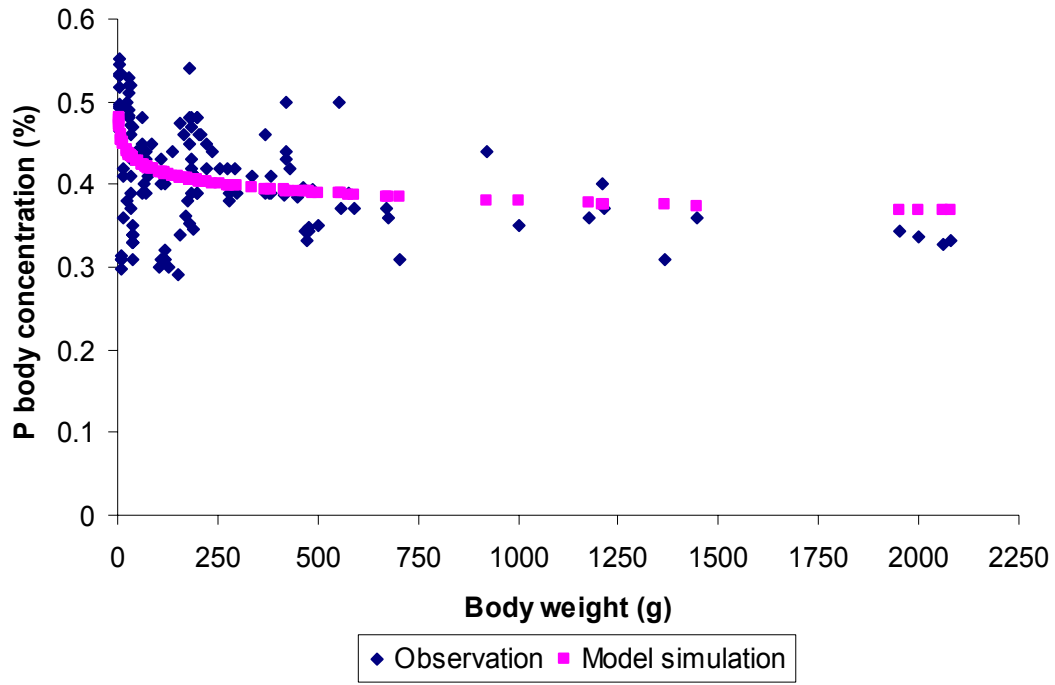


Figure 6.4 Model simulated and observed P body concentration (%) of rainbow trout across body weight ($r^2 = 0.14$).

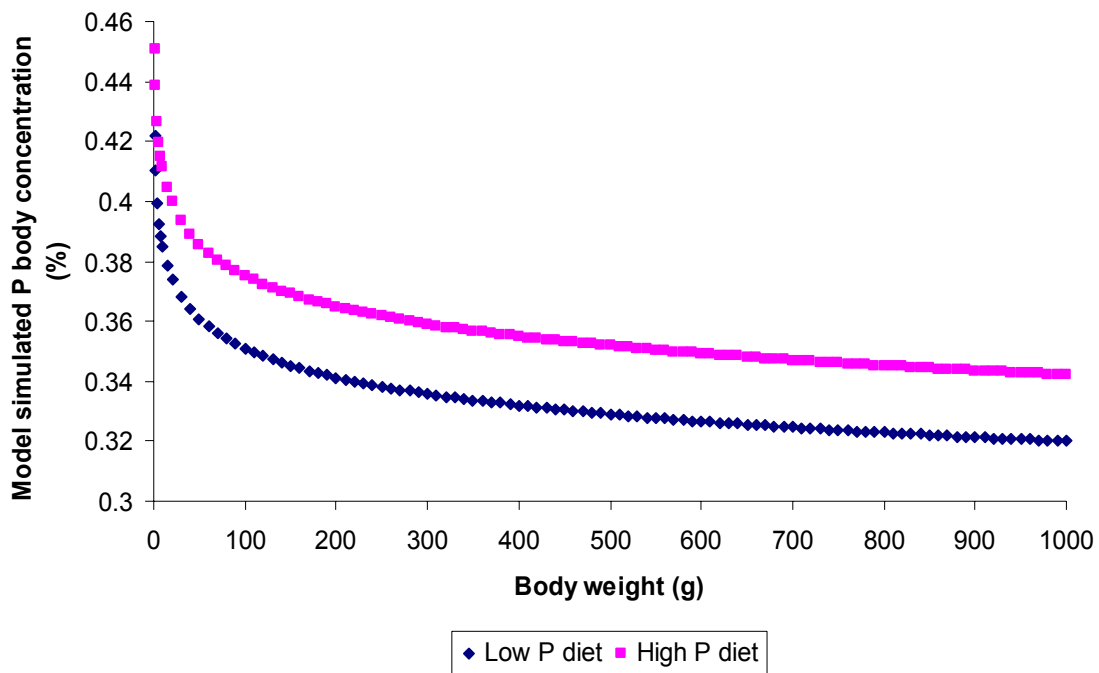


Figure 6.5 Theoretical simulation of model response to a low P diet (digestible P = 0.10 g/MJ DE) versus a high P diet (digestible P = 0.25 g/MJ DE).

6.4.4 Further Delineation of the Variations of P Concentration

The model explained 99% of the variance in P content of the dataset (Figure 6.3), however, only 14% of the variance in P concentration (Figure 6.4). The P content estimate provided by the model appears to be accurate and practical to be used in the mass balance approach for practical diets that are formulated with sufficient dietary P. However, the variation of P concentration should be better explained and the affecting factors should be further delineated and quantified. Since the majority of P exists in bone, change in the proportion of bone and soft tissues can significantly affect P concentration of the whole body, even when dietary P supply is sufficient for maximum bone mineralization. Therefore, P concentration is subject to variation of other body components.

Genetic effect may be one of the contributing factors to the variability of P concentration. There has been no study on genetic variation of P concentration in rainbow trout, although significant genotypic difference in body protein and ash content between strains of rainbow trout was observed (Reinitz et al., 1979). It can be hypothesized that lean fish may have higher proportion of bone and consequently higher P concentration, in comparison to fatty fish. The demonstration of genetic variations in body P concentration requires experiments where P concentration is compared across genetic strains.

Feed intake could potentially result in differences in body composition through the change of the proportion of bone mass and soft tissue mass. Fish fed with restricted rations

may have less body protein mass and more bone mass, thus P concentration would be higher in fish fed at low feeding level. In Sugiura et al. (2000b), fish starved for 8 weeks had significantly higher P body concentration and higher P to nitrogen (N) ratio than fed fish. A highly linear negative relationship between body P concentration and feed intake was also observed in Bureau et al. (unpublished) after 24 weeks of feeding trial (Figure 6.6). A linear relationship was established as follows:

$$Y = -0.29 * X + 1.28 \text{ (} p < 0.005, r^2 = 0.99 \text{)}$$

where Y = ratio of change in P concentration, i.e., ratio of fish body P concentration fed on a certain feeding level to that of fed 100% voluntary feed intake

$$X = \% \text{ of voluntary feed intake}$$

Therefore, in application of the current model, if the feed intake can be estimated, an effect of feed intake can be added to the model by an adjustment of the model parameters, on the assumption that feed intake does not change the curve of model. For examples, as illustrated by Figure 6.7, two feed intake levels (50% versus 100% voluntary feed intake) shifted the curve of low P diet and high P diet upwards, resulting in higher P body concentration. The model simulated that fish fed at 50% of voluntary feed intake with a low P diet can results in higher P body concentration than fish that are fed on a high P diet (sufficient for body P saturation). However, the effect of feed intake level is likely to be dependent on the temporal duration at a certain feed intake level of a diet. For example, extrapolation of the effect of feed intake (obtained after 24 weeks of feeding trial) to zero feed intake (starvation) would suggest a 28% increase in body P concentration. Sugiura et al. (2000b) observed a 14% increase in body P concentration after 8 weeks of starvation. The representation of time effect cannot be achieved by a static model. This requires a dynamic modeling structure.

Discrepancy between model simulation and dataset observation could also be due to variable nutritional composition of the experimental diets and consequent variation in fish body composition. The model dataset were pooled from studies in the literature. The variability within study is more likely due to dietary factors rather than genetic or environmental factors. Reduction in P concentration was observed when body lipid concentration increased, which was resulted from higher dietary lipid level or protein: fat ratio (Ronsholdt, 1995; Green et al., 2002; Lanari et al., 2002). The interaction between diet composition, growth performance, and body composition is a complex subject that is being studied intensively. To add to this complexity, how long the fish are fed a certain diet is likely to affect the previously mentioned interaction between diet composition, growth performance, and body composition as well. The dynamic nature of nutrient deposition cannot be adequately characterized and represented by a static empirical model. The limitations of the static empirical model can only be overcome by a dynamic model taking into account of the dynamics of nutrient deposition over time.

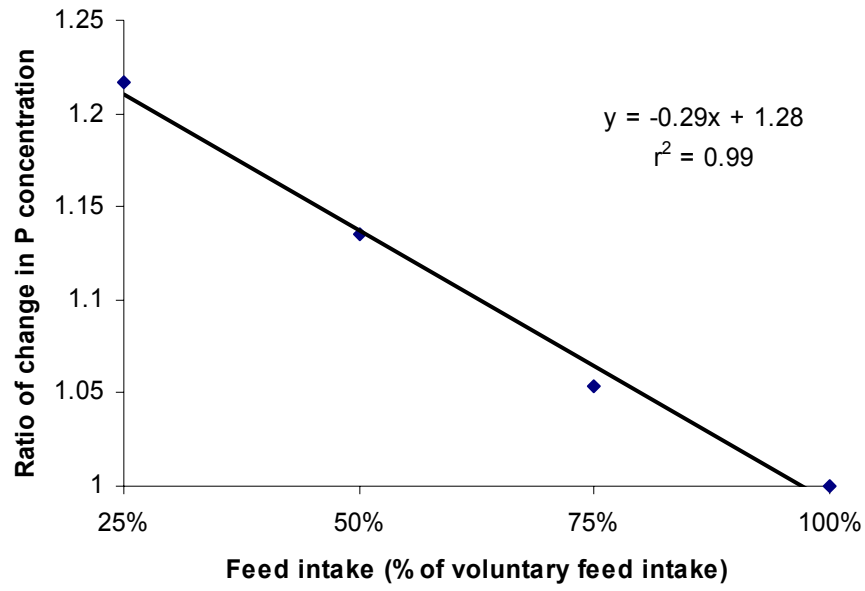


Figure 6.6 Effect of feeding levels on fish body P concentration.

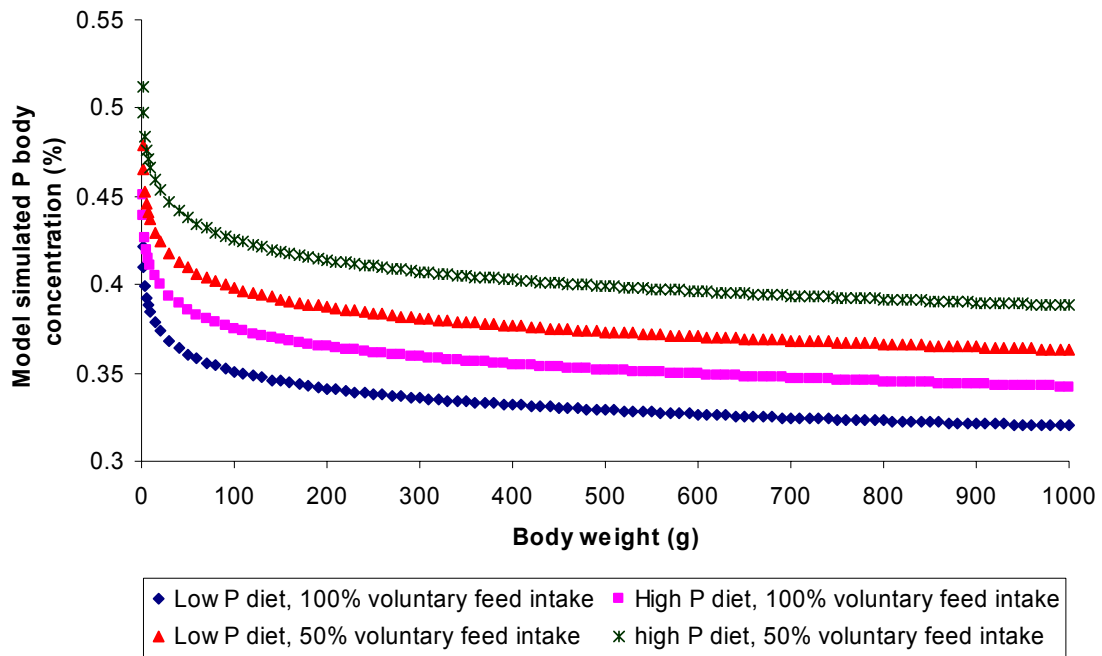


Figure 6.7 Theoretical simulation of the model response to a low P diet (digestible P = 0.10 g/MJ DE) versus a high P diet (digestible P = 0.25 g/MJ DE) at two feed intake levels, 50% and 100% of voluntary feed intake.

6.5 Conclusion

A model to estimate P body content of rainbow trout was constructed based on effects of body weight and dietary digestible P supply. This model represents a preliminary attempt to delineate factors that affect body P concentration. While requiring improvement to better explain the variation of P concentration, it is a practical tool to estimate P content for rainbow trout, and to estimate P retention through a mass balance approach for practical diets that are formulated with sufficient dietary P levels. The model response to deficient P levels should be validated before application. A variety of factors, such as genetic variation and diet composition, remains to be better understood and further quantified. The dynamic nature of the P deposition indicates that better characterization of fish body P concentration perhaps should be achieved through construction of a dynamic model.

CHAPTER 7 INTEGRATION OF A FACTORIAL PHOSPHORUS MODEL TO PREDICT WASTE OUTPUT FROM SALMONID FISH PRODUCTION

7.1 Abstract

Minimizing phosphorus (P) wastes is a key factor for environmental sustainability of freshwater aquaculture operations. A factorial P model was constructed to simulate the effects of different dietary P sources and levels on P digestibility, retention, solid waste output and soluble waste output. This factorial P model consists of three sub-models, P digestibility sub-model, P content sub-model, and waste output sub-model. The factorial P model was further incorporated into a fish bioenergetics model. Four prototypic commercial feeds containing 0.9 to 2.2% dietary P was used to simulate the response of model to rainbow trout growing from 50 to 1000 g reared in cages. Rainbow trout fed the various diets were predicted to have feed efficiency (gain/feed) between 0.74 and 0.83. The apparent digestibility coefficients of P varied from 32 to 60%, retention of P ranged from 14 to 36% of dietary P intake. Solid P waste output varied from 4.5 to 18.9 kg per ton of fish produced, soluble P waste varied from 2.5 to 5.2 kg per ton of fish produced. The factorial P model provided a simple means to estimate P digestion, retention, and waste output for salmonid culture.

KEY WORDS: Phosphorus, Waste, Model, Rainbow trout, Aquaculture

7.2 Introduction

Phosphorus (P) is the first limiting factor for algal growth in freshwater and excessive P can stimulate eutrophication. P waste outputs by fish culture operations is an issue heavily scrutinized by environmental agencies around the world and minimizing P wastes is considered a key factor for the environmental sustainability of freshwater aquaculture operations. The quantity and forms of P waste output are affected by a variety of factors, including dietary P level, digestibility of P, P requirement of fish, feed composition and feed efficiency. Therefore, estimates of P waste outputs vary significantly in the literature. For rainbow trout, total P waste outputs were estimated to be in the range of from 1.4 to 25 g/kg biomass produced (Wiesmann et al., 1988; Gomes et al., 1995; Lanari et al., 1995; Kim et al., 1998; Ruohonen et al., 1999; Vielma et al., 2000; Green et al., 2002; Lanari and D'Agaro, 2002; Vielma et al., 2002).

Biological method of estimating waste outputs based on nutrient balances have been shown to be an accurate, economical, and flexible approach for fish culture operations (Cho et al. 1991, 1994). This method is a mass balance approach, which estimates solid waste output based on apparent digestibility of nutrients and estimates soluble waste output based on comparative carcass analyses. This biological method is particularly useful for practical applications, such as in cage farming conditions where chemical or limnological method often

yield variable estimates yet are very difficult or costly to apply on a routine basis (Cho et al. 1991, 1994). The dietary P supply that is not digested by fish is excreted as largely as solid waste output, whereas the digested portion that is not retained in fish body is excreted as soluble waste output. Soluble P waste is more available to algae and can, consequently, has more immediate environmental impact than solid P waste (Cho and Bureau, 2001). The chemical characteristics of the solid P components determine whether they are mineralized or solubilized in the environment. For example, bone-P excreted as solid P waste is largely inert under normal environmental condition, whereas organic P such as phytate-P may be hydrolyzed by certain organisms in the environment (Persson 1991). Therefore, it is necessary to estimate total P waste output accurately, and more importantly, the quantification of the forms of P wastes released can provide information on the differences in their potential environmental impact.

A series of models to predict digestible P content of fish feed and P body content was developed in the previous chapters of this thesis (Chapter 4, 6). The P digestibility model estimates digestible P contents of fish feed based on the dietary inclusion level of different P chemical compounds, which were characterized into five categories: bone-P, phytate-P, organic P, Ca monobasic / Na / K Pi supplement, Ca dibasic Pi supplement. P content model estimates P body content based on body weight and dietary digestible P supply. A waste output model to estimate solid waste output and soluble waste output could be constructed based on information provided by P digestibility model and P content model. These models could be integrated into a factorial P utilization model that takes into consideration of the various factors affecting P digestibility, retention and waste output in salmonid fish culture.

The objective of study was to construct a factorial P model by integration of sub-models of P digestibility, content, and waste output, and the Fish-PrFEQ bioenergetics model that estimates feed requirement based on diet composition and growth rate.

7.3 Materials and Methods

7.3.1 Model Description

A factorial P model was constructed consisting of a P digestibility sub-model, a P content sub-model, and a P waste output sub-model (Figure 7.1). The factorial P model was further integrated into bioenergetics model of salmonid fish (Cho, 1992; Cho and Bureau, 1998; Bureau et al., 2002, Bureau et al., 2003).

The P digestibility sub-model was previously described (Chapter 4). Different P chemical compounds present in fish feed were characterized into broad categories of bone-P, phytate-P, organic P, Ca monobasic / Na / K Pi supplement, Ca dibasic Pi supplement. The P digestibility sub-model was expressed as: digestible P = 0.68 bone-P + 0 phytate-P + 0.84 organic P + 0.89 Ca monobasic / Na / K Pi supplement + 0.64 Ca dibasic Pi supplement + 0.51 phytase/phytate - 0.02 (phytase/phytate)² - 0.03 (bone-P)² - 0.14 bone-P * Ca

monobasic / Na / K Pi supplement ($p < 0.0001$, $R^2 = 0.96$). The units for all variables are g/kg diet, except for phytase/phytate ratio the unit is 100 FTU phytase/g phytate.

Feed intake of fish was generated by Fish-PrFEQ bioenergetics model of salmonid fish based on diet composition and growth rate (Cho, 1992; Cho and Bureau, 1998; Bureau et al., 2002, Bureau et al., 2003). Growth rate was represented by thermal growth coefficient (TGC) (Iwama and Tautz, 1981; Cho, 1992). Output of this sub-model was dietary digestible P intake.

Digestible P intake was the input to the P content sub-model (Chapter 6). This sub-model was constructed integrating effects of body weight (BW, g) and dietary digestible P (DPhos, per unit of digestible energy, g/MJ DE). The P content model for rainbow trout was described as follows:

$$P \text{ (g)} = (0.005 - 0.0015 / (1 + (Dphos/0.11)^{8.80})) * BW^{0.96}$$

P retention over a growth period was estimated by the difference of P content at final and initial body weight. Output of the sub-model was the retained dietary digestible P in fish body over the growth period.

The P waste output sub-model utilized model outputs from P digestibility sub-model and P content sub-model, and estimated solid waste and soluble waste outputs. The difference between dietary P intake and digestible P intake (information generated by P digestibility sub-model) was the solid P waste output. The difference between P retained and

P digested (information generated by P content sub-model) was the soluble P waste output.

Total waste output was the sum of solid waste and soluble waste outputs.

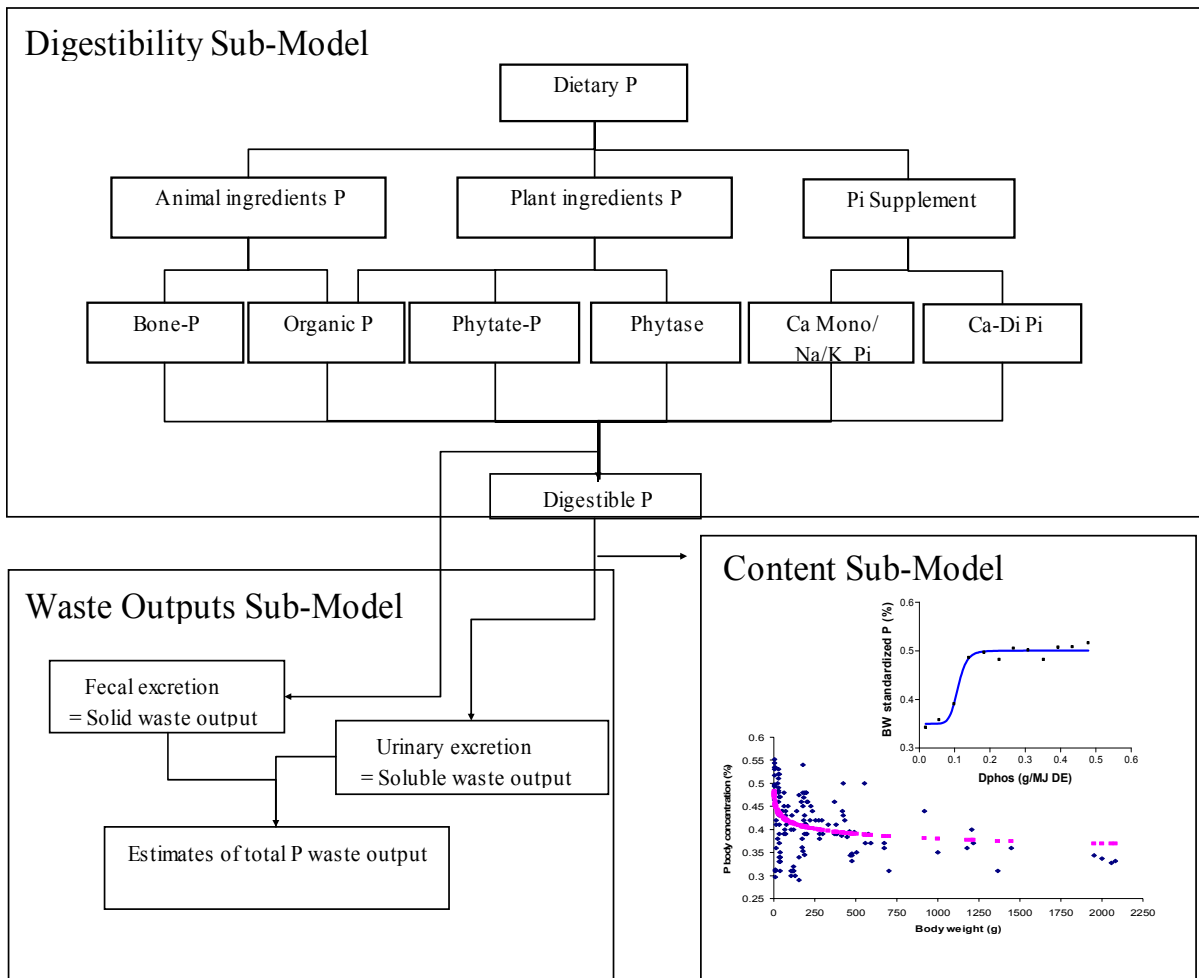


Figure 7.1 Components of a factorial P model for salmonid fish.

7.3.2 Model Simulation

A prototypical commercial feed and a series of theoretical diets were formulated based on this prototypical commercial feed was used to evaluate the response of the model for rainbow trout (Table 7.1). The prototypical commercial feed was formulated with 18% fish meal, 13% poultry byproduct meal, 9% whey, 37.6% corn gluten meal, and 20% fish oil. The dietary total P content was estimated to be 1.15%, including 0.61% of bone-P, 0.12% of phytate-P, and 0.42% of organic P. No inorganic phosphate supplement was used. To test the model response to the effect of total dietary P and types of P on P utilization in fish, five sets of diets were theoretically formulated as follows: 1) dietary bone-P content was increased from 0.61% in the prototypical commercial feed to 1.01% by an increment of 0.1%, while organic P and phytate-P contents were held the same as in the prototypical commercial feed (Diets 1 to 4); 2) dietary organic P content was increased from 0.42% in the prototypical feed to 0.82% while bone-P and phytate-P contents were held constant (Diets 5 to 8); and 3) dietary phytate-P content was increased from 0.12% in the prototypical feed to 0.52% while bone-P and organic P contents were held constant (Diets 9 to 12). In these three sets of diets, the dietary total P contents increased from 1.15% to 1.55% in correspondence to increasing contents of different P types. 4) In addition, a fourth set of diets (Diets 13 to 18) was formulated by holding total P contents constant, while varying the contents of bone-P, organic P, and phytate-P by 0.1% in pairs. 5) A fifth set of diets were formulated to test the model in response to low dietary P: phytate-P was held constant at 0.02% and bone-P content

constant at 0.11%, while organic P content was increased from 0.12% to 0.62% by an increment of 0.1%, dietary total P contents increased from 0.25% to 0.75% correspondingly (Diet 19 to 24). Optimal growth condition was assumed. TGC and temperature were assumed to be 0.2 and 15°C, respectively. The modeled period was from body weight of 50 g to 500 g.

Phosphorus utilization by fish fed practical diets in cage farming conditions was further simulated. A series of prototypical commercial feeds (Diet A, B, C, D) containing dietary P from 0.9 to 2.2% were used to simulate P utilization by rainbow trout growing from 50 to 1000 g. Diet composition was presented in Table 7.2. Rearing condition of rainbow trout cage farm was assumed as follows: TGC was 0.18, and average water temperature was 8.5°C.

Table 7.1 Contents (%) of total P and types of P in a prototypical commercial feed and five sets of diets used in the simulation of model response.

Diet	Total P	Bone-P	Organic P	Phytate-P
Prototypical feed	1.15	0.61	0.42	0.12
Set 1				
Diet 1	1.25	0.71	0.42	0.12
Diet 2	1.35	0.81	0.42	0.12
Diet 3	1.45	0.91	0.42	0.12
Diet 4	1.55	1.01	0.42	0.12
Set 2				
Diet 5	1.25	0.61	0.52	0.12
Diet 6	1.35	0.61	0.62	0.12
Diet 7	1.45	0.61	0.72	0.12
Diet 8	1.55	0.61	0.82	0.12
Set 3				
Diet 9	1.25	0.61	0.42	0.22
Diet 10	1.35	0.61	0.42	0.32
Diet 11	1.45	0.61	0.42	0.42
Diet 12	1.55	0.61	0.42	0.52
Set 4				
Diet 13	1.15	0.51	0.52	0.12
Diet 14	1.15	0.51	0.42	0.22
Diet 15	1.15	0.71	0.32	0.12
Diet 16	1.15	0.71	0.42	0.02
Diet 17	1.15	0.61	0.52	0.02
Diet 18	1.15	0.61	0.32	0.22
Set 5				
Diet 19	0.25	0.11	0.12	0.02
Diet 20	0.35	0.11	0.22	0.02
Diet 21	0.45	0.11	0.32	0.02
Diet 22	0.55	0.11	0.42	0.02
Diet 23	0.65	0.11	0.52	0.02
Diet 24	0.75	0.11	0.62	0.02

Table 7.2 Prototypical commercial feeds used in the simulation of phosphorus utilization by rainbow trout under cage farming conditions.

Diet	A	B	C	D
Fish meal, herring, 68% CP	28	24	20	20
Corn gluten meal, 60% CP	28	24	11	11
Blood meal, spray-dried, 81% CP	6	-	5	5
Feather meal, disc-dried, 71% CP	-	-	17	17
Meat and bone meal, 46% CP	-	-	25	-
Poultry by-product meal, 64% CP	-	20	-	16
P fractions				
Total P, %	1.0	1.3	2.3	1.3
Bone-P, %	0.5	0.8	1.7	0.7
Phytate-P, %	0.1	0.1	0.0	0.1
Organic P, %	0.4	0.4	0.6	0.5

7.4 Results and Discussion

Table 7.3 presents the model response to the five sets of diets theoretically formulated based on a prototypic commercial feed. In general, the model responded appropriately to different types of P compounds and contents. Diet Set 1, 2, and 3 had same increment of total P resulted from increment of different P compounds. Among the three diet sets, increment of organic P in Diet Set 2 resulted in most urinary P excretion but least fecal excretion, suggesting organic P is highly digestible. Increment of bone-P in Diet Set 1 resulted in more fecal but less urinary P excretion than increment of organic P. Increment of phytate-P in Diet Set 3 resulted in most fecal P excretion, consistent with low digestibility of phytate-P. Diet Set 4 illustrated that different combination of P compounds can affect P utilization significantly, even at the same total dietary P contents. Therefore, accurate estimates of P utilization of the diets can only be achieved based on differentiation of P compounds rather than aggregates of total dietary P. Diet Set 5 tested the model response to low P diets. The model estimated unrealistic retention values for low dietary P. This is likely because TGC was assumed constant at 0.2 across all diets in the model simulation. This assumption is apparently unsuitable for the low P diets since P deficiency impairs growth and metabolism of fish. Phosphorus deposition is not an isolated event; it is highly dependent on growth rate and deposition of other nutrients such as protein, lipid and carbohydrate. It is apparent that the factorial P model in the current framework of Fish-PrFEQ bioenergetics model of salmonid is not capable of solving the interaction between low digestible P supply and fish growth and metabolism. Fish-PrFEQ bioenergetics model is not intended as a growth or

nutrient utilization model, rather, it estimates feed intake based on growth rate as model input. The interaction of diet composition and growth performance would be better represented by a nutrient utilization model. Therefore, the current model is not applicable to the situation where P deficiency impairs fish growth and metabolism. It appears that the model is not suitable for the dietary P level below 0.55% total P or 0.4% digestible P.

Table 7.3 Model simulated P utilization of a prototypical commercial feed and five sets of diets for rainbow trout.

Diet	P retention	P urinary excretion % dietary P	P fecal excretion
Prototypical feed	40	16	44
Set 1			
Diet 1	37	16	47
Diet 2	34	16	49
Diet 3	32	16	52
Diet 4	30	15	55
Set 2			
Diet 5	37	21	42
Diet 6	34	26	40
Diet 7	32	30	38
Diet 8	30	33	37
Set 3			
Diet 9	37	16	49
Diet 10	34	14	52
Diet 11	32	13	56
Diet 12	30	12	59
Set 4			
Diet 13	40	20	40
Diet 14	40	13	47
Diet 15	40	11	49
Diet 16	40	18	42
Diet 17	40	23	37
Diet 18	40	9	51
Set 5			
Diet 19	>100	0	32
Diet 20	>100	0	28
Diet 21	>100	0	25
Diet 22	83	2	24
Diet 23	70	9	22
Diet 24	61	17	22

Table 7.4 presents the model simulation of P utilization and waste output of fish in cage farming conditions. The simulation assumed that the fish were fed four prototypical commercial feeds containing 0.9 to 2.2% dietary P growing from 50 to 1000 g. The fish were predicted to have feed efficiency (gain/feed) ranging from 0.74 to 0.83. The apparent digestibility coefficients (ADC) of P varied from 32 to 60%, retention of P ranged from 14 to 36% of dietary P intake.

The model simulation suggested that P utilization in fish varies significantly according to diet formulation. Ingredient selection had significant impact on P dietary content and P digestibility. Many rendered animal protein ingredients (meat and bone meals, poultry by-products meals) generally have a high P content. Phosphorus is present in different chemical compounds in fish feed and their digestibility differs significantly. Furthermore, the digestibility of bone-P, the primary form of P in animal protein ingredients, is not additive and it decreases with inclusion level. The diet formulated with 25% meat and bone meal (Diet C) had highest levels of total P and bone-P contents, and the lowest P digestibility among these four diets. Diet D, formulated with poultry by-products meal and feather meal, had similar digestibility to Diet A, which is a fish meal/corn gluten meal based diet. Diet formulation also affected P retention. Although Diet A and D had similar digestibility, Diet A was simulated to have highest P retention due to its lower total dietary P content. Diet C, the diet that was estimated to have the lowest P digestibility, was also simulated to have the lowest P retention among the four diets.

As presented in Table 7.4, model simulation of solid P waste output varied from 4.5 to 18.9 kg per ton of fish produced, soluble P waste varied from 2.4 to 5.2 kg per ton of fish produced, and total P wastes between 7.0 and 24.1 kg per ton of fish produced. The model simulation illustrated that P waste outputs were highly variable and affected by a variety of factors, such as total dietary P, P digestibility, and feed efficiency. The makeup of the solid waste output also differs. Bone-P apparently accounted for a majority of P compounds in the composition of feces. The diets with 25% meat and bone meal (Diet C) had lowest P digestibility and high total P content, and consequently produced most solid P waste output. Diet C also produced large amount of soluble P waste in spite of low P digestibility, due to its high level of total dietary P. Diet C was estimated to produce feces that composed of 93% bone-P and 7% organic-P (including phytate-P). By comparison, Diet A had highest digestible P retention and produced lowest amount of soluble P waste outputs. It was estimated to produce feces composed of 58% bone-P and 32% organic-P (including phytate-P).

The differentiation and accurate estimate of forms of P waste outputs would be useful in constructing nutrient management strategies to minimize the environmental impact of P waste outputs, particularly, soluble P waste outputs. While solid waste can be reduced by selection of highly digestible ingredients, reduction in soluble waste can be achieved by reducing digestible P inclusion level in feed. By formulating feeds with ingredients of low total P content and high P digestibility, it is possible to minimize both solid waste and soluble waste. The model generated information regarding the fecal P composition can be useful to those who are interested in the investigating the impact of different P compounds to the

aquatic ecosystem, since the chemical characteristics of the solid P components determine whether they are mineralized or solubilized and their eventual impact to the environment (Persson 1991).

The estimates of P utilization and P waste outputs generated by the current factorial P model are theoretical and require experimental validation. Nevertheless, it has demonstrated that feed formulation significantly affects P utilization and waste output, and nutrient management can be an effective approach to minimize P environmental impact. The factorial P model can be a simple yet useful tool in nutrient management of aquaculture operations.

Table 7.4 Simulated P utilization and waste output by rainbow trout under cage farming conditions.

Diet	A	B	C	D
Feed efficiency (gain/feed)	0.82	0.83	0.74	0.78
ADC of P (% dietary P)	59	55	32	60
P body retention (% dietary P)	36	27	14	27
P waste output				
Solid P waste (kg per ton of fish produced)	4.5	6.5	18.9	5.8
Soluble P waste (kg per ton of fish produced)	2.5	4.1	5.2	4.7
Total P waste (kg per ton of fish produced)	7.0	10.6	24.1	10.5
Feces composition				
% bone-P in feces	58	77	93	70
% organic P including phytate in feces	32	23	7	30

7.5 Conclusion

A factorial P model was constructed based on sub-models of digestibility, retention, and waste output. The factorial P model provides a simple means to estimate P utilization in practical salmonid culture operations. The differentiation of P chemical compounds in ingredients and feed not only provides accurate estimates of the digestibility of dietary P, but also allows quantification of forms of P waste output. This model could be a useful tool in practical feed formulation and nutrient management.

CHAPTER 8 DYNAMIC SIMULATION OF PHOSPHORUS UTILIZATION IN SALMONID FISH[†]

8.1 Abstract

Minimizing phosphorus (P) wastes is a key factor for environmental sustainability of freshwater aquaculture operations. A dynamic model was constructed to simulate P utilization in salmonid fish through digestion, body deposition, and excretion into urine and feces. Dietary P was classified into pools of bone-P, phytate-P, organic P, Ca monobasic / Na / K phosphate and Ca dibasic phosphate. Hydrolysis of P from Ca monobasic / Na / K phosphate and Ca dibasic phosphate pools was represented by linear equations, whereas hydrolysis of P from bone-P, phytate-P, and organic P pools was simulated by Michaelis-Menten equations. The available P pool was the sink for these hydrolyzed portions. Absorption from the available P pool was simulated by passive and active uptake of P into the blood pool. Indigestible and unabsorbed P was excreted through feces. Clearance of blood P was simulated by deposition into bone and soft tissues, excretion through urine as well as endogenous secretion into feces. Bone deposition and soft tissue deposition in relation to blood P concentration were represented by Michaelis-Menten equations. Urinary excretion was the difference between glomerular filtration and saturable tubular reabsorption

[†] This chapter has been accepted for publication in the Proceedings of the 6th International Workshop on Modeling Nutrient Utilisation in Farm Animals.

and was regulated by blood P concentration. The model was made dynamic by incorporating a body weight growth function. Response analysis was performed for fish weighing between 50 g and 500 g, and the model responded appropriately to dietary P sources and levels. Retention of P ranged from 25 to 28% of intake for a prototypical commercial feed containing 1.15% dietary P. The model was also tested for parameter sensitivity. The most sensitive parameters were those related to body deposition and urinary excretion. This model can be used to simulate the effects of different dietary P sources and levels on P digestibility, retention, solid waste output (fecal excretion) and soluble waste output (urinary excretion). It could be useful for formulating strategies to improve production efficiency and reduce waste output.

8.2 Introduction

Phosphorus (P) is the first limiting factor for algal growth in freshwater and excessive P stimulates eutrophication. Phosphorus waste output by fish culture operations is an issue heavily scrutinized by environmental agencies around the world and minimizing P waste is considered a key factor for the environmental sustainability of freshwater aquaculture operations. Nutritional management has been shown to be the most effective approach to reduce P waste output by fish culture operations. Further reduction of P waste output using this type of approach requires a better understanding of P utilization in fish.

Practical fish feeds are generally composed of fish meal and other animal products and a variety of plant ingredients. Phosphorus is a component of different chemical compounds in these ingredients. In animal by-products, P exists primarily in bone as hydroxyapatite, which is fairly digestible to fish (Lall, 1991; Sugiura et al., 2000c). In plant ingredients, 60% to 80% of the total P is bound in phytate (Ravindran et al., 1995). Since fish do not possess phytase in the digestive tract, digestibility of phytate is very poor (Ogino et al., 1979; Lall, 1991). Organic P covalently linked to protein, lipid and sugar is easily hydrolyzed and presumably highly digestible. The digestibility of inorganic phosphate supplements is believed to be affected by their solubility. Monobasic Ca phosphate is, for example, more digestible than dibasic Ca phosphate because of its higher solubility (Lall, 1991). Ingredient selection and quality determine the content and digestibility of P in finished feeds and this, in turn, affects P utilization, the forms of P waste released, and the potential environmental impact or mitigation measures required. It is consequently important to take into consideration the relative contribution and fate of the different forms of dietary P when constructing frameworks aimed at better understanding of P utilization in fish.

Once digested and absorbed, P is deposited in body to support biological functions, tissue growth, and bone mineralization. Bone is the major storage site of P, whereas in soft tissues, P serves as a component of organic compounds for synthesis of cell structures and biological functions. Similar to mammals, urinary phosphate excretion in fish is determined mostly by plasma phosphate concentration. A threshold exists below which P excretion is minimal and above which P excretion is proportional to the increase in plasma phosphate concentration (Bureau and Cho, 1999). Urinary P excretion and fecal P excretion make up

total P waste output. However, urinary excreted P (soluble P waste) is more available to algae and can, consequently, have more immediate environmental impact than fecal excreted P (solid P waste), which can be settled or filtered out (Cho and Bureau, 2001).

A number of kinetic and dynamic models of P utilization have been developed for sheep (Grace, 1981; Schneider et al., 1987), pigs (Fernández, 1995), goats (Vitti et al., 2000), and dairy cows (Kebreab et al., 2004), but there has been no attempt to model P utilization, in a mechanistic and dynamic manner, for fish. The objective of this model was, therefore, to dynamically simulate the partitioning of dietary P through digestion, body deposition, fecal excretion and urinary excretion in salmonid fish over growth stages, and to examine the effect of P chemical forms and their inclusion levels on P utilization and waste output.

8.3 Model Description

The model diagram is illustrated by Figure 8.1. The modeling process comprises of digestion of P (release of Pi, orthophosphates HPO_4^{2-} and H_2PO_4^- , from P chemical compound pools and absorption of Pi from available P pool), retention of blood P into soft tissue and bone, and excretion through urine and feces.

Dietary P intake was the driving variable of the model. Amount of feed intake (g/day) was generated by feed requirement model based on bioenergetics of salmonid fish (Cho and Bureau, 1998; Bureau et al., 2002), which takes account of growth rate and diet composition

(digestible energy, digestible protein). Growth rate was represented by thermal-unit growth coefficient (TGC, %), which was model input. Initial body weight and water temperature were also model inputs. TGC allows comparison of growth at different temperatures and is defined as follows (Iwama and Tautz, 1981; Cho, 1992):

$$\text{TGC} = 100 * (\text{BW}^{1/3} - \text{IBW}^{1/3}) / \Sigma (\text{T} * \text{D})$$

where BW = final body weight (g), IBW = initial body weight (g), T = water temperature (°C), and D = number of days.

Therefore, BW (g, converted to kg in modeling process) was calculated from TGC and initial body weight as follows:

$$\text{BW} = (\text{TGC} * \Sigma (\text{T} * \text{D}) * 100 + \text{IBW}^{1/3})^3$$

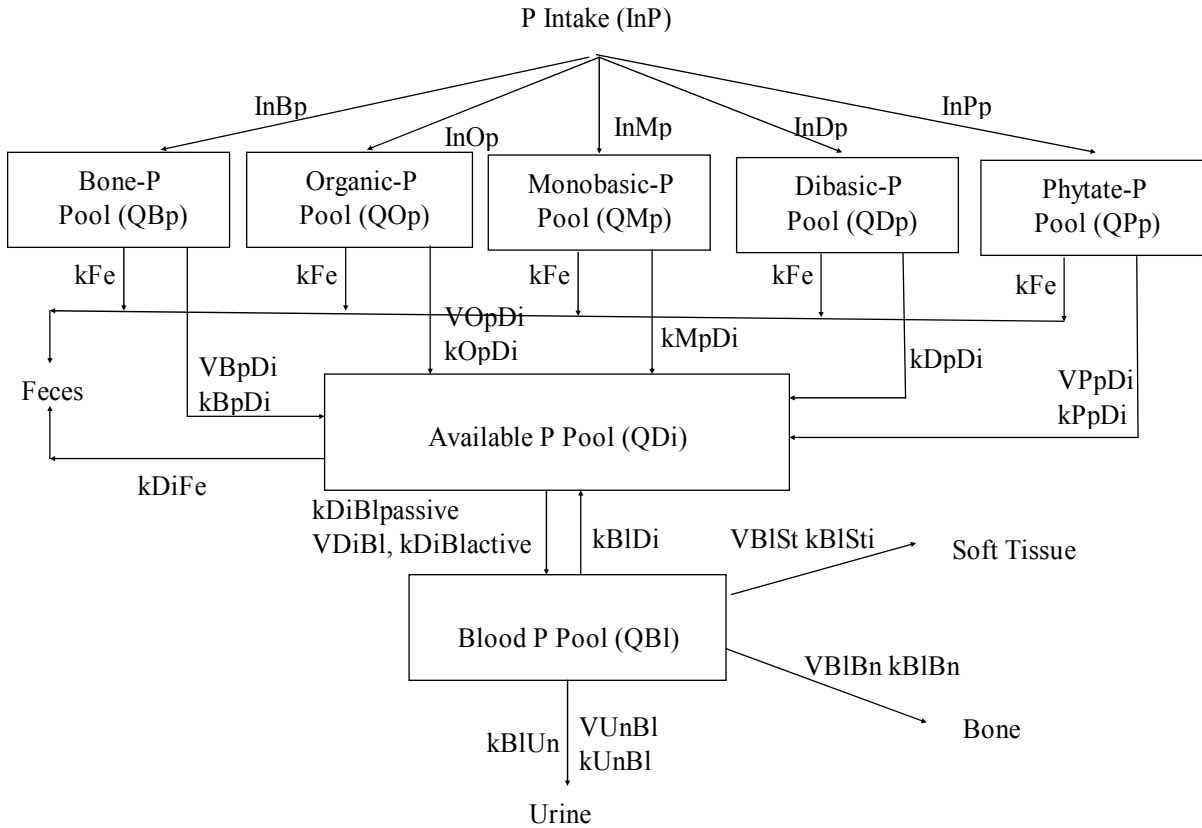


Figure 8.1 Diagram of dynamic model of P utilization in salmonid fish. Boxes indicate pools and arrows indicate fluxes.

8.3.1 Phosphorus Chemical Compound Pools, QBp, QOp, QMp, QDp, QPp (mg)

Dietary P chemical compounds were classified according to their chemical characteristics into bone-P, phytate-P, organic P, Ca monobasic / Na / K inorganic P supplement, and Ca dibasic inorganic P supplement. Amount of daily intakes of these P compounds (mg/day) were the product of feed intake and dietary concentration of these P chemical compounds, which were inputs to the P chemical compound pools, QBp, QOp, QMp, QDp, QPp. Orthophosphate Pi has to be released from these compounds in order to be rendered available for absorption, therefore, the first step in modeling of digestion was to simulate hydrolysis of P compounds in stomach. There is evidence that gastric acid output limits bone-P digestibility. Agastric fish species such as carp are unable to utilize bone phosphorus due to lack of gastric acid secretion (Ogino et al., 1979). In stomached fish species, such as salmonids, digestibility of bone-P is dependent on the inclusion level and decreases significantly as inclusion increases (Sugiura et al., 2000c). Therefore, dissolution of bone-P was simulated by a Michaelis-Menten equation. Similarly, Michaelis-Menten equations were also used to describe the hydrolysis of Pi from organic P and phytate-P since enzymatic actions are involved. Maximum releases of Pi from bone-P, organic P and phytate-P were VBpDi (mg/kg/day), VOpDi (mg/kg/day), and VPPDi (mg/kg/day), whereas affinity constants were kBpDi (mg), kOpDi (mg), and kPPDi (mg), respectively. The release of Pi from Ca monobasic / Na / K and Ca dibasic inorganic phosphate supplement was both assumed to be linearly related to dietary supply with fractional rates of kMpDi (/day) and kDpDi (/day). Derivation of these parameters values is presented in the section “Auxiliary

equations". The outputs of the chemical compound pools were the available fractions of these chemical compounds to the available P pool (QDi). The indigestible fractions were the flow (mg/day) from the chemical compound pools to feces based on gastric empty rate, $k_{Fe} = 0.9$ (/day) (Kristiansen, 1998).

8.3.2 Available P Pool (QDi)

Inputs to available P pool were the outputs from each chemical compound pool and P endogenous secretion. Absorption of P from QDi to blood was simulated by active and passive uptakes. Passive and active P uptake mechanism was identified in rainbow trout along intestine (Avila et al., 2000). The active uptake of P based on P_i concentration followed a Michaelis-Menten type equation with maximum absorption of V_{DiBl} (mg/day) and affinity of $k_{DiBlactive}$ (mg/L). Passive diffusion was linearly related to available P concentration c_{QDi} (mg/L) with rate of $k_{DiBlpassive}$ (L/day). Values of V_{DiBl} , $k_{DiBlactive}$, and $k_{DiBlpassive}$ were derived from Avila et al. (2000) and were 34.5 (mg/day), 37.2 (mg/L), and 0.067 (L/day), respectively. The portion of available P that was not absorbed to blood pool was excreted through feces at gut emptying rate of $k_{DiFe} = 2.36$ (/day) (Storebakken et al., 1999). Total gut volume was exponentially related to body weight (BW) based on the study of Burley and Vigg (1989) and was described as gut volume (L) = $1/1000 * e^{(2.854+1.2*BW)}$.

8.3.3 Blood P Pool, QBI (mg)

Phosphorus absorbed from available P pool was the input to the blood P pool. From the blood P pool, there were four outputs: endogenous secretion, soft tissue deposition, bone deposition, and urinary excretion. Endogenous secretion was derived from Riche and Brown (1996) and Rodehutscord et al. (2000), and was set at $kBIDi = 3.5$ (/day).

Depositions into soft tissues and bone at steady state were modeled at constant body weight, being described by a Michaelis-Menten type equation, with maximum deposition of $VBIS_t$ (mg/kg/day) into soft tissue and $VBIB_n$ (mg/kg/day) into bone, and affinity constants of $kBIS_t$ (mg/L) and $kBIB_n$ (mg/L) for soft tissue and bone deposition, respectively. These parameters were calculated separately with four different body weights at 5 g (Skonberg et al., 1997), 90 g (Rodehutscord et al., 2000), 125 g (Rodehutscord, 1996) and 210 g (Baeverfjord et al., 1998). When only whole body deposition data were available, bone weight was assumed to be 10% of body weight (Gingerich et al., 1990), and partitioning between bone and soft tissue deposition were calculated from whole body deposition. Because there is no evidence in fish nutrition literature that plasma P_i concentration is affected by body weight, the parameters were further adjusted by assuming similar $cQBI$ across these four body weights.

Relationship of the deposition parameters across body weight (BW) was described by the following equations:

$$VBIS_t = 17.866 * BW^{-0.2170} \quad (R^2 = 0.95)$$

$$kBlSt = 0.142 * BW^{-0.1914} (R^2 = 0.97)$$

$$VBIBn = 14.368 * BW^{-0.3605} (R^2 = 0.98)$$

$$kBlBn = 0.085 * BW^{-0.3876} (R^2 = 0.98)$$

Phosphorus urinary excretion was the difference between two processes regulated by plasma Pi concentration: linear glomerular filtration with rate $kBlUn$ (L/kg/day), and tubular reabsorption of the filtrates, which follows Michaelis-Menten mechanism with maximum reabsorption capacity $VUnBl$ (mg/kg/day), and affinity constant $kUnBl$ (mg/L). Fish has been known to show net tubular P_i secretion (Renfro and Gupta, 1990). However, recent development in molecular identification has suggested that tubular secretion may be limited to saltwater fish species. The location of active transporters indicates that renal handling of trout is the result of glomerular filtration and tubular reabsorption, which follows the mechanism rather similar to monogastric mammals (Sugiura et al., 2003). The equation parameters were calculated based on the work of Bureau and Cho (1999). Glomerular filtration rate $kBlUn$ was calculated as 4.2 (L/kg/day), maximum tubular reabsorption capacity $VUnBl$ as 1250 (mg/kg/day), and affinity constant $kUnBl$ as 225 (mg/L). Plasma volume was assumed to be 3.5% of the body weight (Gingerich et al., 1990).

8.3.4 Auxiliary Equations

Body deposition was calculated as the sum of deposition in soft tissue and bone. Body deposition coefficient was the percentage of dietary P intake deposited in body. Fecal P

output (solid waste output) was the sum of indigestible P from each P chemical compound pool, QBp, QOp, QMp, QDp, QPp, and unabsorbed P from available P pool (QDi). Urinary excretion coefficient was the percentage of dietary P intake that was excreted through urine (soluble waste output). Apparent digestibility was the difference between dietary P and P fecal excretion expressed as a percentage of dietary P. True digestibility was the percentage of dietary P intake that was absorbed P from available P pool (QDi).

Apparent digestibility values of different P chemical compounds, estimated from P apparent digestibility of feed ingredients and inorganic phosphates to salmonids in the literature (Lall, 1991), were used to calculate the parameters in P chemical pools QBp, QOp, QPp, QMp, QDp, after taking account of active and passive absorption from available P pool QDi into blood P pool QBI as well as endogenous loss. Digestibility was set constant across body weight modeled (from 50 g to 500 g). The maximum releases of Pi from bone-P, organic P, and phytate-P (VBpDi, VOpDi, and VPPDi) were 77.5 (mg/kg/day), 223 (mg/kg/day), and 9.7 (mg/kg/day) and the affinity constants, kBpDi, kOpDi, and kPpDi, were 2.88 (mg), 0.45 (mg), and 0.06 (mg), respectively. For Ca monobasic / Na / K and Ca dibasic inorganic phosphate supplement, the fractional rates, kMpDi and kDpDi, were 1.5 (/day) and 1.1 (/day), respectively.

The model was written in Advanced Continuous Simulation Language (ACSL) (MGA software, Concord, MA).

8.4 Model Evaluation

To test the model response to the effect of total dietary P and types of P on P utilization in fish, five sets of diets were theoretically formulated as presented in Table 7.1 (Chapter 7). Optimal growth condition was assumed. TGC and temperature were assumed to be 0.2 and 15°C, respectively. The modeled period was from body weight of 50 g to 500 g.

All parameters were tested for sensitivity by examining model outputs when varying individual parameters from half to double the values.

8.5 Results and Discussion

For the prototypical commercial feed, apparent digestibility coefficient (ADC) of P was proximately 60%, urinary excretion ranged from 29 - 36%, whereas body deposition coefficient was 25 - 28%. These values are comparable to reports for typical commercial diets (Heinen et al., 1993; Ketola and Harland, 1993; Cho and Bureau, 1998). This indicates that the model responded appropriately over the growth period. The model could aid in the strategies of reduction of P waste output through feed formulation. For the commercial feed, the model simulated that about 40% of dietary P was excreted as solid waste, and soluble P waste exceeded retention in body. While solid waste can be reduced by selection of highly digestible ingredients, reduction in soluble waste can be achieved by decreasing digestible P level in feed. The level resulting in minimal soluble P waste is around 0.4% digestible P for

rainbow trout (Rodehutscord et al., 2000). There was about 0.7% digestible P in this feed according to model simulation, which can be reduced to the level where growth is maximized, acceptable deposition is achieved, and soluble waste is minimized through feed formulation (Cho and Bureau, 2001).

From model simulations of P flows on the five sets of diets, average values of P retention, urinary excretion, and fecal excretion over the growth period are presented (Table 8.1). Increasing bone-P contents in Diet Set 1 resulted in a higher proportion of dietary P being excreted through feces compared to the prototypical commercial feed, whereas P retention and urinary excretion, as a percentage of dietary P, were reduced. This illustrates the limited capability of fish to digest bone-P. Increasing levels of organic P resulted in a proportion of fecal excretion similar to that in the prototypical commercial feed (Diet Set 2). However, elevated proportions of dietary P were excreted through urine and less dietary P was retained in the fish body. This suggests that organic P is highly digestible, and as more digestible P is included in diets, P retention coefficient decreases, and more P is excreted as soluble metabolic waste in urine. Simulations of Diet Set 3 indicated that increasing phytate-P contents greatly depressed P digestibility of the diets. Consequently, fecal P excretion increased, and P body retention and urinary excretion decreased. This is in agreement with the poor digestibility of phytate-P to fish and consistent with the physiology of fish which do not appear to possess phytase within the gastrointestinal tract (Ogino et al., 1979; Lall, 1991).

Results from Diet Set 4 further illustrate that the proportion of different types of P has great impact on P utilization even at a given dietary P level. This suggests fish utilize

different types of P to a varying degree. Accurate estimates of P utilization of the diets can only be achieved based on differentiation of P compounds rather than aggregates of total dietary P. Identical P retention coefficients were simulated across Diet 15 to 18, but urinary and fecal P excretions varied and were negatively correlated. This illustrates that all six diets were sufficient in digestible P level, although their digestibility differed. Once requirement for maximum body saturation (maximum P retention) is met, surplus digestible P is excreted as soluble P (urinary P) into environment. Corresponding strategies could be constructed through feed formulation to reduce solid waste output or soluble waste output depending on objectives. The model also responded appropriately to low dietary P levels as in Diet Set 5 (Diet 19 to 24). At very low dietary P level, essentially all digestible P is retained in the body and the urinary P excretion remains minimal. As dietary P levels increase, more P is excreted through urine and less P is retained in the body. The theoretical simulation illustrates that the model responds well to different levels and types of P compounds. This dynamic P model could potentially be a useful tool to estimate P digestibility, retention, and waste output.

Table 8.1 Model simulated P utilization of a prototypical commercial feed and five sets of diets for rainbow trout.

Diet	P retention	P urinary excretion % dietary P	P fecal excretion
Prototypical feed	27	33	40
Set 1			
Diet 1	25	31	43
Diet 2	23	30	46
Diet 3	22	29	49
Diet 4	20	27	52
Set 2			
Diet 5	25	36	39
Diet 6	23	38	39
Diet 7	22	40	38
Diet 8	20	41	38
Set 3			
Diet 9	25	30	44
Diet 10	23	28	48
Diet 11	22	26	52
Diet 12	20	24	55
Set 4			
Diet 13	27	37	36
Diet 14	27	31	42
Diet 15	27	28	44
Diet 16	27	32	40
Diet 17	27	37	36
Diet 18	27	27	46
Set 5			
Diet 19	85	0	15
Diet 20	85	0	15
Diet 21	69	15	16
Diet 22	56	27	17
Diet 23	48	34	18
Diet 24	41	40	19

Sensitivity tests suggested that k_{Fe} , k_{BIUn} , V_{UnBl} , V_{BlBn} , and V_{BlSt} were sensitive parameters. Model outputs were affected by over 15% when varying these parameters from half to double the values. This suggests that gastric emptying rate could considerably affect digestibility. Sensitivity of body deposition and urinary excretion warrants optimization of these parameters using a large dataset. Estimates of body deposition and urinary excretion could be confounded by experimental conditions and physiological status of the fish. Plasma Pi concentration is important to the estimates of these parameters; however, measurements of plasma Pi concentration are variable among studies and affected by sampling protocols. Radioisotopes and compartment analysis have been used in ruminants and monogastric mammals to study and model P metabolism (Grace, 1981; Schneider et al., 1987; Fernández, 1995; Vitti et al., 2000). This technique enables profiling of P distribution in different pools in the body and monitoring of kinetics of P flows between these pools, and can be applied to fish.

In this model, a simple growth equation was incorporated and growth was assumed to be independent of nutritional composition of the diet. The interaction between diet composition and growth performance could potentially affect the utilization of P, especially P deposition. Furthermore, P deficient diets would depress feed intake and growth. Therefore, the P utilization model should eventually be integrated within a nutrient flow fish growth model in order to investigate the effect of digestion, metabolism and utilization of major nutrients on phosphorus utilization, and conversely, the effect of digestible P supply on growth and utilization of other nutrients.

Future work should include optimization of parameters and validation of the model using independent experimental data. The effect of different combination of P chemical forms on P utilization and waste output should be further evaluated with a wide range of diets fed to fish under controlled experimental conditions. The effect of microbial phytase should also be incorporated in the model.

8.6 Conclusion

This model simulates P utilization in salmonid fish through digestion, body deposition, and excretion into urine and feces. The model responded appropriately for a prototypical commercial feed containing 1.15% dietary P. This model could be a useful tool in diet formulation and nutrient management. It could aid in selection of highly digestible ingredients and help in formulating diets to just meet, but not exceed P requirement of fish, and consequently improve production efficiency and reduce waste output.

CHAPTER 9 GENERAL DISCUSSION

This thesis presents two models constructed to simulate phosphorus utilization by salmonid fish, a factorial P model and a dynamic P model.

The factorial P model represents a statistical approach to simulate P utilization. Integrating sub-models of P digestibility, P content, and P waste output, and the Fish-PrFEQ bioenergetics model, this factorial P model provides a simple and practical tool to estimate the effects of different dietary P sources and levels on P digestibility, retention, and waste output.

The P digestibility sub-model was constructed based on P digestibility values of diets formulated with a wide range of ingredients from various experiments published in the literature. It estimates the digestible P content of diets based on inclusion levels of different P chemical compounds. The model was validated by the subsequent digestibility trial that suggested a good agreement between the model predicted and experimental observed digestible P contents. However, the experimental diets only contained bone-P, organic P and phytate-P, the common P compounds in practical feed formulation. The complete validation of the model should be done in the future with respect to various Pi supplement and exogenous phytase supplements.

The current P digestibility sub-model focused on the primary factor that affects P digestibility in fish, the content of different P chemical compounds. In addition, a number of other factors could also affect P digestibility to fish. Feces collection methods for P digestibility studies in fish nutrition research generally fall into 4 major methods/collection systems: the Guelph system (Cho et al., 1982), the St Pée system (Choubert et al., 1982), the Tokyo University of Fisheries (TUF) system (Ogino et al., 1973), and the stripping method (Austreng, 1978). It has been reported that P digestibility values estimated by different feces collection system may differ. Lall (1991) argues that stripping is a better method to estimate P digestibility, because it avoids the leaching of soluble P and yields values that are more consistent. However, the contamination of urine, or undigested feed, with feces may result in underestimates of the digestibility (Hardy, 1997; Sugiura et al., 2001). Stripping also inflicts a certain degree of stress to fish. On the other hand, the Guelph and TUF systems were observed to produce similar P digestibility estimates (Satoh et al., 1992a). Comparison across studies is difficult due to the differences in experimental and environmental conditions. The non-differentiation of feces collection methods in the current model is a generalization of these methods, but appears to be adequate, as suggested by the digestibility trial validation. However, in the future, if sufficient and reliable information can be collected, feces collection system could be incorporated as a nonparametric variable into the P digestibility model.

It is well known that Ca/P ratio in diets of terrestrial monogastric animals is important for their utilization. Fish can absorb Ca from aquatic environment to meet their most of their entire requirement for Ca; therefore, Ca/P imbalance rarely presents a problem for fish

(NRC, 1993). Increment of dietary Ca was observed to depress dietary P digestibility in rainbow trout (Satoh et al., 1993; Encarnacao, 1997), and in carp (Nakamura, 1982). However, tissue mineralization or fish growth does not seem to be affected by Ca/P ratio as long as dietary P is sufficient and Ca is present in rearing water (Lovell, 1978; Watanabe et al., 1980; Wilson et al., 1982; Shim and Ho, 1989; Vielma and Lall 1998b, Chavez-Sanchez et al., 2000). The quantification of Ca effect on P utilization in fish is questionable except for fish reared in experimental controlled Ca-free systems. The extrapolation to practical situation is problematic as Ca is always present in practical rearing systems.

Phosphorus digestibility estimates could also be affected by environmental conditions. There is evidence that salinity could possibly affect nutrient digestibility. Salinity depressed protein digestibility in Atlantic salmon smolts (Usher et al. 1990). MacLeod (1977) observed salinity significantly and linearly decreased absorption efficiency of dry matter, energy, and nitrogen. Brown trout raised in fresh water had higher P availability (76%) than in brown trout raised in seawater (65%) (Dosdat et al., 1998). Therefore, the current modeling dataset only included P digestibility values for rainbow trout reared in freshwater. Extrapolation to seawater condition should be preceded by incorporation of salinity factor in the model.

The current P digestibility sub-model focused on the primary factor that affects P digestibility in fish, and it explained 96% of the variance of the modeling dataset. The digestibility sub-model was further validated by digestibility trial. Although this sub-model could be refined in the future by incorporating the above-mentioned factors when relevant

data become available, it provides a simple means to estimate digestible P contents of salmonid fish feeds and can be a useful tool in practical feed formulation.

The P content sub-model investigated the effect of body weight and dietary digestible P level on P content. While the P content model can be a useful practical model, the variation of P concentration should be better explained and the affecting factors such as genetic variation and diet composition, remain to better understood and further quantified. As a first attempt to account for the various factors affecting P content and concentration through a quantitative modeling approach, this sub-model focused on current available literature information. The further improvement of the sub-model has to rely on experiments to generate data, for example, the comparison of genetic variation across strains. Alternatively, it is possible to extract useful information from literature data of chemical body composition such as contents of protein, lipid, ash, and phosphorus, and anatomical body composition such as bone/muscle ratio and dressed carcass yield, across fish genetic stains. Furthermore, the P content sub-model was constructed as a static model, which focuses on the delineation of various factors contributing to the variances of P concentration at a certain body weight. This static approach is probably inadequate for some of the factors whose effects on P concentration are of a dynamic, temporal nature, such as the interaction between diet composition, feeding level, and growth performance. Such factors could possibly be more adequately characterized and represented by a dynamic modeling structure.

The integration of sub-models of P digestibility, content and waste output sub-model into the framework of the Fish-PrFEQ bioenergetics model enables the factorial P model to

provide information on the P retention, partition solid P waste output and soluble P waste output, as well as detailed composition of solid P waste, throughout growth stages. This method is a mass balance approach, which estimates solid waste output based on apparent digestibility of P and estimates soluble waste output based on body P content over the growth period. The simulation of model response suggested that the model could be a useful tool in simulating P utilization and in formulating nutritional management strategies to minimize P waste outputs under practical farming conditions. However, it is currently not applicable to very low P diets since it results in unrealistic P retention and soluble P waste output estimates. Phosphorus is an essential dietary element for fish to maintain proper body function and adequate growth. Phosphorus deficiency not only results in lower bone mineralization, but also impairs growth and depresses feed intake. Phosphorus retention over a period is not isolated; it is accompanied by growth and deposition of other nutrients such as protein, lipid and carbohydrate. It is apparent that the factorial P model in the current framework of Fish-PrFEQ bioenergetics model of salmonid is not capable of solving the interaction between low digestible P supply and growth/metabolism. Fish-PrFEQ bioenergetics model is not intended as a growth or nutrient utilization model, rather, it estimates feed intake based on growth rate and dietary composition/energy as model inputs. The interaction between diet composition, nutrient utilization, and growth performance is not accounted for in the current framework. The response of fish to nutritional factors would be better represented by a nutrient utilization model that explicitly evaluates the utilization of specific nutrients and their interactions. Before such improvement, the current model is not applicable to the situation where P deficiency impairs fish growth and metabolism.

The differentiation of P compounds offered a semi-mechanistic feature to this empirical model, and enables this model to be more physiologically relevant and biologically meaningful than a pure empirical model. However, the characteristics of static empirical models imply that the model can only be applied with confidence to conditions similar to those used to develop the model; extrapolation beyond the data range should be exercised with caution. The model was constructed based on literature data covering a broad variety of feed ingredients and diets, as well as a wide range of fish body weight/growth stages. Therefore, it can be applied to the majority of situations encountered in practical feed formulation and aquaculture operations. However, the limitations of the model should always be recognized and made aware to the users in application.

An empirical model is an essentially black-box approach that describes input and output of a system, whereas a mechanistic model investigates the underlying cause and effect relationship. The dynamic P model represents a mechanistic and dynamic approach to simulate P metabolism. The dynamic P model integrates current biological principles of intestinal absorption, renal handling, and body deposition. It quantitatively evaluates the partitioning of dietary P and their interaction over time. Therefore, it is more flexible and has more explanatory powers. The mechanistic, dynamic nature of this dynamic P model potentially allows extrapolation of model simulations to a wider range of conditions upon its full development in the future.

However, the current dynamic P model is at its preliminary stage of development, namely the quantitative assessments of model response. The model has been tested for its

capacity to simulate response patterns of the modeled system, not the actual accuracy. The model should be further improved and validated. The effect of phytase supplementation should be incorporated in the model. The model parameters should be optimized and the model should be validated before its application. Preliminary comparison between the model simulations from the factorial P model (Chapter 7) and from the dynamic P model (Chapter 8) suggests that dynamic P model was able to respond to low dietary P levels, but in general estimated lower P digestibility, lower P retention coefficients, and consequently more urinary P excretion. Precision or accuracy does not necessarily always accompany increased details and complexity in the description of a system; rather, it is possible that increased complexity may result in temporary decrease in precision or accuracy until sufficient knowledge and observations of the system are known (McNamara, 2004). The next step in the development of the dynamic P model is to optimize and recalibrate the parameters of the model. The optimization of parameters requires a relatively large number of data, especially in the aspect of P deposition over growth stage. The relevant data are few at present. This issue is further complicated by the fact that phosphorus utilization cannot be separated from the utilization of other nutrients, as mentioned above in the discussion of the factorial P model. The model should be put into the framework of a fish nutrient utilization model in order to investigate the effect of digestion, metabolism and utilization of major nutrients on phosphorus utilization, and conversely, the effect of digestible P supply on growth and utilization of other nutrients. In this aspect, a mechanistic, dynamic model is probably advantageous over a static, factorial model since the response of animals to changes to dietary composition is of a dynamic nature. A dynamic model can examine not only the temporal effect of the response, but also the interaction of factors involved in the response. Preferably, the model should be

able to predict anatomical compositions along with chemical compositions of the fish. Environmental factors (such as water temperature) and genetic variations in addition to nutritional factors should also be incorporated into the model to extend its applicability. This conceptual mechanistic, dynamic model, if current available data are sufficient for its development, will likely be too sophisticated for practical application and probably remain as a research support tool.

Most applied models currently in use in agricultural science are empirical, static, and deterministic (Baldwin, 1995). Despite their limitations, these models have been proven useful (Baldwin, 1995; McNamara, 2004). However, to broaden the applicability of empirical models, incorporation of mechanistic elements is necessary (Baldwin, 1995). Advance in the understanding of animal digestion and metabolism and explanation of quantitative variations in animal performance is the ultimate goal of agricultural scientific research, which eventually has to be achieved through mechanistic, dynamic, stochastic models (Baldwin, 1995; McNamara, 2004). The presented P factorial model is practice oriented. It is semi-mechanistic, direct, and simple to use in practical feed formulation and aquaculture operations. To broaden its application beyond practical situations, such as in the case of low dietary P, requires further improvement, rigorous testing, and validation of the model. The dynamic P model is more research oriented. It can be further improved and used as a research tool in the P metabolism and the interaction between P and other nutrients. Potentially it can also be used in practical application, provided a user-friendly interface is devised.

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