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dissertation entitled

Effect of Phytic Acid on Nitrogen Retention in Tilapia (Oreochromis niloticus)

presented by

Martin Alan Riche

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Fish. & Wildl.

Major professor

Date May 23, 2000

EFFECT OF PHYTIC ACID ON NITROGEN RETENTION IN TILAPIA (*OREOCHROMIS NILOTICUS*)

By

Martin Alan Riche

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Fisheries and Wildlife

2000

ABSTRACT

EFFECT OF PHYTIC ACID ON NITROGEN RETENTION IN TILAPIA

(OREOCHROMIS NILOTICUS)

By

Martin Alan Riche

Culturing tilapia in temperate regions requires the use of intensive recirculating systems and formulated diets. Diets used typically contain fish meal (FM). The expense associated with feeding FM based diets has resulted in efforts to find alternative proteins such as soybean meal (SBM). However, replacement of FM with SBM has met with mixed success. SBM contains anti-nutritional factors (ANF) that reduce its biological value. One such ANF is phytic acid (PA). PA has the potential to reduce availability of amino acids. PA can be removed from SBM with the enzyme phytase at considerable expense. A series of experiments were conducted to determine the effect of PA on growth and efficiency in Nile tilapia, and whether phytase treatment is warranted for increasing nitrogen (N) retention.

Two preliminary studies were conducted to determine the optimal feeding frequency and interval between feedings to maximize growth and efficiency. Growth, whole body proximate composition, and efficiency parameters were evaluated in fish fed 1, 2, 3, or 5 feedings day $^{-1}$ to apparent satiation. Three feedings day $^{-1}$ led to optimal growth and efficiency.

Fish eat food at intervals determined by rate of gastric emptying. In a second study, gastric evacuation rate (GER) in fish fed 3 or 5 meals day $^{-1}$ was determined by following movement of feed containing colored dyes over time. Equations describing GER were $V_T = 67.0\ e^{-0.153(x)}$, and $V_T = 85.0\ e^{-0.149(x)}$ for fish fed 3, and 5 meals day $^{-1}$, respectively. The optimal interval between feedings was 4 – 5 hours.

A series of four experiments were conducted to determine the effect of PA on growth and N retention. An eight week growth study was conducted with phytase treated, or untreated SBM substituted into a FM based diet at 0, 25, 50, 75, or 100 % of the crude protein (CP). This was followed with a study utilizing the same diets to determine CP and amino acid digestibility. Untreated SBM can replace 75 % of the CP, and phytase treated SBM 25 % of the CP, without significantly depressing growth and efficiency. Growth models suggest restricting untreated SBM, and phytase treated SBM, to 30 %, and 15 % of the dry diet, respectively. Lysine (Lys) and methionine (Met) became limiting with increasing levels of SBM, regardless of treatment. Availability of Lys and Met from phytase treated SBM appears to be responsible for reduction in performance in fish fed phytase treated SBM beyond 25 % of the CP.

Tilapia were fed FM based diets supplemented with graded levels of PA during an eight week growth study, and a digestibility study. Growth, efficiency, and digestibility were independent of PA concentration. PA removal from SBM with phytase is not efficacious or warranted for increasing N retention in tilapia.

Measurements of pH were taken of the food and mucosa of the stomach and intestine before feeding and at 0.5, 1, 2, 4, 6 and 8 hours postprandially. The pH values in the gastric region were not as low as previously reported.

This body of work is dedicated to the memory of my mother, Shirley Anne Casavant; my father, Walter Thomas Riche, Jr.; and my stepfather Jack Edward Casavant; all of whom passed away after its inception, but before its completion Although they were unable to see its culmination, I know they were with me in spirit, even to the last word.

ACKNOWLEDGMENTS

I wish to extend my deepest and sincerest gratitude to Dr. Donald Garling who had the faith and patience to guide me through this program and this research. He has been a mentor and true friend while providing me with the encouragement and opportunities to grow and develop both personally and professionally. I would also like to express my appreciation to the other members of my graduate committee Dr. Nathalie Trottier, Dr. Weiming Li, and Dr. Michael Allen for their helpful advice, without which this dissertation would not be a reality.

I give a big thank you to my dear friends Dave Haley, Betsy Haley, Jon Amberg, and Mike Oetker for the many hours of their tireless assistance in collecting and analyzing samples. I acknowledge Dr. Pao Ku for his technical assistance and advice both of which, without, I could not have finished. I also acknowledge the assistance of Patty LeChance, Tiffani Smith, and Sarah Garbrecht.

To my beautiful new bride I say thank you for the love, support, and endless hours of work you have given me. I promise to pay you back in full for the patience, kindness, and support you have displayed through this process. Without you I would not have made it.

Lastly I express my appreciation to Purdue University, Illinois State University, Zeeland Farm Services, BASF, and Zapata Proteins for their donations. Thanks also to the CANR for providing me the financial means to complete this dissertation. This research was supported in part by the North Central Regional Aquaculture Center and the Michigan Soybean Promotion Committee.

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LIST OF ABBREVIATIONS

AA – Amino acids

Ala – Alanine

ACPD – Apparent crude protein digestibility

ANF – Anti-nutritional factor

ANPU – Apparent net protein utilization

Arg – Arginine

Asp – Asaprtic acid

BAPNA - Nα-benzoyl-DL-arginine-p-nitroanilide hydrochloride

BOD – Biological oxygen demand

BW – Body weight

CO_2 – Carbon dioxide

CP – Crude protein

Cys – Cysteine

DE – Digestible energy

DDI – Distilled deionized water

DM – Dry matter

DO – Dissolved oxygen

DMSO – Dimethyl sulfoxide

FCR – Feed conversion ratio

FE – Feed efficiency

FM – Fish meal

GE – Gross energy

GER – Gastric evacuation rate

GET – Gastric evacuation time

GI - Gastrointestinal

Glu – Glutamic acid

Gly – Glycine

His – Histidine

HROM – Hydrolysis resistant organic matter

IAA – Indispensable amino acid

Ile – Isoleucine

IU- Internaional Unit of enzyme activity

Leu – Leucine

Lys – Lysine

MDI – Mean daily intake

Met – Methionine

MS-222 - Tricainemethane sulfonate

N – Nitrogen

NH_3/NH_4^+ - Ammonia/ammonium

NO_2^- - Nitrite

NO_3^- - Nitrate

P:DE – Crude protein:digestible energy ratio (g/MJ)

PER – Protein efficiency ratio.

Pro – Proline

Phe – Phenyalanine

SD – Standard deviation

SEM – Stanard error of the mean

SGR – Specific growth rate

Ser – Serine

Thr – Threonine

TIA – Trypsin inhibitor activity

TIU – Trypsin inhibitor units

Tris- Tris(hydroxymethyl) aminomethane

Trp – Tryptophan

TSAA – Total sulfur amino acids

Tyr – Tyrosine

Val – Valine

INTRODUCTION

Tilapia is a generic term applied to the collection of Cichlidae species within the genera *Oreochromis*, *Tilapia*, and *Sarotherodon*. They are warmwater species originating on the African continent, and are found in a diverse range of habitats. Many of the Tilapine species and their hybrids are cultured worldwide as a food source.

Tilapia are well suited for culturing. They exhibit rapid growth, high resistance to stress and disease, endure submarginal water quality, readily utilize relatively low quality feedstuffs, and are highly prolific. Current conventional wisdom dictates tilapia culture requires recirculating systems for economic viability. Recirculating systems allow for intensive culture of organisms while maintaining temperature and removing waste products from the aquatic environment (Appendix 1). In much of the temperate regions, such systems are imperative for maintaining the warm water temperatures required by tilapia. The Nile tilapia (*Oreochromis niloticus*) were chosen for this study because it is the foremost species of tilapia cultured domestically, as well as world-wide.

A major limiting constraint associated with recirculating systems is the accumulation of nutrients, particularly nitrogenous products, and solids. Nitrogenous wastes associated with feeds and feeding are divided into a solid fecal fraction, and a soluble fraction associated with gill and urinary excretions. A number of mechanical methods for the separation and removal of the solid fraction exist, and this area continues to be a rich topic for aquacultural engineers. While numerous biofiltration devices exist for handling the soluble fraction, they all utilize nitrifying bacteria for the oxidation of NH_3/NH_4^+ to the end product NO_3^-. However, the efficacy of nitrifying bacteria is variable and contingent upon relatively unpredictable and often hard to manage

parameters such as dissolved oxygen (DO), pH, temperature, CO_2, biological oxygen demand (BOD), and competitive heterotrophic bacteria, as well as circulating NH_3/NH_4^+, NO_2^-, and NO_3^- levels (Wheaton 1993).

The difficulty and expense associated with nitrogen and solids removal makes reducing wastes entering the system the most viable and desirable alternative. It is estimated 10 % of ingested nitrogen is excreted in the feces with as much as 66 % excreted as ammonia (Cho 1993). These values are based on feeding high quality fish meal proteins. The values are higher with lower quality plant protein substituted diets. Despite these statistics the industry is moving towards replacing expensive, and sometimes unavailable, fish meal products with less expensive plant protein feedstuffs. Solids reduction and increased nitrogen retention are obvious starting points for reducing nitrogen inputs into the system.

The most promising plant protein alternatives identified to date are soybean products. However, partial or complete replacement of fishmeal (FM) with soy products has met with mixed success in tilapia species (Davis and Stickney 1978; Jackson et al. 1982; Viola and Arieli 1983; Shiau et al. 1987; 1989; 1990; Davies et al. 1989; De Silva and Gunasekera 1989; El-Dahhar and El-Shazly 1993). Generally, poor performance is in direct relationship to the level of FM replacement (Shiau et al. 1990). Reasons cited for decreased performance (reduced growth, protein efficiency ratio, apparent net protein utilization, and increased feed conversion ratio) were residual trypsin inhibitors, unbalanced amino acids, methionine deficiency, or undigestible polysaccharide anti-nutritional factors. However, evidence to support these conclusions is lacking.

Soybean meal (SBM) based diets supplemented with methionine (Shiau et al. 1987; Shiau et al. 1989) or methionine and lysine (El-Dahhar and El-Shazly 1993) did not increase performance to the level of FM control diets. Methionine was found not limiting in diets with 24 % CP, but limiting in diets with 32 % CP, and methionine supplementation only had a significant effect on performance parameters when diets contained SBM as the sole source of protein (Shiau et al. 1989). However, methionine levels in these studies were above reported requirements determined for tilapia (Jackson and Capper 1982). Substitution of SBM for FM at 30 % of the CP had no significant effect on total protein or dry matter digestibility, but led to a significant reduction at greater than 33 % substitution (Shiau et al. 1987; Shiau et al. 1989). These findings would indicate bioavailability of methionine is less than expected, or the available amino acids are unbalanced. However, increasing the ratio of soy flour in a blend from 25:75 to 75:25 (soyflour:feather meal) had no effect on apparent availability of methionine (Sadiku and Jauncey 1995). The currently accepted maximum replacement of fishmeal with SBM in tilapia diets is 30 % of the crude protein (Shiau et al. 1990).

Soybeans have anti-nutritional factors (ANF) with the potential to reduce their biological value and result in pathological states in monogastric animals (Rackis 1974; Liener 1994). Phytic acid (1,2,3,5/4,6-hexakis dihydrogen phosphate), one such ANF, exists as a salt of mono- and divalent cations in legumes and cereals. Phytate, the salt of phytic acid, binds divalent cations making them unavailable, resulting in mineral deficiencies. Reduced mineral bioavailability has been demonstrated in rainbow trout (Spinelli et al. 1983; Cain and Garling 1995; Riche and Brown 1996), channel catfish

(Satoh et al. 1989), carp (Hossain and Jauncey 1993), and tilapia (McClain and Gatlin 1988).

Phytate also complexes with proteins. These complexes occur via direct bonding with phytic acid forming a binary complex and through mineral-phytate-protein ternary complexes (Cheryan 1980; Reddy et al. 1989). In acidic environments, such as Tilapine stomachs (pH 1.0-2.0), half of the phosphorus moieties of phytic acid are negatively charged creating an environment favorable for binding proteins with ε-amino groups on lysine, imidazole groups on histidine, and guanidyl groups on arginine. In alkaline environments, such as Tilapine intestine (pH 8.5-8.8) protein-cation-phytate complexes are favored. These protein-phytate and protein-mineral-phytate complexes are more resistant to proteolytic digestion *in vitro* and *in vivo* (Singh and Krikorian 1982; Satterlee and Abdul-Kadir 1983; Grabner and Hofer 1985; Knuckles et al. 1985; Carnovale et al. 1988; Vaintraub and Bulmaga 1991; Caldwell 1992).

In vitro studies indicate phytic acid inhibits pepsin and trypsin activity. Decreased pepsin activity is linearly related to phytate level and independent of digestion time (Knuckles et al. 1985). Inhibition is inversely correlated to the degree of phytate hydrolysis, and is strongly affected by pH. Maximal inhibition occurs near pH 2.0 (Camus and Laporte 1976; Vaintraub and Bulmaga 1991) suggesting the potential for decreased proteolytic digestion efficiency in tilapia. Decreased activity is likely due to complex formation making sites on the protein less susceptible to enzymatic attack.

As with pepsin, the inhibition of trypsin is dependent on phytate concentration. The inhibition is a function of temperature, calcium concentration, and contact time (Singh and Krikorian 1982). The mechanism by which this inhibition occurs is ill

defined. Possible explanations for the inhibition are decreased activation of the zymogen form, increased autolysis of trypsin, formation of the ternary complex, or competitive sequestration of Ca^{2+} ions between phytate and trypsin (Singh and Krikorian 1982; Vaintraub and Bulmaga 1991; Caldwell 1992).

Decreased protein digestibility of diets supplemented with salts of phytic acid led to depressed growth and poor performance in rainbow trout (Spinelli et al. 1983), Chinook salmon (Richardson et al. 1985), and carp (Hossain and Jauncey 1993). Carp are stomachless fish and do not produce pepsin, providing further evidence that trypsin activity may be altered.

The addition of microbial phytase to swine diets significantly increased total tract digestibility of CP, and all amino acids except cystine and proline. Ileal digestibilty of methionine and arginine were also significantly increased (Mroz et al. 1994). Nitrogen retention was increased and daily nitrogen excretion was reduced 20-25 % suggesting improved amino acid balance. Nitrogen balance studies indicated significantly higher fecal and urinary nitrogen losses in rats fed a high phytate bran flour diet (Satterlee and Abdul-Kadir 1983). These authors also demonstrated increased protein digestibility, higher biological value, and better protein efficiency ratios with diets containing lower phytate levels using both *in vitro* and *in vivo* techniques.

Monogastric animals, including fish, are unable to hydrolyze the complexing phosphate groups on phytate due to a lack of intestinal secretions of the enzyme phytase. However, diets prepared with phytase either as an enzymatic pretreatment, or as an additive, result in better performance. Cain and Garling (1995) found rainbow trout fed diets pretreated with phytase exhibited superior growth compared to fish fed the same

diets without pretreatment. The authors suggested the increased performance may be attributable to improved protein quality.

In early work, it was suggested tilapia did not possess a functional stomach. The investigators suggested tilapia contain an intestinal bulb, an enlarged region of the anterior intestine found in stomachless fishes (Bowen 1982). However, recent evidence suggests tilapia contain the histological, morphological and physiological characteristics required for gastric digestion. Tilapia exhibit tremendous plasticity of the GI tract, which lends itself to adaptability and a high degree of variability (Smith 1989; Boujard and Leatherland 1992). It is this characteristic which was likely responsible for the confusion.

Morphologically, tilapia exhibit distinct muscular cardiac and pyloric sphincters (Moriarity 1973) as well as endodermal epithelium (Smith 1989). Histologically, chief cells, oxyntic cells (Kapoor et al. 1975), and gastric glands (Al-Hussaini and Kholy 1953) have been identified. Physiologically, acid secretion is under neuronal control (Fish 1960) and tilapia exhibit peristaltic movement (Moriarity 1973).

Acidic proteases have been identified in tilapia (Fish 1960; Moriarity 1973). One such protease has been isolated and characterized from *O. niloticus* stomach mucosa (Yamada et al 1993). Kinetic analysis indicated the K_m was 5.4 mg/mL utilizing hemoglobin as substrate. Maximum activity was observed at 50°C and pH 3.5 which was similar to eel and ayu, but higher than rainbow trout, dace, and bonito (Yamada et al. 1993). The enzyme had a molecular weight of 54,000 and an isoelectric point of 3.7. Substrate and inhibitor assays indicated the enzyme was an aspartic acid protease with pepsin-like activity similar to swine pepsin (Yamada et al. 1993).

In addition to gastric digestion, tilapia exhibit intestinal proteolytic activity (Nagase 1964; Hofer and Scheimer 1981). Proteolysis has been attributed to both trypsin- and chymotrypsin-like enzymes (Fish 1960; Moriarity 1973). It was speculated tilapia lack carboxypeptidase activity (Moriarity 1973). However, this seems unlikely since tilapia have been found to exhibit the full complement of enzymes found in other fish (Nagase 1964), including endoprolylpeptidase, leucinaminopeptidase, cysteinaminopeptidase, prolylamino peptidase, and aminopeptidase (Barth et al. 1995).

Histological evidence supports the existence of exocrine pancreatic enzymes. Acinar cells containing zymogen granules have been identified in *O. niloticus* (Kugler and Pequignot 1988). In addition, two pancreatic proteolytic enzymes from *O. niloticus* intestine have been isolated and characterized (Yamada et al. 1991). Kinetic analysis indicated the K_m for the two enzymes was 0.03 mg/mL utilizing casein as the substrate. Maximum activity was observed at 55°C and pH 8.5-9.0. The two enzymes, labeled PA-3 and PB-3 had molecular weights of 32,000 and 21,000, respectively. The effects of various inhibitors led the authors to predict PA-3 was a serine protease, and PB-3 was a cysteine protease (Yamada et al. 1991). Trypsin-like enzymes from tilapia behave similarly to porcine trypsin (El-Shemy and Levin 1997) indicating the potential for inhibition by phytic acid.

Pretreatment of plant proteins with phytase should render phytate incapable of sequestering proteins and minerals, potentially increasing their availability to the animal. The hydrolysis of phytate should decrease the inhibitory effect observed on gastric and intestinal proteolytic enzymes thereby increasing CP digestibility. Assuming energy is not a limiting factor, increased amino acid availability would potentially increase protein

accretion, and decrease amino acid catabolism and ammonia excretion. Both solid and soluble nitrogen would be reduced minimizing nitrogen inputs into the system, while also allowing for greater substitution of soybean products for fishmeal.

Therefore, the overall objective of this study is to determine whether phytic acid associated with soybeans is a causative agent responsible for reduced growth and performance in tilapia fed SBM based diets. More specifically, the objectives of this study are two fold. The first is to determine what rate of SBM incorporation leads to equivalent growth and performance relative to a fish meal control diet, and whether removal of phytic acid can increase the rate of SBM incorporation. The second objective is to determine whether phytic acid inhibits the activity and function of gastric and intestinal proteases in tilapia.

HYPOTHESIS STATEMENTS

Experiment 1 – Optimal Feeding Frequency

In fish nutrition studies, it is imperative that food availability, in itself, does not act as a factor in limiting growth and efficiency. Nutrient utilization efficiencies should be calculated at the feeding frequency at which rates of growth and efficiency are not suppressed (Jobling 1983). Optimum feeding frequencies vary with physiology, diet, behavior, species, size, and temperature. There is some contention as to optimal feeding frequencies for tilapia. Additionally, recommendations do not differentiate between species, feed type, or rearing system (NRC 1993). Based on feeding behavior, physiology, and gastrointestinal morphology of wild fish it has been reported tilapia require many frequent small meals to achieve greatest efficiency (Moriarity 1973; Jauncey and Ross 1982). However fish reared in confinement have different requirements than wild fish.

Hypothesis: There is an optimum feeding frequency for *O. niloticus* reared in recirculating systems beyond which growth and efficiency are reduced.

Experiment 2 – Tilapia Gastric Evacuation Rate

The rate at which food can be consumed and efficiently utilized is a prime factor in determining growth rate. The rate of consumption is a function of environmental conditions, meal size, fish size, and feeding frequency (Holmgren et al. 1983). Feeding frequency has been shown to be strongly correlated with gastric evacuation time (Holmgren et al. 1983).

Utilizing an inert undigestible marker the rate at which food traverses the GI tract can be determined (Fange and Grove 1979). The assumption is fish will eat available food in amounts depending on stomach fullness and at intervals determined by the rate of emptying (Holmgren et al. 1983). Additionally, the rate at which food traverses the GI tract dictates the contact time the intestinal milieu has with digestive enzymes.

Hypothesis: The rate of passage through the GI tract of *O. niloticus* is affected by feeding frequency.

Experiment 3 – Solvent Extracted SBM Growth Trial

Phytate complexes with proteins (Cheryan 1980; Reddy et al. 1989). This complex formation potentially reduces protein digestion and availability of amino acids for uptake. Increased protein digestibility, higher biological value, and better protein efficiency ratios have been demonstrated with diets containing lower phytate levels (Satterlee and Abdul-Kadir 1983). Cain and Garling (1995) found rainbow trout fed diets pretreated with phytase exhibited superior growth compared to fish fed the same diets without pretreatment. The authors suggested the increased performance may have been attributable to improved protein quality.

Hypothesis: Removing phytate from SBM with the use of phytase will increase the biological value of the SBM, increasing growth and efficiency. Conversely, feeding increasing graded levels of untreated SBM will decrease the biological value of the SBM, decreasing growth and

efficiency in a dose-response manner.

Experiment 4 – Phytic Acid Supplemented Fish Meal Growth Trial

If phytic acid is the causative agent for differences in growth and efficiency in *O. niloticus* fed phytase treated and untreated diets, the effects should be mimicked by purified phytic acid. Incorporating purified phytic acid into a control diet, in graded levels reflecting those in the SBM diets, should mimic a dose-response relationship on growth and efficiency similar to one observed in experiment 3. Such a relationship would lend support to the hypothesis in experiment 3.

Hypothesis: Incorporating graded levels of purified phytic acid into a control diet will decrease the biological value of the control diet decreasing growth and efficiency in a dose-response relationship.

Experiment 5 – Solvent Extracted SBM Digestibility Trial

Decreased protein digestibility of diets supplemented with salts of phytic acid led to depressed growth and poor performance in rainbow trout (Spinelli et al. 1983), Chinook salmon (Richardson et al. 1985), and carp (Hossain and Jauncey 1993). The addition of microbial phytase to swine diets significantly increased total tract digestibility of CP, and all amino acids except cystine and proline. Ileal digestibilty of methionine and arginine were also significantly increased (Mroz et al. 1994). Pretreatment of plant proteins with phytase should render phytate incapable of sequestering proteins and minerals, potentially increasing their availability to tilapia.

Hypothesis: Removing phytate from SBM with the use of phytase will increase the digestibility of total N and individual amino acids. Conversely, feeding increasing graded levels of untreated SBM will decrease digestibility of total N and individual amino acids in a dose-response relationship.

Experiment 6 – Phytic Acid Supplemented Fish Meal Digestibility Trial

As in experiment 4, if phytic acid is the causative agent for differences in total N and individual amino acid digestibilities in *O. niloticus* fed phytase treated and untreated diets, the effects should be mimicked by purified phytic acid. Incorporating purified phytic acid into a control diet, in graded levels reflecting those in the SBM diets, should mimic a dose-response relationship on N and individual amino acid digestibilities in tilapia.

Hypothesis: Incorporating increasing graded levels of purified phytic acid into a control diet will decrease digestibility of total N and individual amino acids in a dose-response relationship.

Experiment 7 – Tilapia Gastrointestinal Tract pH Profile

Tilapia exhibit tremendous plasticity of the GI tract, which lends itself to adaptability and a high degree of variability (Smith 1989). There is evidence to suggest Tilapine stomachs are highly acidic, with pH values as low as 1.0-2.0 (Moriarity 1973). Maximal inhibition of enzymes by phytic acid occurs near pH 2.0 (Camus and Laporte 1976; Vaintraub and Bulmaga 1991) suggesting the potential for decreased proteolytic

digestion efficiency in tilapia. However, maximum activity of a protease isolated from *O. niloticus* gastric mucosa was observed at pH 3.5 with much reduced activity at pH values as low as 2.0, and only 10 % activity at pH 5.5 (Yamada et al. 1993).

In alkaline environments, such as Tilapine intestine (pH 8.5-8.8) protein-cation-phytate complexes are favored. These protein-phytate and protein-mineral-phytate complexes are more resistant to proteolytic digestion *in vitro* and *in vivo* (Singh and Krikorian 1982; Satterlee and Abdul-Kadir 1983; Grabner and Hofer 1985; Knuckles et al. 1985; Carnovale et al. 1988; Vaintraub and Bulmaga 1991; Caldwell 1992). In addition to demonstrating tilapia manifest a suitable environment for protein-phytic acid complexation, the data gathered can be used to mimic *in vivo* pH conditions in the *in vitro* enzyme assays.

Hypothesis: The pH values of the *O. niloticus* GI tract are optimal for protein-phytic acid complex formation.

METHODS AND MATERIALS

Experiment 1 – Optimal Feeding Frequency

A mixed sex population of *O. niloticus* was obtained from Illinois State University (Normal, Illinois) and transported to Michigan State University's Fisheries Research Laboratory. Fish were held in flow-through well water heated to 25±1.0°C until stocking.

Experimental Design

Fish were fed a standard commercial trout diet for a four-week preliminary period prior to stocking in experimental units. The diet was analyzed for gross energy (GE), and proximate components. The diet contained 46.3 % crude protein (CP); 3.86 % lipid; 18.4 % ash; and 18.02 kJ/g, on a dry matter basis.

During the preliminary period, fish were fed 2 % wet body weight per day $^{-1}$ (BW/day) divided between two feedings. After four weeks, five fish were randomly selected for analysis. All analyses were performed in triplicate. Efficiency parameters were determined by difference between fish analyzed before the experiment and at its termination.

Five fish each were stocked into 12 experimental units. Experimental units were defined as 40 L tanks. The system was a parallel flow-through system receiving water from a common head tank. Well water in the head tank was heated to 27°C. Fish were maintained on a 16:8 light:dark cycle. Mean weight of fish at stocking was 34.4±5.4 g.

Prior to the experimental period, fish were allowed a one-week acclimation period and fed as above. The experimental period lasted 29 days.

Experimental units were randomly assigned a daily feeding regimen. The feeding regimens were once daily (8:00 h); twice daily (8:00 and 17:00 h); three times daily (8:00, 12:00, and 17:00 h); and five times daily (8:00, 10:00, 12:00, 15:00, and 17:00 h). Fish were fed 6 days a week and weighed on the seventh. Fish in each unit were fed to apparent satiation at each feeding.

Feeding followed the same regimen for each tank and meal. Fish initially received pellets until they lost interest. After all tanks received feed, a second and third pass was made offering additional pellets. Satiation was defined as the point at which a single pellet remained uneaten for 1 min after the third pass. Consumption was recorded.

Temperature and dissolved oxygen were monitored. Parameters were within acceptable ranges for tilapia (Cherivinski 1982; Papoutsoglou and Tziah 1996).

Proximate Analysis

At termination of the experimental period, fish were weighed collectively and euthanized in tricainemethane sulfonate (MS-222) at a concentration of 500 mg/L (Post 1983). Euthanized fish were weighed individually, dissected, and sexed. Visceral tissue was removed and weighed. Visceral tissue was defined as all tissues within the body cavity including gonadal tissue. Perivisceral fat was removed from the gastrointestinal tract and weighed. Whole fish, including tissues and perivisceral fat, were frozen at -20°C until analyzed.

Frozen whole fish were passed through a meat grinder and further homogenized by pulverizing with mortar and pestle. Ground tissues were dried at 105°C after which they were further ground to pass a 850 μm screen. A 2.500±0.003 g subsample was taken from each fish within an experimental unit and pooled. Pooled samples were blended by hand mixing until the sample appeared homogenous, but not less than one minute. The blended samples were used for the remaining proximate analyses. All analyses were performed in triplicate on pooled samples.

Dry matter was determined by standard methods (AOAC 1990). Feed and whole body nitrogen content were determined by the Kjeldahl method (AOAC 1990). Crude protein was calculated as N x 6.25. Gross energy of feed and whole fish were determined by bomb calorimetery using the isoperibol method (Parr Instruments, Moline, IL). Crude lipid content of feed and whole fish were determined by difference following lipid extraction with diethyl ether (AOAC 1990). Ash was determined by combustion at 550°C (AOAC 1990).

Calculations

Fish within treatments were analyzed for growth and efficiency parameters. Efficiency parameters evaluated were feed efficiency (FE), protein efficiency ratio (PER), specific growth rate (SGR), and apparent net protein utilization (ANPU). Efficiency parameters were calculated using the standard equations of Jauncey and Ross (1982).

Feed Efficiency:

$$FE = \text{wet weight gain} / \text{total feed fed}$$

Protein Efficiency Ratio:

$$PER = \text{wet weight gain} / \text{total protein fed}$$

Energy Retention:

$$ER = [(W_f \times E_f) - (W_0 \times E_0)] / EI \times 100$$

Specific Growth Rate (%/day):

$$SGR = [(\ln W_f - \ln W_0) / (T_2 - T_1)] \times 100$$

Apparent Net Protein Utilization:

$$ANPU = [(W_f \times P_f) - (W_0 \times P_0)] / PI \times 100$$

where :

W_f = final wet weight

W_0 = beginning wet weight

P_f = final whole body protein

P_0 = beginning whole body protein

E_f = final whole body energy

E_0 = beginning whole body energy

PI = total protein intake

EI = total energy intake

$(T_2 - T_1)$ = number of days during the experiment

Statistical Analysis

A completely randomized design was employed with number of feedings day^{-1} as the main effect. Proximate components, FCR, ANPU, PER, and SGR were analyzed as a one-way ANOVA using the general linear method of SAS (SAS, 1979).

When significant differences were detected, means were separated using Duncan's multiple range test. Significance was reported at $P<0.05$ controlling for the type I comparisonwise error rate.

Cumulative feed fed was analyzed by ANOVA using the regression procedure of SAS. Slopes were tested with t-tests, and significance reported at $P<0.05$.

Multivariate regression analyses were performed with visceral wet weight and weight of perivisceral fat as dependent variables, and total wet weight, gender, and number of feedings a day as the mutlivariate regressors (SAS 1979). The same procedure was also used with weight of perivisceral fat as the dependent variable, and total wet weight, gender, and number of daily feedings as the independent variables. Results were reported in partitioned ANOVA tables. F-statistics and probabilities were reported and the null hypotheses rejected at $P<0.01$.

Experiment 2 – Tilapia Gastric Evacuation Rate

Nile tilapia were obtained from Purdue University and transported to Michigan State University's Fisheries Research Laboratory. Fish were held in a recirculating system at 28°C until stocking. Fish were fed a standard commercial catfish diet during a four-week acclimation period prior to stocking in experimental tanks.

Experimental Diet Preparation

The experimental diet formulation is given in Table 1. Herring meal was obtained from Zeigler Brothers, Inc. (Gardners, Pennsylvania). Solvent extracted SBM was obtained from Zeeland Farm Services (Zeeland, Michigan) (Appendix 2). Dextrin (type

Table 1. Composition of experimental diets fed to tilapia (*Oreochromis niloticus*) to determine gastric evacuation rate and intestinal motility.

Ingredient	Cr_2O_3 diet (g/kg dry diet)	Fe_2O_3 diet (g/kg dry diet)
Herring meal	259.7	259.7
Soybean meal (solv. ext.)	277.5	277.5
Wheat Bran	100.0	100.0
Dextrin	100.0	100.0
Mineral Premix [1]	60.0	60.0
Vitamin Premix [2]	3.0	3.0
Carboxymethyl cellulose	20.0	20.0
Cellulose	68.0	68.0
Ascorbic acid	1.0	1.0
Choline chloride	0.8	0.8
Menhaden oil	75.0	75.0
Soy oil	25.0	25.0
Chromic Oxide	10.0	-------
Ferric Oxide	-------	10.0
Total	1,000.0	1,000.0

[1] Mineral premix contained (g/kg dry mix): $CaSO_4$, 350.0; NaH_2PO_4, 250.0; KH_2PO_4, 250.0; $MgCO_3 \cdot 5H_2O$, 20.0; $ZnSO_4 \cdot 7H_2O$, 3.0; $FeSO_4 \cdot 7H_2O$, 2.8; $MnSO_4 \cdot H_2O$, 2.0; $CuCl_2 \cdot 2H_2O$, 1.0; $AlCl_3 \cdot 6H_2O$, 1.0; KF, 0.5; KI, 0.1; Na_2SeO_3 0.1; $CoCl_2 \cdot 6H_2O$, 0.1; $NaMoO_4 \cdot 2H_2O$, 0.1.

[2] Warmwater fish performance premix (Hoffmann-La Roche, Inc.,Nutley, NJ) – as incorporated in the diet: vitamin A, 10,582 IU; vitamin D3, 2,381 IU; vitamin E, 132 IU; vitamin K, 2 mg; B12, 4.4 µg; folic acid, 5.3 mg; riboflavin, 17.2 mg; pantothenic acid, 42.3 mg; niacin, 105.8 mg; choline-Cl, 529.1 mg; thiamin, 11.9 mg; pyridoxine, 13.2 mg; biotin, 165 µg.

II from corn), chromic oxide, ferric oxide, and L-ascorbic acid were obtained from Sigma Chemical Co. (St. Louis, Missouri). Choline chloride, CMC, and α-cellulose were obtained from ICN Biochemicals (Cleveland, Ohio). Alkali refined, bleached, and pressed menhaden oil stabilized with 200 ppm Coviox was supplied by Zapata Protein, Inc. (Reedville, Virginia). Feed grade soybean oil was purchased from a local retailer.

All dry ingredients were mixed in a liquid-solids V-mixer for a minimum of 12 hours. Ingredients were then transferred to a Univex mixer (Univex Corp, Salem, New Hampshire) where water and lipids were added under continuous mixing. The moist diet was cold extruded using the appropriate die cast for the experimental fish. Pelleted diets were dried in a forced air oven at 60°C for 12 hours. Dried diets were stored at –20°C until fed.

Experimental Design

The experimental system was a 4,200 L recirculating system containing eighteen 150 L tanks. The recirculating system was similar in scope and design as in Appendix 1. Space limitations required the use of three complete blocks. Each block represented a replicate. One fish was stocked into each of the 18 tanks. Individual fish were considered experimental units. Experimental units were randomly assigned to one of two feeding regimens, and one of eight sample collection periods. The two feeding regimens consisted of feeding to satiation either 3 times day $^{-1}$ (8:00, 12:00, and 17:00 hrs), or 5 times day $^{-1}$ (8:00, 10:00, 12:00, 15:00, and 17:00 hrs). The eight sample collection periods were 1, 2, 4, 6, 8, 12, 18, and 24 hrs following the 8:00 meal on the experimental day. The remaining two tanks in each block were used to sample fish at 0 (before

feeding) and 0.5 hr following the 8:00 feeding and were not used for statistical analysis, but for descriptive purposes only.

Fish were fed an experimental diet containing chromic oxide (Cr_2O_3) at 1.0 % of the dry diet (Table 1). The Cr_2O_3 diet was fed for a four day preliminary period prior to commencement of a one day experimental period. Following the fourth day, fish were given one meal at 8:00 hrs of a similar diet containing ferric oxide (Fe_2O_3) at 1.0 % of the dry diet. Ferric oxide was substituted for Cr_2O_3 (Table 1). During all meals, fish were fed to apparent satiation, which was defined as the point at which a single pellet remained uneaten for 1 min. Consumption was recorded.

During the 1 day experimental period, fish were serially dissected at 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 hrs following the 8:00 hr feeding. Fish were euthanized in MS-222 at a concentration of 500 mg/L before dissection (Post 1983). The fish remaining in the treatments receiving 3 meals day $^{-1}$ were fed to satiation again at 12:00 hrs and 17:00 hrs. The fish remaining in the treatments receiving 5 meals day $^{-1}$ were fed to satiation again at 10:00, 12:00, 15:00, and 17:00 hrs. After the initial feeding with the Fe_2O_3 diet all subsequent feedings were with the Cr_2O_3 diet.

The entire GI tract was removed and rinsed with cold distilled deionized water (DDI). Visceral fat and other tissues were removed. The GI tract was divided into seven segments. The segments consisted of the stomach, and two equal size segments each from the anterior, middle, and posterior intestine. The segments were excised and visually inspected for iron containing digesta. All digesta and feces from each segment were removed and rinsed with cold DDI water to remove any potential blood

contaminants. The samples were dried at 105°C for 24 hrs. Dried feces were ground and homogenized.

Iron Analysis

Ground samples ≤ 100 mg were wet ashed in 5 mL concentrated H_2SO_4 until charred. Samples were allowed to cool slightly before adding 30 % H_2O_2 dropwise until remaining carbonaceous material was completely oxidized. Samples were returned to heat. After heating for approximately 5 minutes, samples were again allowed to cool slightly before adding 5 mL DDI water. Samples were again returned to heat for 2 minutes. After cooling, samples were brought to 100 mL with DDI water and filtered through Whatman # 1 filter paper.

Filtered samples were analyzed for ferric iron colorimetrically at λ=535nm (Davies, Bush, and Motzok 1972). The assay was slightly modified by substituting bathophenanthroline disulfonic acid for 4,7-diphenyl-1,10-phenanthroline thereby obviating the need for a sulfonation step.

Statistical Analysis

ANOVA using the general linear method (SAS 1979) was performed on iron consumption, total consumption, and amount of iron appearing in the terminal segment. The data were analyzed as a randomized complete block design with all factors fixed using the model statement:

$$Y_{ijkl} = \mu + B_i + T_j + S_k + \varepsilon_{ijkl}$$

where B = block; T = time; and S = segment.

Contrasts between the two feeding regimens were performed on iron appearing in the terminal segment of the posterior intestine.

Data utilized for determining gastric evacuation rate (GER) were transformed. The transformed data (natural log cumulative iron) was plotted versus time to generate a linear relationship and the slopes tested by t-test. Similarly a t-test was used to test the slopes defining the relationship between rate of iron appearance in the terminal segment and time following initial feeding. Significance was reported at $P<0.05$.

Experiment 3 – Solvent Extracted SBM Growth Trial

Nile tilapia were obtained from Purdue and transported to Michigan State University. These fish were spawned at Michigan State University and reared in aquaria until stocked for the experiment. Second generation fish were used for an eight-week trial to evaluate the effects of graded levels of SBM inclusion on growth and performance. Fish not utilized during this experiment were saved for spawning.

Solvent Extracted SBM Diet Preparation

Solvent extracted SBM was obtained from Zeeland Farm Services (Zeeland, Michigan) (Appendix 2). Herring meal was obtained from Zeigler Brothers, Inc. (Gardners, Pennsylvania). The SBM and herring meal were ground to pass an 850 μm mesh screen.

Dextrin (type II from corn), L-methionine, and L-ascorbic acid were obtained from Sigma Chemical Co. (St. Louis, Missouri). Choline chloride, CMC, and α-cellulose were obtained from ICN Biochemicals (Cleveland, Ohio). Alkali refined, bleached, and

pressed menhaden oil stabilized with 200 ppm Coviox was supplied by Zapata Protein, Inc. (Reedville, Virginia). Feed grade soybean oil was purchased from a local retailer.

Soybean meal was substituted, for an isonitrogenous mixture of herring meal and cellulose, to provide 25, 50, 75, or 100 % of the crude protein (Table 2). Crystalline L-methionine was added to diets containing SBM substituted at 50, 75, and 100 % of the CP to meet the Met and total sulfur amino acid (TSAA) requirements for *O. niloticus* (Santiago and Lovell 1988). Methionine addition was at the expense of cellulose. All diets were formulated to contain 33 % CP on a dry matter basis, and a P:DE of 25.0 g/MJ based on predicted digestible energy values for *O. niloticus* (Anderson et al. 1991; NRC 1993). All diets were supplemented with a complete mineral premix (Table 3), and Roche warmwater fish performance vitamin premix (Table2).

Microbial phytase (BASF, 5,000 IU/g) was activated by hydration in a 0.1 *M* citrate solution (pH 5.0) at room temperature. The enzyme solution was mixed thoroughly for 15 min. Soybean meal was wetted with phytase solution 1:1 (w/v) and mixed thoroughly for one hour at room temperature. The resultant mash was covered and incubated for 6 hrs at 50°C. Following incubation, the SBM was dried in a forced air convection oven at 60°C. The re-dried SBM was reground to pass an 850 μm mesh screen. The SBM used in diets without phytase treatment was wetted 1:1 (w/v) with a 0.1 *M* citrate buffer (pH 5.0). The sham treated SBM was mixed, incubated, dried, and reground in the same manner as the phytase treated SBM.

All dry ingredients for each experimental diet were mixed in a liquid-solids V-mixer for a minimum of 12 hours. Ingredients were then transferred to a Univex mixer (Univex Corp, Salem, New Hampshire) where water and lipids were added under

Table 2. Composition of solvent extracted SBM experimental diets used in grow out and digestibility trials. Substitution of SBM into the herring meal control diet provided 25, 50, 75, and 100 % of the crude protein. Values shown are g/kg of the dry diet.

			Percent Soybean Meal Substitution			
Ingredient	**International Feed #**	**Control**	**25**	**50**	**75**	**100**
Herring meal	5-02-000	463.7	347.8	231.9	115.9	-----
Solvent extracted SBM	5-04-612	-----	166.7	333.4	500.0	666.7
L-Methionine		-----	-----	0.5	1.5	2.5
Dextrin	4-08-023	125.0	125.0	125.0	125.0	125.0
Mineral premix [1]		60.0	60.0	60.0	60.0	60.0
Vitamin premix [2]		3.0	3.0	3.0	3.0	3.0
Carboxymethyl cellulose		20.0	20.0	20.0	20.0	20.0
Ascorbic acid		1.0	1.0	1.0	1.0	1.0
Choline chloride		0.8	0.8	0.8	0.8	0.8
α-Cellulose		226.5	175.7	124.4	72.8	21.0
Menhaden oil	7-08-049	75.0	75.0	75.0	75.0	75.0
Soybean oil	4-07-983	25.0	25.0	25.0	25.0	25.0
Total		1,000.0	1,000.0	1,000.0	1,000.0	1,000.0

Table 2 (cont'd).

[1] Table 3

[2] Roche warmwater fish performance premix (Hoffman-La Roche, Inc., Nutley, NJ) supplied the following per kg dry diet: vitamin A, 10,582 IU; vitamin D_3, 2,381 IU; vitamin E, 132, IU; menadione, 2 mg; folic acid, 5.3 mg; riboflavin , 17.2 mg; pantothenic acid, 42.3 mg; niacin, 105.8 mg; choline-Cl, 529.1 mg; thiamin, 11.9 mg; pyridoxine, 13.2 mg; biotin, 0.165 mg; cyanocobalamine, 0.0044 mg.

Table 3. Mineral premix composition and dietary mineral supplementation in both the soybean meal and phytic acid supplemented experimental diets fed to tilapia

Mineral Salt	Premix Salt Concentration (g/kg dry premix)	Dietary Mineral Supplementation (mg/kg dry diet)
$CaSO_4$	350.0	6,182
NaH_2PO_4	250.0	3,366
KH_2PO_4	250.0	3,414
$MgCO_3 \cdot 5H_2O$	20.0	167
$ZnSO_4 \cdot 7H_2O$	3.0	41
$FeSO_4 \cdot 7H_2O$	2.8	34
$MnSO_4 \cdot H_2O$	2.0	39
$CuCl_2 \cdot 2H_2O$	1.0	22
$AlCl_3 \cdot 6H_2O$	1.0	6.7
KF	0.5	9.8
KI	0.1	4.5
Na_2SeO_3	0.1	2.8
$CoCl_2 \cdot 6H_2O$	0.1	2.6
$NaMoO_4 \cdot 2H_2O$	0.1	1.5

continuous mixing. The moist diet was cold extruded using the appropriate die cast for the experimental fish. Pelleted diets were dried in a forced air oven at 60°C for 12 hours. Dried diets were stored at –20°C until fed.

Experimental Design

The mixed sex population of *O. niloticus* spawned from fish obtained from Purdue University were reared in aquaria until reaching approximately 1-1.5 g. Fish held during this period were fed a standard commercial trout diet (Purina Mills, St. Louis, Missouri).

The experimental system was a 3,750 L recirculating system, with 27 experimental tanks, and similarly configured as in experiment 2. Temperature was maintained at 28 ±1.0°C and flow rate to each tank maintained at 1-2 L/min. Fish were maintained on a 16:8 light:dark cycle. Dissolved oxygen was monitored three times daily. Ammonia, nitrite, and nitrate were measured 3 times weekly with a Hach chemical test kit (Hach Co., Loveland, Colorado). All water quality parameters were within acceptable limits for tilapia throughout the trial (Cherivinski 1982; Daud et al. 1988; Papoutsoglou and Tziah 1996).

A completely randomized 2 x 4 factorial design was employed, in addition to a control group. Phytase treatment and level of SBM substitution served as main effects. Eight fish, each, were randomly stocked into 40 L tanks. Each tank was defined as an experimental unit. Experimental units were randomly assigned a dietary treatment. Three replicates were run for each treatment and the control.

Mean weight at stocking was 1.29 g (0.04 g SEM, n=216). Fish were allowed a two-week acclimation period during which they were fed the control diet. Fish were fed to satiation three times per day. Maximum intake was empirically determined as 7.5 % (SEM 0.15, n=189) BW/day on a dry matter basis. In an attempt to optimize utilization, fish were offered 80 % of maximum consumption. This rate corresponded to suggested feeding rates for tilapia (Jauncey and Ross 1982; NRC 1993).

Following acclimation, the fish were fed the experimental diets at 6.0 % BW/day on a dry matter basis, divided between three equal meals. Weights were recorded at two week intervals to adjust feed rates. Fish were fed 7 days a week, except on days fish were weighed.

The experiment was terminated after eight weeks. Fish were euthanized in MS-222 at a concentration of 500 mg/L (Post 1983). Euthanized fish were weighed, pooled by experimental unit, and stored at –20°C for analysis. In addition to the pooled samples, 25 fish were randomly selected from the population at stocking, and stored at –20°C for CP, dry matter, and lipid analysis.

Phytate Analysis

Phytate was determined colorimetrically (Latta and Eskin 1980) on phytase treated and untreated SBM, as well as the experimental diets. Approximately 100 mg samples were placed in 5.0 mL of 2.4 % HCl in sealed vials. Samples were extracted overnight on a shaker bath maintained at room temperature. Samples were filtered through Whatman #1 filter paper under vacuum. Samples were rinsed with DDI water and brought to 20 mL.

Phytate was separated via anion exchange chromatography using 200-400 mesh AG1-X4 chloride exchange resin (Bio-Rad Laboratories, Richmond, California). Charged resin was eluted with 5.0 mL of 0.05 *M* NaCl to remove organic phosphorus. The resin was recharged with 10.0mL 0.7 *M* NaCl and rinsed with 15.0 mL DDI water. Filtered samples were passed over the column and rinsed with 15.0 mL DDI water. The rinsed resin was eluted with 0.05 *M* NaCl to remove inorganic phosphorus. The column was eluted with 15.0 mL 0.7 *M* NaCl and eluent collected for analysis.

Standard solutions were prepared from sodium phytate (Sigma Chemical Company, St. Louis, Missouri). A 1.0 mL aliquot of Wade reagent (0.03 % $FeCl \cdot 6 H_2O$ and 0.3 % sulfosalicylic acid) was added to 3.0 mL aliquots of standards and samples for phytate determination. Absorbance was read at λ=500 nm.

Trypsin Inhibitor Activity

Residual trypsin inhibitor activity was determined on solvent extracted SBM using a modification of American Association of Cereal Chemists Method 71-10 (AACC 1983). A 1.0 g sample of SBM was extracted with 50 mL 0.01 *N* NaOH for 3 hr. Samples were transferred to 50 mL scintillation tubes and centrifuged at 4000 RPM for 10 min. Aliquots of 0, 0.6, 1.0, 1.4, and 1.8 mL of extract were brought to 2.0 mL, mixed with 2.0 mL trypsin solution (4 mg Type I-from bovine pancreas, (Sigma Chemical Co., St. Louis, Missouri) in 200 mL 0.001 *M* HCl), and warmed to 37°C. Following addition of 5.0 mL BAPNA (40 mg BAPNA dissolved in 1.0 mL DMSO and diluted to 100 mL with 0.05 *M* tris buffer (pH 8.2)) the solution was incubated at 37°C for exactly 10 min before stopping the reaction with 1.0 mL 30 % acetic acid. The solution was twice

filtered through Whatman # 2 filter paper and absorbance read at $\lambda = 410$ nm. Activity was expressed as TIU which was defined as an increase of 0.01 absorbance units at 410 nm per 10.0 mL reaction volume. TIU was converted to trypsin inhibitor activity (TIA) expressed as mg/g sample (Hamerstrand et al. 1981).

Proximate Analysis

Frozen whole fish, pooled by experimental unit, were passed through a meat grinder and homogenized by pulverizing with mortar and pestle. Ground tissues were dried at 105°C and further ground to pass an 850 μm screen. Feed and whole body nitrogen contents were determined by the Kjeldahl method (AOAC 1990). Crude protein was calculated as N x 6.25. Crude lipid was determined by difference following diethyl ether extraction (AOAC 1990). All analyses were performed in triplicate on pooled samples.

Efficiency Calculations

Fish within treatments were analyzed for growth and efficiency parameters. Efficiency parameters evaluated were feed efficiency, protein efficiency ratio, specific growth rate, and apparent net protein utilization. Efficiency parameters were calculated using the standard equations of Jauncey and Ross (1982) as described in experiment 1.

Statistical Analysis

A completely randomized 2 x 4 factorial design, with a control group was employed. Phytase treatment and level of SBM substitution served as main effects.

Growth, SGR, selected proximate components, and efficiency parameters were analyzed as a two-way ANOVA utilizing the model statement

$$Y_{ijk} = \mu + C_i + P_j + CP_{ij} + \varepsilon_{ijk}$$

where C = SBM concentration, and P = phytase treatment.

When significant differences were detected, means were separated using Student-Newman-Keuls multiple range test controlling for the type I experimentwise error rate. In addition all SBM containing diets, whether phytase treated or not, were tested against the control group using Dunnett's t-test. Significant differences were reported at the 0.05 level unless otherwise indicated. To evaluate the effect of phytase treatment at each level of incorporation, orthogonal contrasts were run between treated and untreated groups at each level (Cody and Smith 1997). All analyses were performed using SAS statistical software (SAS 1979). Additionally, linear regression and non-linear regression (quadratic model) were performed on growth and efficiency parameters.

Experiment 4 – Phytic Acid Supplemented Fish Meal Growth Trial

Third generation Nile tilapia originating from Purdue University were used for an eight-week trial to evaluate growth and performance of fish fed fish meal based diets supplemented with graded levels of phytic acid.

Phytic Acid Supplemented Fish Meal Diets

The fish meal control diet used in the SBM growth trial served as the formulation for the phytic acid supplemented diets. All ingredients used were the same as previously described.

Dodecasodium salt of phytic acid from corn (Sigma Chemical Co., St. Louis, Missouri) was incorporated into the control diet in graded levels (Table 4). Levels incorporated were equivalent to phytic acid concentrations in diets that contain SBM providing 0, 25, 50, 75, 100, and 200 % of the CP (Table 5). Diets were formulated, prepared, and stored as previously described for experiment 3.

Experimental Design

Fry were reared in aquaria until reaching approximately 2.0 g. During this period, they were fed the same standard commercial trout diet as used above. The experimental system and conditions were the same as for the SBM growth trial.

A completely randomized design was employed with a control, and five diets incorporating graded levels of phytic acid. All treatments were run in triplicate. Eight fish, each, were randomly stocked into 40 L tanks. Each tank was defined as an experimental unit. Experimental units were randomly assigned a dietary treatment.

Mean weight at stocking was 2.04 g (0.04 g SEM, n=144). This experiment followed the same feeding, sampling, and experimental protocol as in experiment 3. At initiation of the experiment, 33 fish were collected from the population at stocking and stored at –20°C for CP, dry matter, and lipid analysis.

Statistical Analysis

A completely randomized design was employed with concentration of phytic acid supplementation as the main effect. Growth, SGR, selected proximate components, and efficiency parameters were analyzed as a one-way ANOVA (SAS 1979). When significant differences were detected, means were separated using Student-Newman-

Table 4. Composition of phytic acid supplemented experimental diets used in grow out and digestibility trials. Phytic acid incorporation was equivalent to phytic acid concentrations in diets that contain SBM providing 25, 50, 75, 100, and 200 % of the crude protein. Also shown is composition of the N-free diet fed to correct apparent digestibility coefficients to true digestibility coefficients. Values shown are g/kg of the dry diet.

	International			Percent Soybean Meal Substitution				
Ingredient	**Feed #**	**N-Free**	**Control**	**25**	**50**	**75**	**100**	**200**
Herring Meal	5-02-000	-----	463.7	463.7	463.7	463.7	463.7	463.7
Solvent Extracted SBM	5-04-612	-----	-----	-----	-----	-----	-----	-----
Corn Starch		333.3	-----	-----	-----	-----	-----	-----
Dextrin	4-08-023	333.3	125.0	125.0	125.0	125.0	125.0	125.0
Mineral Premix [1]		60.0	60.0	60.0	60.0	60.0	60.0	60.0
Vitamin Premix [2]		3.0	3.0	3.0	3.0	3.0	3.0	3.0
CMC [3]		20.0	20.0	20.0	20.0	20.0	20.0	20.0
Ascorbic Acid		1.0	1.0	1.0	1.0	1.0	1.0	1.0
Choline Chloride		0.8	0.8	0.8	0.8	0.8	0.8	0.8
α-Cellulose		148.6	226.5	223.1	220.0	216.8	213.6	200.7
Na-Phytate		-----	-----	3.4	6.5	9.7	12.9	25.8
Menhaden Oil	7-08-049	75.0	75.0	75.0	75.0	75.0	75.0	75.0
Soybean Oil	4-07-983	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Total		1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0

Table 4 (cont'd).

[1] Table 3

[2] Roche warmwater fish performance premix (Hoffman-La Roche, Inc., Nutley, NJ) supplied the following per kg dry diet: vitamin A, 10,582 IU; vitamin D_3, 2,381 IU; vitamin E, 132, IU; menadione, 2 mg; folic acid, 5.3 mg; riboflavin , 17.2 mg; pantothenic acid, 42.3 mg; niacin, 105.8 mg; choline-Cl, 529.1 mg; thiamin, 11.9 mg; pyridoxine, 13.2 mg; biotin, 0.165 mg; cyanocobalamine, 0.0044 mg.

[3] Carboxymethyl cellulose

Table 5. Phytic acid concentrations (% DM) in diets incorporating graded levels of solvent extracted SBM substituted into a herring meal control diet on a percent protein basis. Diets contained either untreated or phytase treated SBM. Also shown is the Na-phytate equivalent incorporated into the herring meal control/phytic acid supplemented diets.

Level of SBM Substitution (% crude protein)	SBM Incorporation (% dry diet)	Phytase Treated SBM (% dry diet)	Untreated SBM (% dry diet)	Na-phytate Supplementation (g/kg dry diet) [1]
0	0	ND [2]	ND	--------
25	16.7	ND	0.20	3.4
50	33.3	ND	0.39	6.5
75	50.0	ND	0.58	9.7
100	66.7	ND	0.77	12.9
200	--------	--------	ND	25.8
Solvent Extracted SBM	--------	ND	1.12	--------

[1] Calculated to meet phytic acid content of SBM diets based on 9 mol H_20/mol Na-phytate.
[2] ND indicates none detected.

Keuls multiple range test. In addition, all levels of phytic acid supplementation were tested against the control group using Dunnett's t-test. Significant differences were reported at the 0.05 level unless otherwise indicated (SAS 1979).

Experiment 5 – Solvent Extracted SBM Digestibility Trial

Nile tilapia were obtained from Illinois State University (Normal, Illinois) and transported to Michigan State University. These fish were spawned and reared at Michigan State University until stocking. Second generation fish were used to determine amino acid and total N digestibility of the SBM diets used in experiment 3.

Experimental design

The mixed sex population of *O. niloticus* spawned from fish obtained from Illinois State University were held in flow-through well water heated to 27± 1.0°C until stocking. Fish held during this period were fed a standard commercial trout diet.

The experimental system was a 4,700 L recirculating system similarly configured as in experiment 2. Temperature was maintained at 28°C, and flow rate to each tank maintained at 2-3 L/min. Fish were maintained on a 16:8 light:dark cycle. Dissolved oxygen, ammonia, nitrite, and nitrate remained within acceptable limits for tilapia throughout the trial (Cherivinski 1982; Daud et al. 1988; Papoutsoglou and Tziah 1996).

The experiment was designed as a randomized complete block design. Two replicates, for each experimental diet, were run in each of two blocks. Fifteen fish, each, were randomly stocked into one of eighteen 125 L tanks. Each tank was defined as an experimental unit. Experimental units were randomly assigned a dietary treatment.

Mean weight at stocking was 69.9 g (1.04 g SEM, n=270), and 66.9 g (0.63 g SEM, n=270), for block one and two, respectively. Fish were allowed a four-day acclimation period during which they were fed a commercial trout diet. Following acclimation, the fish were fed the experimental diets for a 10-day preliminary period. The preliminary period allowed for dietary adjustment. The experimental diets were the same SBM diets used in experiment 3 (Table 2). Fish were fed 2.4 % BW/day on a dry matter basis, divided between three equal meals (9:00, 13:00, and 18:00). Following the preliminary period, the experimental period ensued two hours following the afternoon feeding.

Fish were euthanized in MS-222 at a concentration of 500 mg/L (Post 1983). Incisions were made along the mid-ventral line anteriorly from the anus. The exposed GI tract was clamped 10 cm from the anus, and the posterior section excised. All fecal material within the excised section was collected and pooled by experimental unit. Pooled fecal samples were stored at –20°C for subsequent analysis. Pooled fecal samples, and feed samples, were freeze dried for amino acid, nitrogen, and hydrolysis resistant organic matter (HROM) determinations.

Digestibility coefficients were calculated utilizing the indirect method of Jobling (1983), with HROM serving as an internal marker.

$$ADC = 100 - (100)\left[\frac{\%\ \text{HROM in feed}}{\%\ \text{HROM in feces}} \times \frac{\%\ \text{Nutrient in feces}}{\%\ \text{Nutrient in feed}}\right]$$

where ADC = Apparent Digestibility Coefficient

HROM and Crude Protein

Freeze dried feed and fecal samples were analyzed for HROM with slight modifications to a method described by Buddington (1980). A standard curve was prepared using α-cellulose (ICN Biochemicals, Cleveland, Ohio). Approximately 50 mg fecal samples, or 100 mg feed samples were placed in 15.0 mL of 80 % acetic acid and 1.5 mL HNO_3 and gently boiled for 20 min. Samples were filtered under vacuum with glass microfiber filter paper (Whatman GF/B) with a pore size of 1μm. Samples were sequentially washed and filtered under vacuum with 6 mL hot ethanol, 6 mL hot benzene, 6 mL petroleum ether, and 6 mL ethanol to remove residual organic solvents. Filtered samples were dried at 105°C for 12 hr. Dried samples were weighed and ashed at 525°C for 16 hr before re-weighing. Hydrolysis resistant organic matter was calculated as amount of material lost on ignition expressed as a percentage of the original sample weight.

Approximately 15 mg freeze dried fecal samples were analyzed for N with a N-analyzer (Leco FP-2000, Leco Corp., St. Joseph, Michigan) following manufacturers specifications. Crude protein was determined as N x 6.25.

Amino Acid Analysis

Feed and feces collected during the digestibility trials were analyzed for amino acids, except tryptophan. Samples were freeze-dried, pulverized, and hydrolyzed with 6 *N* HCl at 110°C for 24 hrs for hydrolysate amino acid analysis. Free amino acids were derivatized with phenylisothiocyanate (PITC) before analysis (Waters Manual 1989). Derivatized amino acids were determined on a C-18 reverse phase HPLC column using a

Waters HPLC separation system (Waters Chromatography Division, Millipore Corp., Milford, Massachusetts).

Statistical Analysis

The design was a randomized complete block design with all factors fixed. The data were analyzed with two levels of SBM treatment (phytase treated and untreated) and four levels of SBM incorporation (25, 50, 75, and 100 % replacement), and a control (0 %) in each of two blocks utilizing the model statement

$$Y_{ijkl} = \mu + B_i + C_j + P_k + CP_{jk} + \varepsilon_{ijkl}$$

where B = block, C = SBM concentration, and P = phytase treatment. There was insufficient material for analytical analysis in some samples. Therefore, means were tested by the least-squares estimates of marginal means (lsmeans) method, by SBM treatment (SAS 1979).

In addition, all SBM containing diets, whether phytase treated or not, were tested against the control group using Dunnett's t-test (SAS 1979). Significant differences were reported at the 0.05 level unless otherwise indicated. To evaluate the effect of phytase treatment at each level of incorporation, orthogonal contrasts were run between treated and untreated groups at each level (Cody and Smith 1997).

Experiment 6 – Phytic Acid Supplemented Fish Meal Digestibility Trial

Cohorts of the fish used in experiment 5 were used to determine amino acid and total N digestibility of the fish meal based diets supplemented with graded levels of phytic acid used in experiment 4.

Experimental Design

The digestibility trial was conducted using the same protocol as in experiment 5. In addition, three replicates in each block were fed a similarly formulated N-free diet (Table 4) to determine endogenous N and amino acids excretion. Mean weight of tilapia at stocking was 64.8 g (0.53 g SEM, n=225) and 63.1 g (0.92 g SEM, n=225) for block one and two, respectively. Fecal collection, sample preparation, and analyses were carried out as described in experiment 5.

Statistical Analysis

Crude protein and individual amino acid digestibilities were analyzed as a randomized complete block design (SAS 1979). Due to insufficient material for analytical analysis in some samples, means were tested by the least-squares estimates of marginal means (lsmeans) method (SAS 1979). Additionally, all levels of phytic acid supplementation were tested against the control group using Dunnett's t-test (SAS 1979). Significant differences were reported at the 0.05 level unless otherwise indicated.

Experiment 7 – Tilapia Gastrointestinal Tract pH Profile

Experimental Design

Third generation fish from those originating from Purdue University were used for pH measurements of the GI tract. Fish were maintained in a 2000 L tank utilizing flow-through well water heated to 27°C. Fish were fed a standard commercial trout feed containing 41.0 % CP, 12.0 % lipid, and 4.0 % fiber for a minimum 10-day preliminary

period prior to initiation of sampling. During the preliminary period fish were fed to satiation three times daily at 4 hour intervals.

On days of sampling, fish were fed to satiation in the morning. Following the morning feeding, five fish each were netted at random at 0.5, 1, 2, 4, 6, and 8 hours post-prandially. Additionally, five fish each were netted at random prior to first feeding and labeled time 0 (control group). Fish were euthanized via hypothermia, by submersion in an ice/water slurry for 15 minutes, prior to dissection.

Euthanized fish were blotted dry, individually weighed, and measured for total length. Incisions were made along the mid-ventral line posteriorly, exposing the coelom. The GI tract was clamped, and severed at the esophagus anteriorly, and at the anus posteriorly. The entire visceral cavity was excised and the GI tract teased away from other visceral components. The external surface of the GI tract was rinsed in distilled water and blotted dry prior to weighing.

All pH measurements were made with a flat membrane pH microelectrode (model MI-406) and external micro-reference electrode (model MI-402) (Microelectrodes, Inc., Bedford, New Hampshire). Values were digitally displayed on an Accumet model 25 pH/ISE meter (Fischer Scientific, Pittsburgh, Pennsylvania) and recorded.

The intestine was clamped just posterior to the pyloric sphincter and the stomach removed. The stomach was slit anteriorly from the pyloric sphincter to the esophagus, and ventrally to expose the contents (Figure 1). Stomach contents were analyzed on the surface, on both sides of the mid-ventral line near the top and bottom of the bolus. Additionally, any food remaining in the esophagus or pylorus was measured for pH. Stomach contents were collected in 50 mL Erlenmeyer flasks with 30 mL distilled water

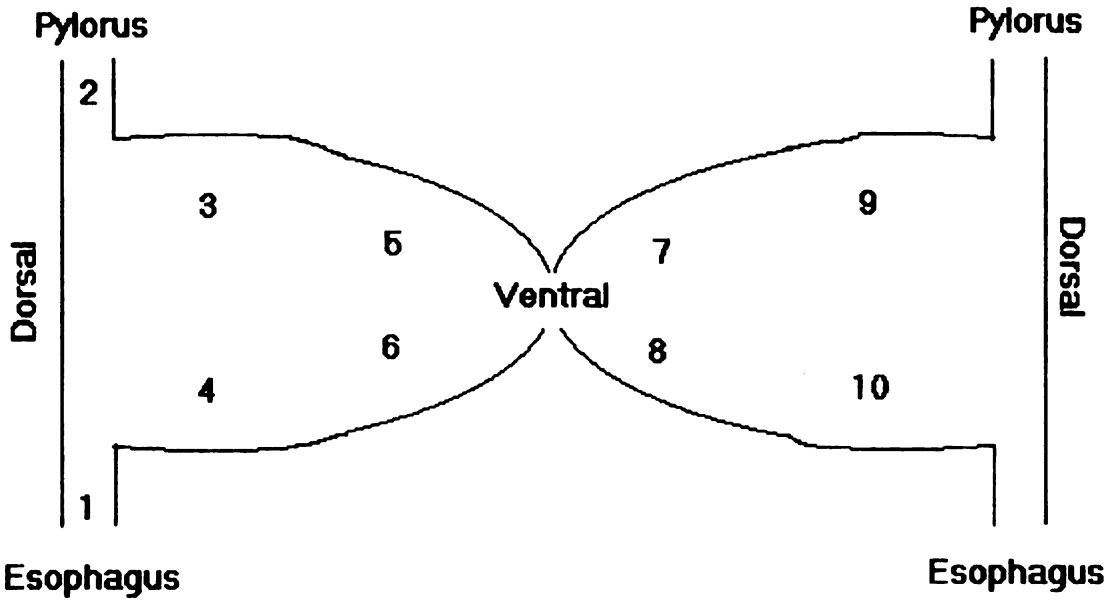

Figure 1. Top view of stomach excised from tilapia (*O. niloticus*). The stomach was slit anteriorly from the pyloric sphincter to the esophagus and ventrally to expose the contents. View indicates 10 sites where pH measurements were made on the surface of the gastric mucosa. The sites corresponding to 1 and 2 represent the esophagus and pylorus, respectively.

and homogenized. Samples were stored at –20°C for subsequent pH analysis of the total contents. The mucosa of the emptied stomach was rinsed under a gentle stream of distilled water to remove any remaining feed particles. The surface of the gastric mucosa was measured for pH at the esophagus (1), the pylorus (2), and four sites on each side of the mid-ventral line (3-6, and 7-10) (Figure 1).

The intestine was measured for total length prior to segmenting into10 equal segments. Each segment was slit along its length and contents exposed. Readings were collected on the contents at three sites along the most anterior segment (segment 1). The first measurement was taken immediately following the pyloric sphincter and anterior to the bile duct. The second measurement was taken in the middle of the segment. The third measurement was taken at the posterior end of the segment (Figure 2). The remaining nine segments were read at the middle of the segment. Following these measurements the intestine was rinsed under a gentle stream of distilled water to remove any remaining digesta. Mucosal readings were taken at sites corresponding to the point digesta readings were measured.

Statistical Analysis

Analyses were performed as a one-way ANOVA with time as a fixed factor. Due to missing values where digesta was not available, means were separated using the least-squares estimates of marginal means (lsmeans) method (SAS 1979).

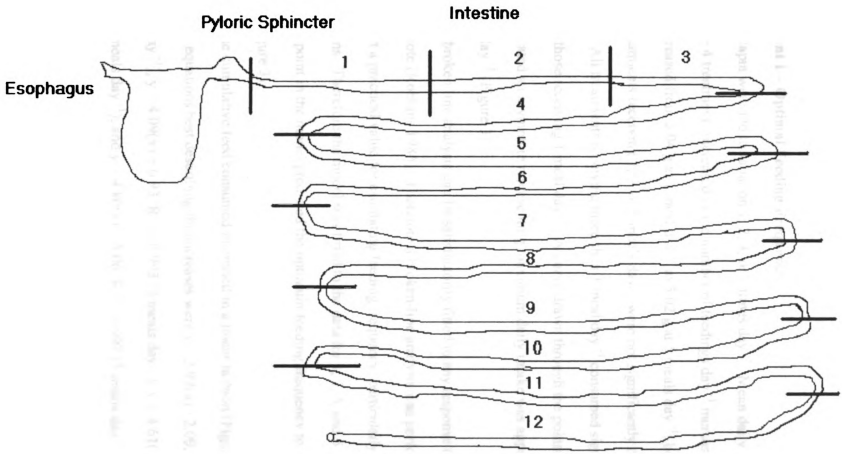

Figure 2. Excised GI tract of tilapia (*O. niloticus*). Numbers indicate intestinal segments where pH measurements were made. Readings were collected on the contents and mucosa at three sites along the most anterior segment; 1) immediately following the pyloric sphincter and anterior to the bile duct; 2) the middle of the segment; 3) the posterior end of the segment. Intestinal contents and mucosa were measured at the middle of the nine remaining segments (sites 4 - 12).

RESULTS

Experiment 1 – Optimal Feeding Frequency

Tilapia were fed to satiation 1, 2, 3, or 5 times day $^{-1}$. Mean daily intake (MDI) among the 4 treatments increased as the number of feedings day $^{-1}$ increased. Mean daily intake increased from 3.04 g at 1 meal day $^{-1}$ to 5.02 g at 5 meals day $^{-1}$; however, intake among treatments receiving 2, 3, or 5 meals day $^{-1}$ were not significantly different ($P<0.05$). All treatments receiving more than 1 meal day $^{-1}$ consumed significantly more feed than those receiving 1 meal day $^{-1}$. A curve drawn through the points representing MDI indicated an asymptote representing maximum daily intake was approached at 3 feedings day $^{-1}$ (Figure 3).

A broken line analysis can be satisfactorily fitted to any response that approaches an asymptote (Robbins 1986). Therefore a broken-line analysis was performed on MDI. Zero is not a practical value for estimating feeding frequency, particularly for long term applications. Therefore the model was fitted to the data for 1, 2, 3, and 5 feedings day $^{-1}$. The breakpoint in the model predicted the optimum feeding frequency to be 3.18 feedings day $^{-1}$ (Figure 3).

The cumulative feed consumed increased in a linear fashion (Figure 4). The predictive equations best describing the increases were $y = 2.97(x) + 2.00$, $R^2 = 0.997$ (1 meal day $^{-1}$); $y = 4.09(x) + 0.93$, $R^2 = 0.998$ (2 meals day $^{-1}$); $y = 4.61(x) + 3.97$, $R^2 = 0.997$ (3 meals day $^{-1}$); and $y = 4.80(x) + 5.06$, $R^2 = 0.997$ (5 meals day $^{-1}$). A slope ratio analysis indicated those fish receiving 1 meal day $^{-1}$ consumed significantly less feed

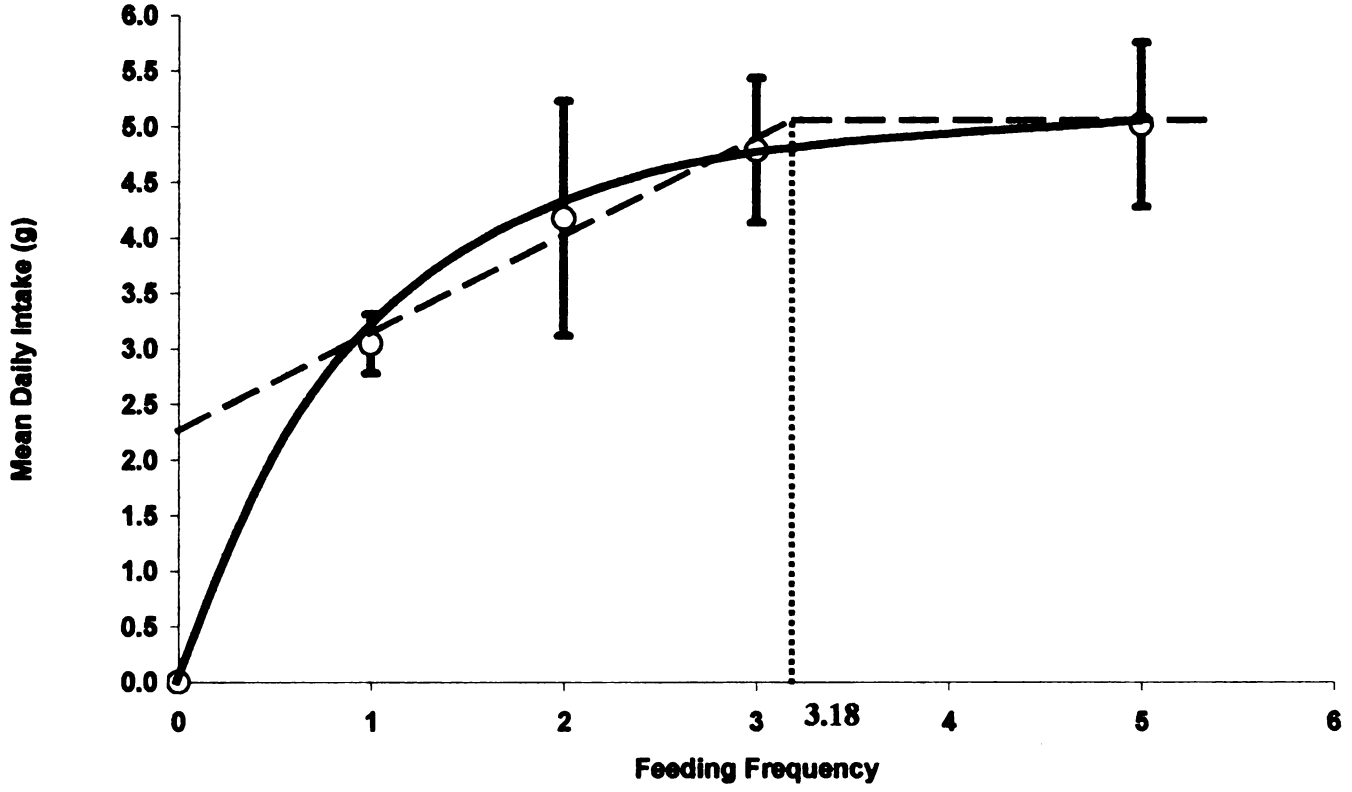

Figure 3. Mean daily intake of tilapia *O. niloticus* fed to satiation 1, 2, 3, or 5 times day $^{-1}$. The curve, as drawn approaches an asymptote representing maximum daily intake at 3 feedings day $^{-1}$. Error bars represent standard deviation of three replicates of pooled samples (n=5) over a 29 day period. A broken-line analysis indicated the breakpoint was 3.18 feedings day $^{-1}$. Mean daily intakes of fish fed 2, 3, or 5 times day $^{-1}$ were significantly greater than fish fed 1 time day $^{-1}$ ($P<0.05$), but were not significantly different from each other.

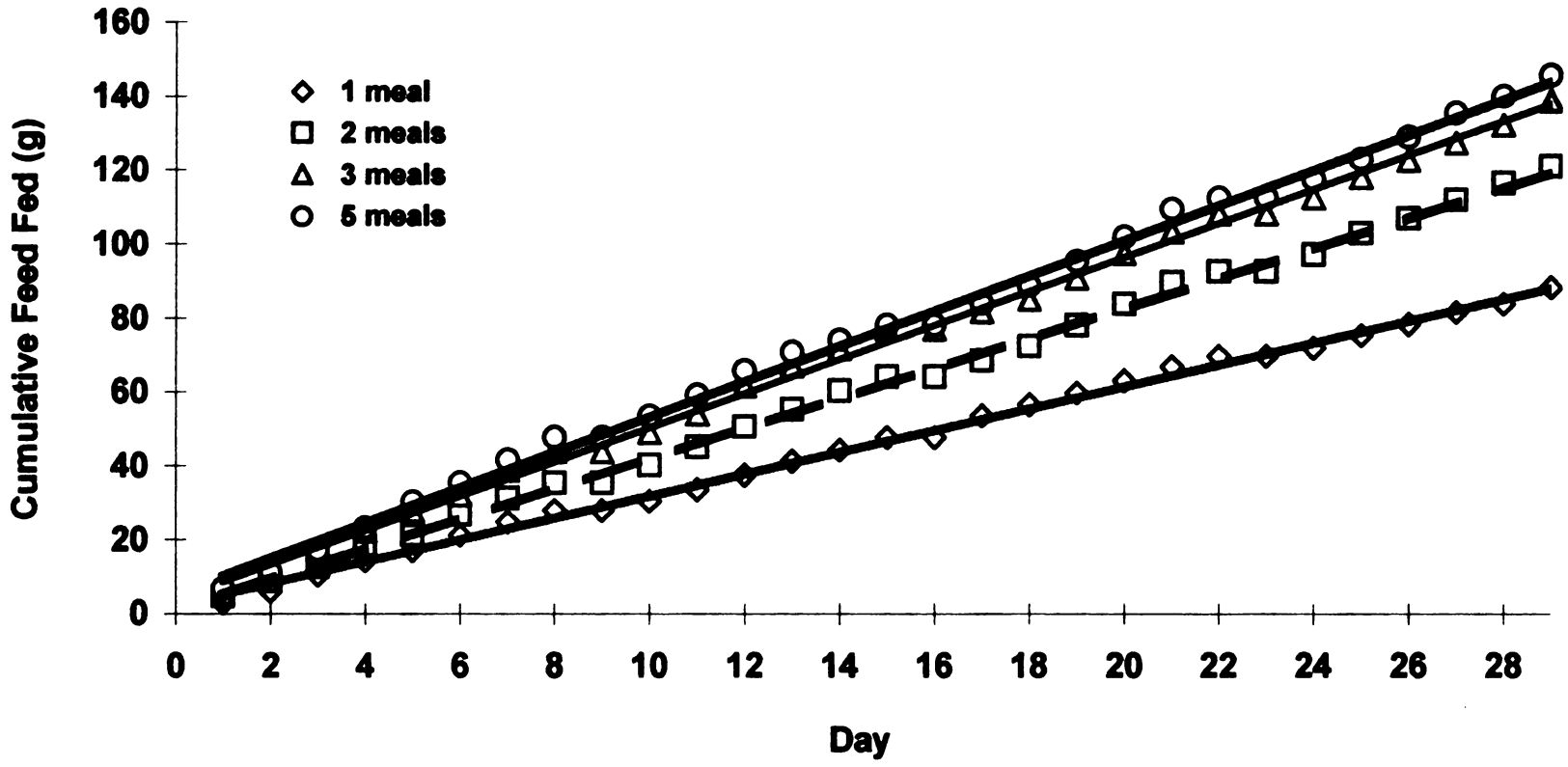

Figure 4. Cumulative feed consumed by *O. niloticus* fed to satiation 1, 2, 3, or 5 times day $^{-1}$. Regression analysis indicated the equations best describing the relationships were; $y=2.97x+2.00$, $R^2=0.997$ (◇); $y=4.09x+0.93$, $R^2=0.998$ (□); $y=4.61x+3.97$, $R^2=0.997$ (Δ); and $y=4.80+5.06$, $R^2=0.997$ (○). Slope ratio analysis indicated cumulative consumption by fish fed 1 meal day $^{-1}$ was significantly less than those fed 3, or 5 meals day $^{-1}$ ($P<0.05$). No other differences were detected.

during the course of the trial than those receiving 3 or 5 meals day $^{-1}$. No other differences were detected ($P<0.05$).

Mean daily intake partitioned on a per meal basis indicated fish receiving 1 meal day $^{-1}$ consumed significantly more feed at the 8:00 hr feeding than did fish receiving multiple meals during the day ($P<0.05$) (Figure 5). Fish receiving 2 meals day $^{-1}$ consumed significantly more feed at 8:00 hr than fish receiving 5 meals day $^{-1}$, but not more than those receiving 3 meals day $^{-1}$. The amount of feed consumed during the first meal by fish receiving 3 meals day $^{-1}$ and 5 meals day $^{-1}$ were not statistically different ($P<0.05$).

Mean daily intake for all fish at 8:00 hr was significantly higher during the first week than during the final week. Although all treatments showed decreased intake at 8:00 hr during the final week, the greatest decrease was in the fish receiving 3 meals day $^{-1}$.

Fish receiving a second meal during the day consumed less feed during that meal the sooner it followed the previous meal. During the second meal, fish receiving 2 meals day $^{-1}$ ($\bar{x} = 2.41$ g) consumed significantly more than did those receiving 3 meals day $^{-1}$ ($\bar{x} = 1.61$ g), which was significantly more than those receiving 5 meals day $^{-1}$ ($\bar{x} = 0.93$ g) ($P<0.05$). Similarly during the third meal fish, receiving 3 meals day $^{-1}$ ($\bar{x} =$ 1.51 g) consumed significantly more than fish receiving 5 meals day $^{-1}$ ($\bar{x} = 0.88$ g).

All treatments exhibited an increase in growth expressed as a percent of original wet weight (Figure 6). Mean increase in weight was 60 %, 49 %, 36 %, and 9 % for fish fed 3, 2, 5, and 1 meal day $^{-1}$, respectively. Weight increase in fish fed one meal a day

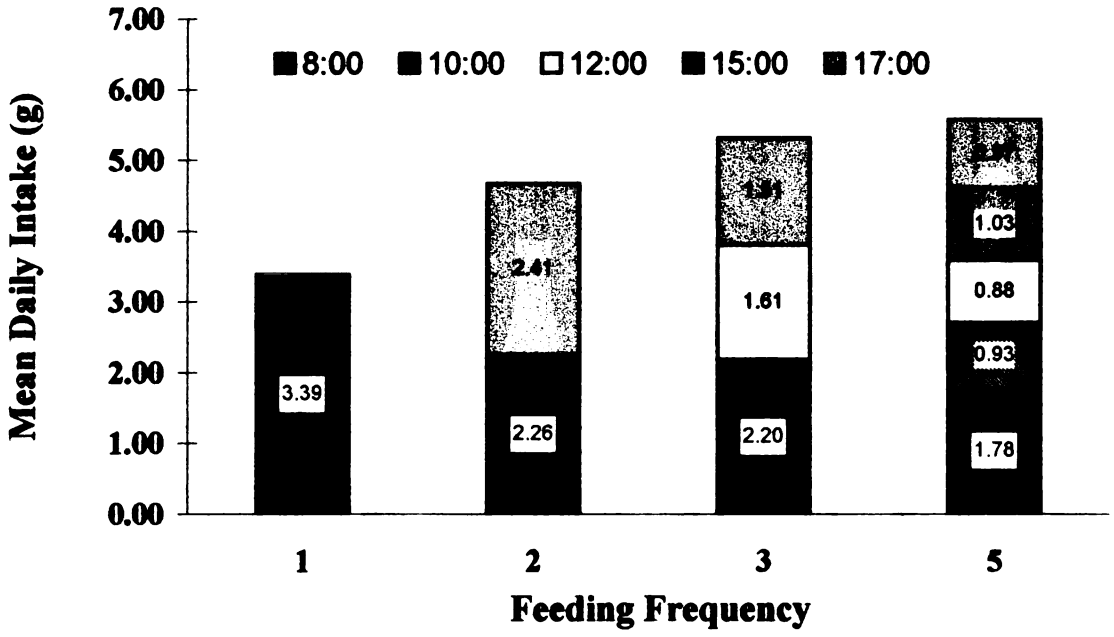

Figure 5. Mean daily intake of *O. niloticus* fed 1, 2, 3, or 5 meals day $^{-1}$ partitioned into mean consumption at each feeding. Values represent the mean consumption of three replicate groups fed during a four week trial.

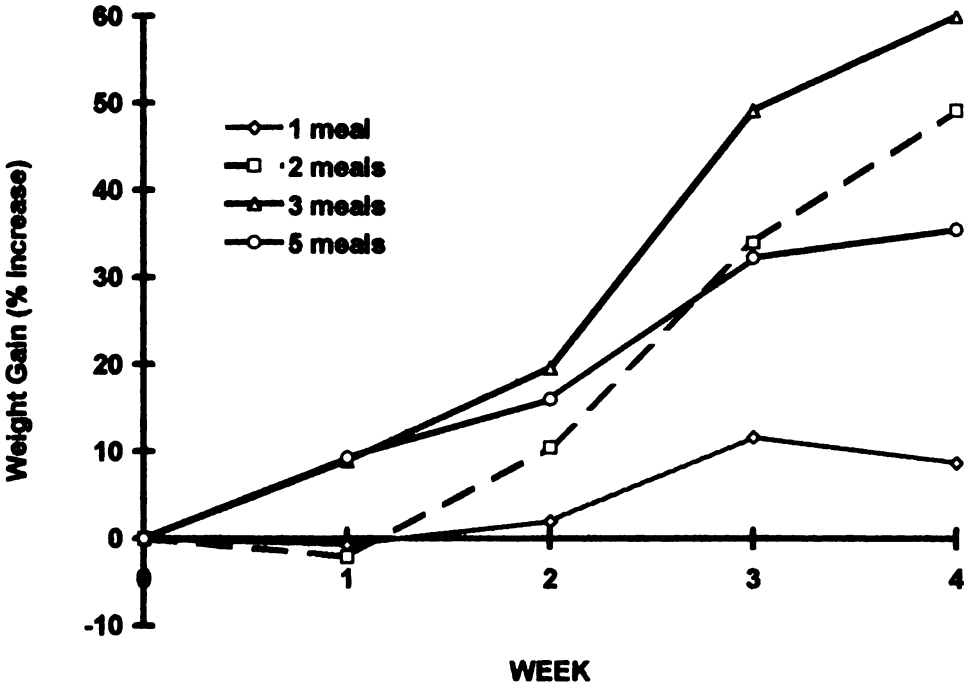

Figure 6. Weight gain in *O. niloticus* fed to satiation 1, 2, 3, or 5 times day $^{-1}$. Fish fed 1 meal day $^{-1}$ exhibited a significantly lower increase in weight than fish fed multiple meals day $^{-1}$, which were not significantly different from each other ($P<0.05$).

was significantly less than in those fed multiple meals ($P<0.05$). The remaining treatments were not significantly different from each other ($P<0.05$).

The initial and final proximate compositions of the fish are summarized in Table 5. Fish fed 3 meals day $^{-1}$ contained significantly more lipid and GE than fish fed either 1, or 5 meals day $^{-1}$, but were not significantly different in GE than fish fed twice day $^{-1}$, even though they contained significantly more lipid (Table 6). Fish fed 3 meals day $^{-1}$ contained significantly less CP than the other treatments ($P<0.05$). Fish fed 1 meal day $^{-1}$ had significantly more CP than fish fed 5 meals $^{-1}$, but were not different from fish fed 2 meals day $^{-1}$.

Performance and efficiency parameters of the fish are summarized in Table 7. Fish fed 2, 3, or 5 meals day $^{-1}$ were not different from each other in performance as measured by total weight gain, SGR, and FE ($P<0.05$). Although fish fed once day $^{-1}$ were not significantly different from fish fed 2 or 5 meals day $^{-1}$ in terms of total weight gain, they did exhibit a significantly lower SGR and FE (Table 7).

The PER indicated fish fed 2, 3, or 5 meals day $^{-1}$ were not different from each other, but all retained significantly more protein than fish fed once day $^{-1}$. The ANPU among the treatments mirrored PER.

Fish fed once day $^{-1}$ performed significantly poorer in terms of ER ($P<0.001$). Energy retention was significantly better in fish fed 3 meals day $^{-1}$ than those fed 5 meals day $^{-1}$, but was not significantly different from those fed 2 meals day $^{-1}$.

Multivariable analysis indicated there was a significant correlation between visceral weight and total wet weight, with an overall correlation coefficient of 0.67 (Table 8 (A)). The relationship was stronger among females than males when the

Table 6. Initial and final whole body proximate composition of *O. niloticus* fed a standard commercial trout diet to apparent satiation 1, 2, 3, or 5 times day $^{-1}$ during a four-week growth trial. All components are reported on a dry matter basis. Values represent the mean and (SEM) of triplicate assays on pooled samples (n=5) from three replicate treatments. Initial values represent the mean and (SEM) of triplicate assays on pooled samples (n=5). Different superscripts within a column indicate significant differences ($P<0.05$).

Feeding Frequency	Gross Energy (kJ/g)	Moisture (%)	Crude Protein (%)	Lipid (%)	Ash (%)
Initial	21.99 (0.09)	73.79 (0.01)	57.84 (0.24)	8.59 (0.09)	18.40 (0.65)
Final					
1	20.37 (0.02) ^{c}	72.04 (0.40)	61.17 (0.66) ^{a}	7.30 (0.20) ^{c}	18.76 (0.26) ^{a}
2	21.50 (0.25) ^{ab}	71.84 (0.52)	60.56 (0.51) ^{ab}	8.19 (0.26) ^{b}	16.52 (0.61) ^{ab}
3	22.33 (0.37) ^{a}	70.63 (0.61)	58.70 (0.56) ^{c}	9.05 (0.29) ^{a}	15.13 (1.34) ^{b}
5	21.31 (0.29) ^{b}	71.80 (1.10)	59.89 (0.97) ^{b}	8.14 (0.43) ^{b}	16.62 (1.71) ^{ab}

Table 7. Weight gain, specific growth rate (SGR), feed efficiency (FE), protein efficiency ratio (PER), energy retention (ER), and apparent net protein utilization (ANPU) in *O. niloticus* fed to satiation 1, 2, 3, or 5 times day $^{-1}$ during a four-week growth trial. Values represent the mean and (SEM) of pooled samples (n=5) from three replicate treatments. Different superscripts within a column indicate significant differences ($P<0.05$).

Feeding Frequency	Weight Gain (g/fish)	SGR (%BW/day)	FE	PER	ER (%)	ANPU (%)
1	3.01 (1.42) ^{b}	0.28 (0.11) ^{b}	0.16 (0.07) ^{b}	0.39 (0.17) ^{b}	0.04 (8.95) ^{c}	16.06 (1.17) ^{b}
2	13.19 (3.89) ^{ab}	1.18 (0.22) ^{a}	0.52 (0.08) a	1.27 (0.19) ^{a}	63.71 (10.57) ^{ab}	27.70 (2.12) ^{a}
3	16.85 (1.87) ^{a}	1.62 (0.09) ^{a}	0.60 (0.02) ^{a}	1.23 (0.19) ^{a}	84.65 (2.56) ^{a}	30.04 (2.04) ^{a}
5	12.69 (4.38) ^{ab}	1.02 (0.30) ^{a}	0.42 (0.11) ^{a}	1.01 (0.26) ^{ab}	49.35 (12.61) ^{b}	22.14 (3.46) ^{ab}

Table 8. Partitioned ANOVA tables and parameter estimates for the multivariable regressions examining (A) visceral weight as a function of total wet weight, gender, and number of daily feedings; and (B) perivisceral weight as a function of visceral weight, gender, and number of daily feedings. Number of feedings evaluated were one, two, three, or five times a day.

(A)

Analysis of Variance						Parameter Estimates		
Source	DF	SS	MS	F value	P	Variable	Prob>|T|	Model R-Square
Model	2	49.70	24.85	53.96	0.0001	Intercept	0.0023	
Error	54	24.87	0.46			Total Weight	0.0001	0.4799
						Gender	0.0001	0.6665
						Feedings	0.4831	0.6696

(B)

Analysis of Variance						Parameter Estimates		
Source	DF	SS	MS	F value	P	Variable	Prob>|T|	Model R-Square
Model	3	0.40	0.13	7.09	0.0004	Intercept	0.3102	
Error	53	1.01	0.019			Visceral Weight	0.0436	0.1610
						Gender	0.0136	0.2290
						Feedings	0.0144	0.2865

analysis was separated by gender. The effects of gender and total weight were confounded with each other. Males were significantly larger, but females had a significantly higher wet visceral weight. However, the female visceral weight was comprised primarily of gonadal tissues, whereas male visceral weight was comprised primarily of liver and intestinal tract. The number of feedings a day had no significant effect on the total wet visceral weight.

Visceral weight, gender, and number of feedings day $^{-1}$ had a significant effect on the amount of perivisceral fat (Table 8 (B)). The overall correlation was low, but was greater for males than for females. When separated by gender, there was no correlation among females, whereas the correlation among males was highly significant. Males had significantly more perivisceral fat than females. Fish fed once day $^{-1}$ had significantly less perivisceral fat than fish fed 3 or 5 meals day $^{-1}$.

Experiment 2 – Tilapia Gastric Evacuation Rate

The rate of gastric evacuation and passage through the GI tract was followed using Fe_2O_3 as an inert marker. There were no significant differences in iron consumption or total consumption between the treatments. Few fish dissected at time 0 (prior to feeding) had digesta remaining from the previous day's feeding. The digesta that was found, was principally localized in segments 5 – 7. No digesta was found in the gastric region. Where digesta was found, it was analyzed for iron and the results used to correct for background iron.

The contrast between the green chromic oxide marker and red ferric oxide marker was readily discernable during dissection. At 2 hrs postprandially, iron was observed

throughout the GI tract. At 4 hrs postprandially, a red/green mixed bolus was noted in segment 4. At 6 hrs postprandially, the mixed bolus was observed in the terminal segment for both treatments. In fish fed 5 meals day $^{-1}$, stomachs at 24 hrs were full of red and green digesta, whereas the stomachs in fish fed 3 meals day $^{-1}$ were empty and flaccid. Visual inspections were verified by analytical determination.

The initial rate of gastric emptying for both treatments was similar during the first hour (Figure 7). Following the first hour, GER of fish fed 5 meals day $^{-1}$ was slower than that of fish fed 3 meals day $^{-1}$. Gastric evacuation rate for both treatments was curvilinear and could be described by the exponential function $V_T = V_0 e^{-b(x)}$; where V_T = volume of feed at time T, V_0 = volume of feed at time 0. The equation describing GER for fish fed 3 meals day $^{-1}$ was $V_T = 67.0\ e^{-0.153(x)}$ with a correlation coefficient of R^2=0.90, and for fish fed 5 meals day $^{-1}$ was $V_T = 85.0\ e^{-0.149(x)}$ with a correlation coefficient of R^2=0.97. A t-test following natural log transformation of cumulative iron suggested the slopes were not significantly different ($P<0.05$).

The rate at which iron appeared at the terminal segment of the intestine was slightly more rapid for fish fed 5 meals day $^{-1}$ over the first hour (Figure 8); however, the treatments were not significantly different. The amount of iron fed appearing in the terminal segment, between the 2 hr sampling and the 4 hr sampling, and between the 4 hr sampling and the 6 hr sampling, was significantly higher in the group fed 3 meals day $^{-1}$ (Figure 8). Conversely, the increase between the 12 hr and 18 hr samplings was significantly higher in the group fed 5 meals day $^{-1}$ ($P<0.05$).

The rate at which iron appeared at the terminus was linear over the first eight hours postprandially (Figure 9). A slope analysis indicated the rates were not

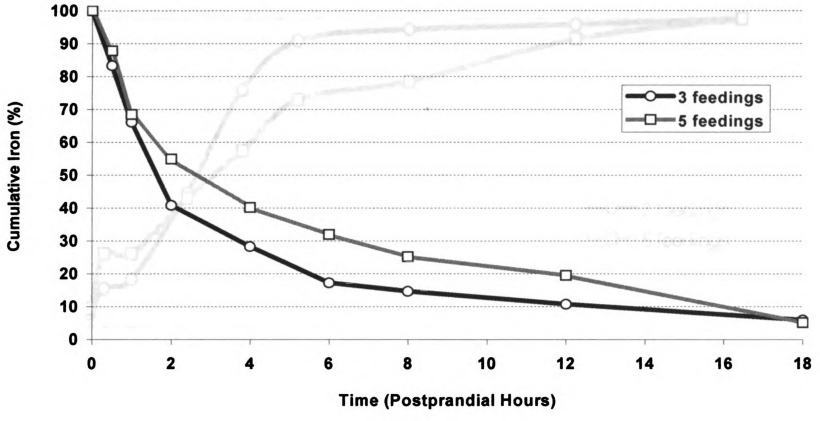

Figure 7. Rate of gastric evacuation in *O. niloticus* fed an experimental diet containing Fe_2O_3 as an external marker at time 0. Fish were fed either 3 feedings day $^{-1}$ to satiation (8:00, 12:00, and 17:00), or 5 feedings day $^{-1}$ to satiation (8:00, 10:00, 12:00, 15:00, and 17:00). Cumulative iron is relative to total iron collected 24 hrs following feeding. The gastric evacuation rates for the two treatments are described as $67.0\ e^{-0.153(x)}$, R^2=0.90 (3 feedings day $^{-1}$); and $85.0\ e^{-0.149(x)}$, R^2=0.97 (5 feedings day $^{-1}$).

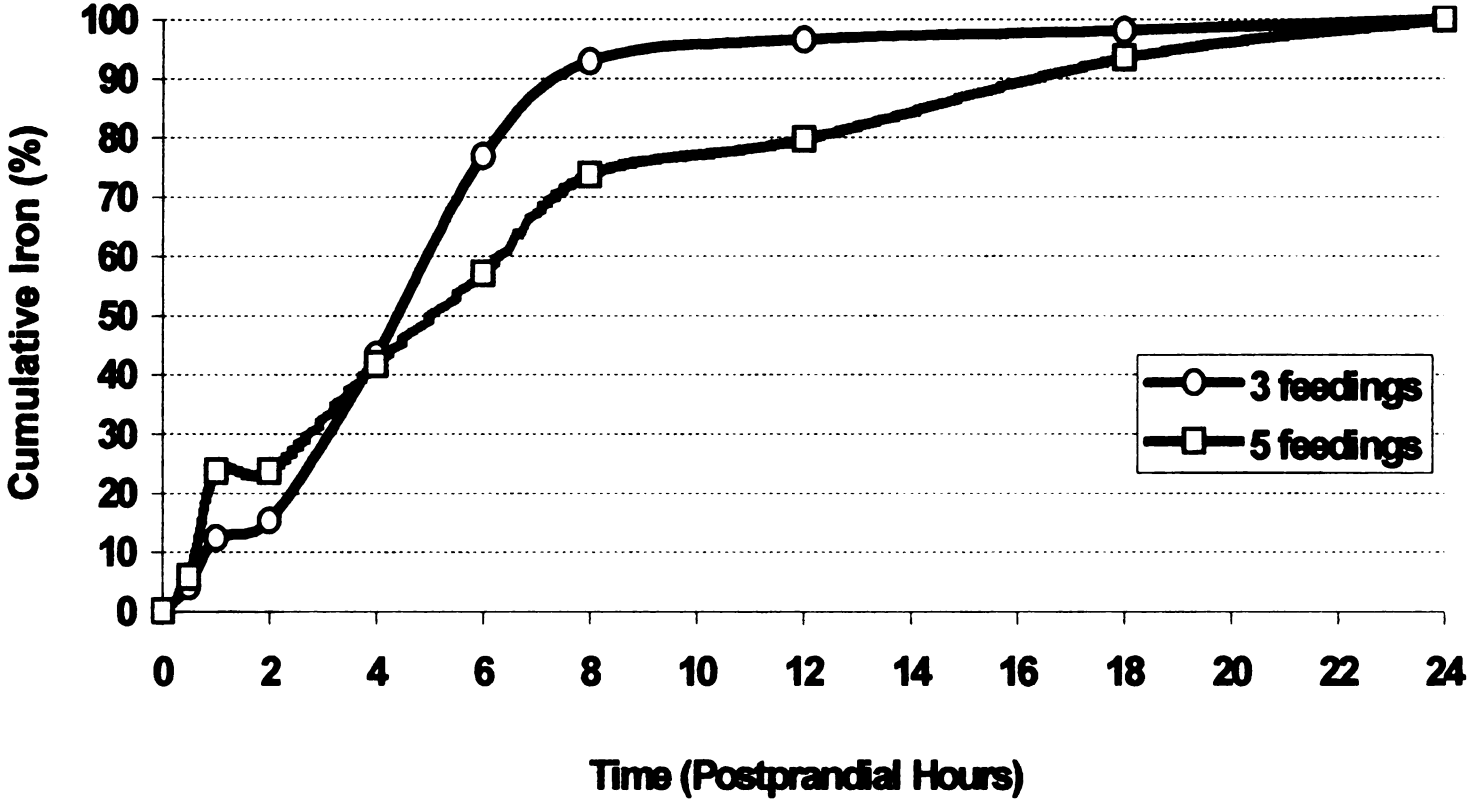

Figure 8. Rate of iron appearance at terminus of *O. niloticus* intestinal tract in fish fed an experimental diet containing Fe_2O_3 as an external marker at time 0. Fish were fed either 3 feedings day $^{-1}$ to satiation (8:00, 12:00, and 17:00), or 5 feedings day $^{-1}$ to satiation (8:00, 10:00, 12:00, 15:00, and 17:00). Following the initial feeding, subsequent meals consisted of a similar diet without Fe_2O_3.

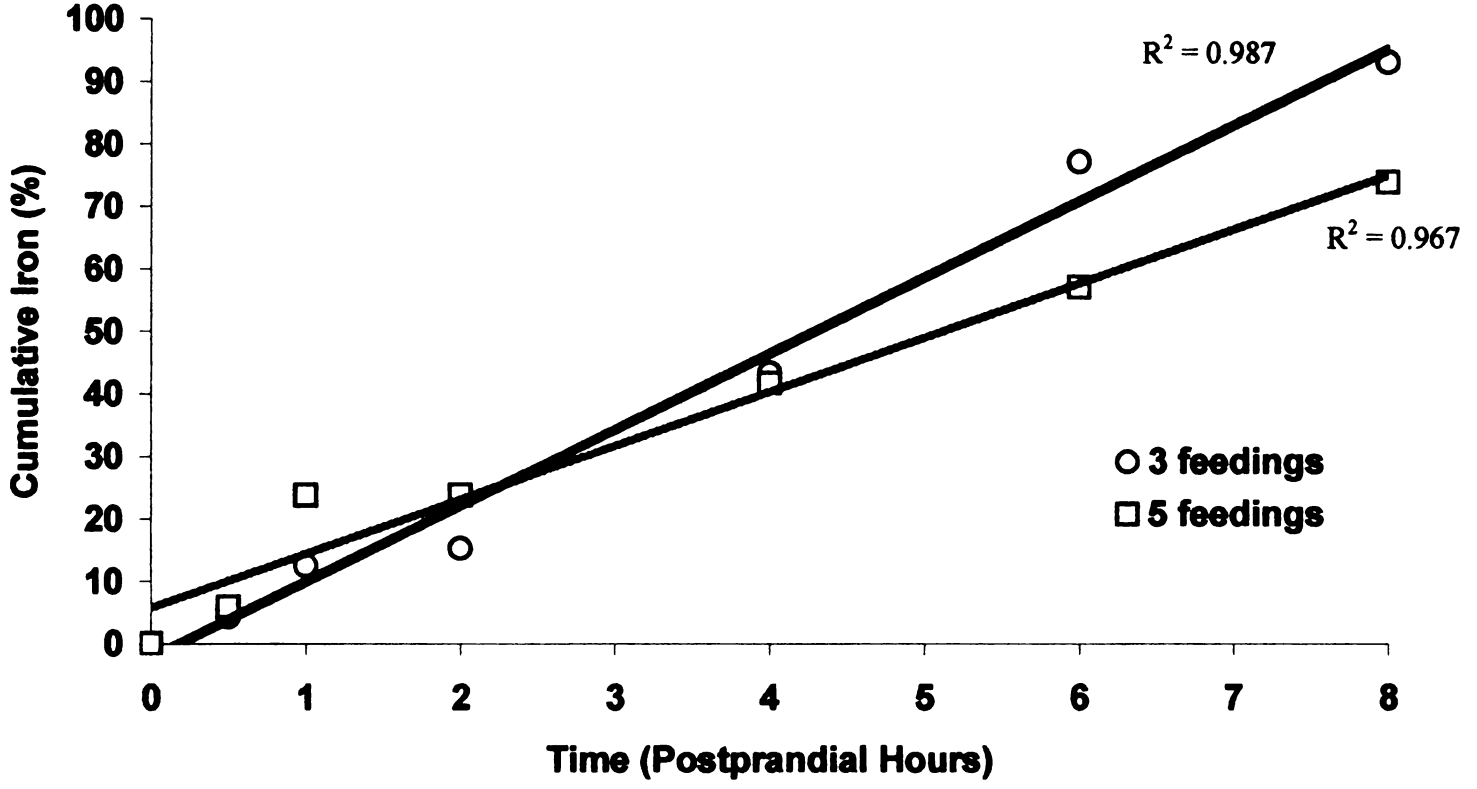

Figure 9. Linear increase in iron appearing at the terminal segment of *O. niloticus* intestinal tract during the first eight hours following the feeding of an experimental diet containing Fe_2O_3 as an external marker. Cumulative iron is relative to total iron collected over 24 hrs.

significantly different. The time required for 90 % of the recovered iron to appear in fish fed 3 meals day $^{-1}$ was 8 hrs, and for fish fed 5 meals day $^{-1}$ was 18 hrs.

Experiment 3 – Solvent Extracted SBM Growth Trial

Graded levels of phytase treated SBM, or untreated SBM, were substituted for FM and in diets fed to juvenile tilapia to evaluate the effects on growth, efficiency, and body composition. Experimental diets were formulated to contain 33 % CP. Analysis showed actual dietary CP ranged from 32 – 36 %. No relationship was observed between growth or performance and dietary CP level.

There was a linear increase in phytic acid with increasing levels of SBM substitution in the untreated diets (Table 5). All diets prepared with fishmeal and phytase treated SBM had phytic acid concentrations below detectable limits. Trypsin inhibitor activity in the solvent extracted SBM was 2.8 mg/g SBM.

During the eight week experimental period, fish fed the phytase treated SBM, and untreated SBM, exhibited similar growth patterns. Increase in weight for fish fed the phytase treated SBM diets ranged from 402 – 771 %, for the 100 % and 25 % CP as SBM, respectively (Table 9). Weight gain in fish fed the untreated SBM diets ranged from 441 – 731 %, for the 100 % and 25 % CP as SBM, respectively (Table 9).

Fish grew slightly better with addition of SBM at 25 % CP regardless of SBM treatment; however, the overall trend was toward lower weight gain with increasing incorporation of SBM (Figure 10). Diets containing phytase treated SBM resulted in significantly lower growth as the percentage of CP as SBM increased above 25 % ($P<0.05$). In contrast, diets containing untreated SBM did not result in significantly

Table 9. Weight gain, whole body crude protein, whole body lipid, and moisture in juvenile tilapia fed diets containing untreated, or phytase treated, solvent extracted SBM substituted into a reference diet at 25, 50, 75, or 100 % of the dietary protein. Values represent the mean of three replicates of pooled samples (n=8). Different superscripts in a column, within the phytase treatment, or within the untreated treatment, represent significant differences within that treatment ($P<0.05$). Asterisks represent significant differences from the control diet ($P<0.05$).

	Weight Gain (% Increase)	Crude Protein (% DM)	Lipid (% DM)	Moisture (%)
Control	694	56.09	17.61	74.32
Phytase Treatment				
25 % SBM	771 ^{a}	58.70	17.51	74.43
50 % SBM	495 ^{b}	60.70 *	14.45	75.17
75 % SBM	561 ^{b}	58.41	14.24	74.88
100 % SBM	402 ^{b*}	61.65 *	15.10	75.04
Untreated Treatment				
25 % SBM	731 ^{a}	54.75 ^{c}	19.45	72.67 ^{b}
50 % SBM	671 ^{ab}	58.21 ^{b}	15.48	73.44 ^{ab}
75 % SBM	490 ^{ab}	59.60 ^{ab}	13.55	74.00 ^{a}
100 % SBM	441 ^{b*}	61.56 ^{a*}	15.36	74.47 ^{a}

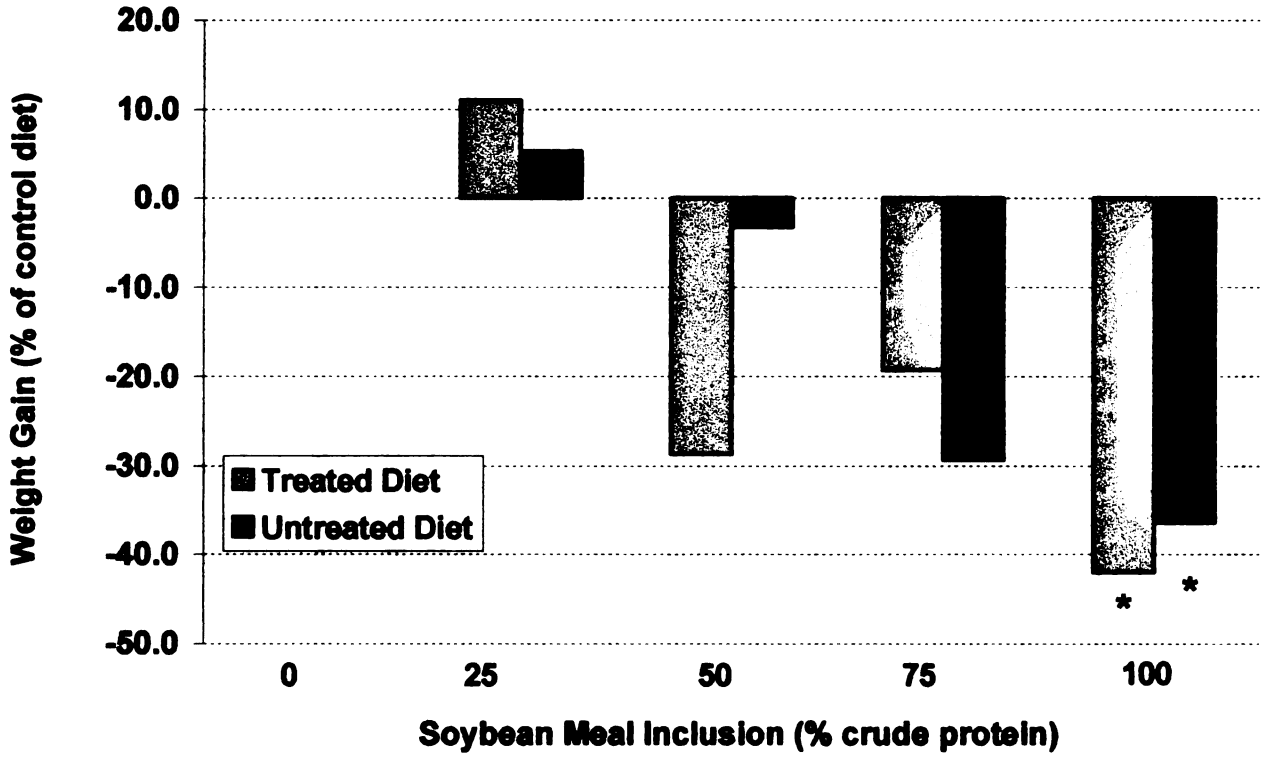

Figure 10. Weight gain of juvenile tilapia fed experimental diets relative to a control diet. Experimental diets contained untreated, or phytase treated, solvent extracted SBM substituted into the control diet at 25, 50, 75, and 100 % of the dietary protein. Asterisks indicate experimental diets resulting in significantly different growth rates relative to the control diet ($P<0.05$).

lower growth until the percentage of CP as SBM surpassed 75 %. Orthogonal contrasts, between phytase treated and untreated diets at the same level of SBM substitution, indicated the diets incorporating SBM at 50 % of the CP were the only diets resulting in significantly different growth (Figure 11). Growth was not significantly reduced relative to the fish meal control diet, for either treatment, until SBM provided all of the dietary protein (Table 9).

Proximate components in fish fed the phytase treated SBM diets were not significantly different from each other. However, relative to fish fed the control diet, fish fed diets containing 50 and 100 % of the CP as phytase treated SBM contained significantly more CP (Table 9). Similarly, fish receiving the diet containing untreated SBM incorporated at 100 % of the CP contained significantly more CP relative to the control group.

In contrast to the phytase treated diets, fish fed diets containing untreated SBM exhibited increasing CP (%DM) with increasing dietary SBM. Fish fed the diet containing 25% CP as SBM had significantly less CP (%DM) than the remaining treatments (Table 9).

Whole body lipid levels (% DM) ranged from 13.55 – 19.45 %. None of the treatments were significantly different from each other, or from those fed the control diet. Moisture followed a similar pattern to CP.

Specific growth rate, feed efficiency, protein efficiency ratio, and apparent net protein utilization are summarized in Table 10. Specific growth rate ranged from 2.86 – 3.86 %/day. Relationships and significant differences within the diets of each SBM treatment mirrored those of weight gain. However, unlike weight gain, the orthogonal

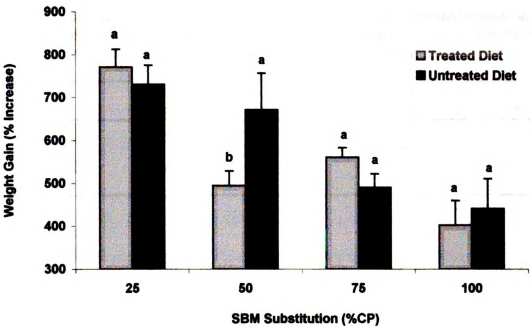

Figure 11. Orthogonal contrasts of weight gain in juvenile tilapia fed diets containing untreated, or phytase treated, solvent extracted SBM substituted into a reference diet at 25, 50, 75, or 100 % of the dietary protein. Values represent the mean of three replicates of pooled samples (n=8). Error bars represent SEM. Different labels within a level of substitution represent significant differences ($P<0.05$).

Table 10. Specific growth rate (SGR), feed efficiency (FE), protein efficiency ratio (PER), and apparent net protein utilization (ANPU) in juvenile tilapia fed diets containing untreated, or phytase treated, solvent extracted SBM substituted into a reference diet at 25, 50, 75, or 100 % of the dietary protein. Values represent the mean of three replicates of pooled samples (n=8). Different superscripts in a column, within the phytase treatment, or within the untreated treatment, represent significant differences within that treatment ($P<0.05$). Asterisks represent significant differences from the control diet ($P<0.05$).

	SGR (% / day)	FE	PER	ANPU (%)
Control	3.68	0.79	2.52	36.79
Phytase Treatment				
25 % SBM	3.86 ^{a}	0.82 ^{a}	2.53 ^{a}	38.65 ^{a}
50 % SBM	3.37 ^{b}	0.69 ^{a}	2.08 ^{b*}	32.15 ^{b}
75 % SBM	3.18 ^{b}	0.71 ^{a}	2.09 ^{b*}	31.19 ^{b*}
100 % SBM	2.86 ^{b*}	0.57 ^{b*}	1.58 ^{c*}	25.17 ^{c*}
Untreated Treatment				
25 % SBM	3.77 ^{a}	0.84 ^{a}	2.51 ^{a}	38.32 ^{a}
50 % SBM	3.63 ^{ab}	0.85 ^{a}	2.49 ^{a}	39.40 ^{a}
75 % SBM	3.17 ^{ab}	0.74 ^{a}	2.21 ^{a}	35.34 ^{a}
100 % SBM	2.99 ^{b*}	0.62 ^{b*}	1.83 ^{b*}	29.91 ^{b*}

contrasts between phytase treated and untreated diets at the same level of SBM substitution indicated no significant differences in SGR (Figure 12).

Feed efficiency values ranged from 0.57 to 0.84 (Table 10). There were no differences in FE between the fish fed the control diet and diets containing 75 % or less of the CP as SBM. Fish fed the diets containing 100 % of the CP as SBM exhibited a significantly lower FE than the other treatments ($P<0.05$). Contrasts between the same rates of SBM incorporation indicated only the two diets at the 50 % level were different from each other ($P<0.01$), with the fish receiving the untreated SBM performing better (Figure 13).

Protein efficiency ratio and ANPU exhibited similar trends (Table 10). Among the groups fed the phytase treated SBM diets, significant differences were detected in PER with incorporation of more than 25 % of the CP as SBM. The fish receiving diets at the 50 % and 75 % rates were more efficient than fish receiving the diet with all the CP supplied by SBM ($P<0.05$). Significant differences were not detected in PER or ANPU among the groups receiving the untreated SBM until the level of incorporation reached 100 % of the CP as SBM.

Relative to the control diet, fish receiving the phytase treated diets showed significantly lower PER with more than 25 % of the CP as SBM, and significantly lower ANPU with more than 50 % of the CP as SBM. Orthogonal contrasts between the same rates of SBM incorporation for PER indicated only the fish fed the 50 % level were different from each other (Figure14). However, contrasts between the same rates of SBM substitution for ANPU indicated significantly lower ANPU among fish fed the phytase treated diets with more than 25 % incorporation of SBM as CP (Figure 15). Results of

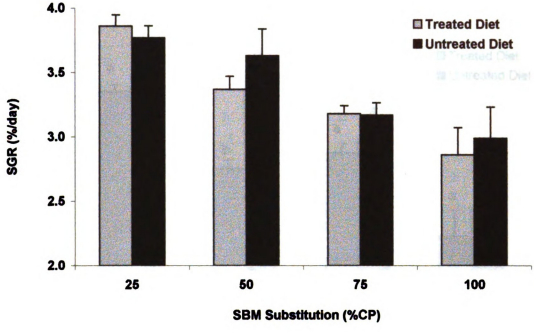

Figure 12. Orthogonal contrasts of specific growth rate (SGR) in juvenile tilapia fed diets containing untreated, or phytase treated, solvent extracted SBM substituted into a reference diet at 25, 50, 75, or 100 % of the dietary protein. Values represent the mean of three replicates of pooled samples (n=8). Error bars represent SEM. No significant differences were detected between treatments at any level of substitution.

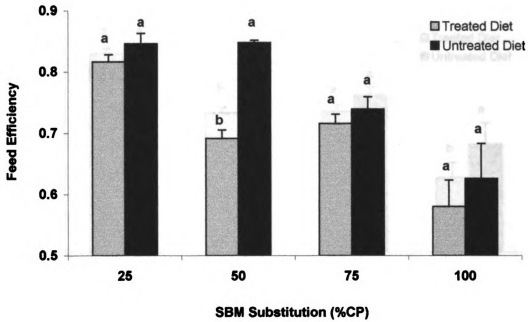

Figure 13. Orthogonal contrasts of feed efficiency in juvenile tilapia fed diets containing untreated, or phytase treated, solvent extracted SBM substituted into a reference diet at 25, 50, 75, or 100 % of the dietary protein. Values represent the mean of three replicates of pooled samples (n=8). Error bars represent SEM. Different labels within a level of substitution represent significant differences ($P<0.01$).

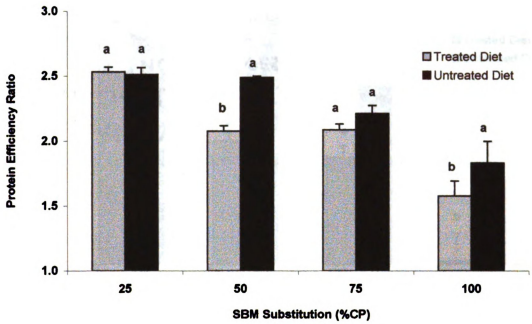

Figure 14. Orthogonal contrasts of protein efficiency ratio in juvenile tilapia fed diets containing untreated, or phytase treated, solvent extracted SBM substituted into a reference diet at 25, 50, 75, or 100 % of the dietary protein. Values represent the mean of three replicates of pooled samples (n=8). Error bars represent SEM. Different labels within a level of substitution represent significant differences ($P<0.05$).

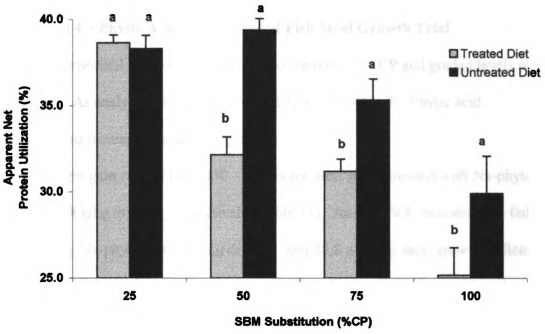

Figure 15. Orthogonal contrasts of apparent net protein utilization in juvenile tilapia fed diets containing untreated, or phytase treated, solvent extracted SBM substituted into a reference diet at 25, 50, 75, or 100 % of the dietary protein. Values represent the mean of three replicates of pooled samples (n=8). Error bars represent SEM. Different labels within a level of substitution represent significant differences ($P<0.05$).

the linear and non-linear regression analyses for growth and efficiency parameters are given in Appendix 3.

Experiment 4 – Phytic Acid Supplemented Fish Meal Growth Trial

Experimental diets were formulated to contain 33 % CP and graded levels of phytic acid. As analyzed, the diets contained 32.6 – 35.4 % CP. Phytic acid concentrations increased linearly (Figure 16).

Weight gain ranged from 430 – 560 % for diets supplemented with Na-phytate at 12.9 and 25.8 g/kg dry diet, respectively (Table 11). An ANOVA indicated fish fed diets incorporating Na-phytate at 3.4 g/kg dry diet, and 25.8 g/kg dry diet, grew significantly slower than the fish fed the diet incorporating 12.9 g/kg dry diet, whereas the other treatments did not ($P<0.01$). The only diet which resulted in significantly slower growth than the control diet was the diet incorporating Na-phytate at 25.8 g/kg dry diet (Table 11). There were no significant differences among any of the treatments in final whole body CP, whole body lipids, or moisture.

Specific growth rate followed a similar trend as weight gain (Table 12). Fish fed the control diet, the diet incorporating Na-phytate at 9.7 g/kg dry diet, and the diet incorporating Na-phytate at 12.9 g/kg dry diet, performed significantly better in terms of SGR than the diet containing Na-phytate at 25.8 g/kg dry diet ($P<0.01$). There were no other differences in terms of SGR (Table 12). Additionally, there were no differences in the performance parameters FE, PER, and ANPU.

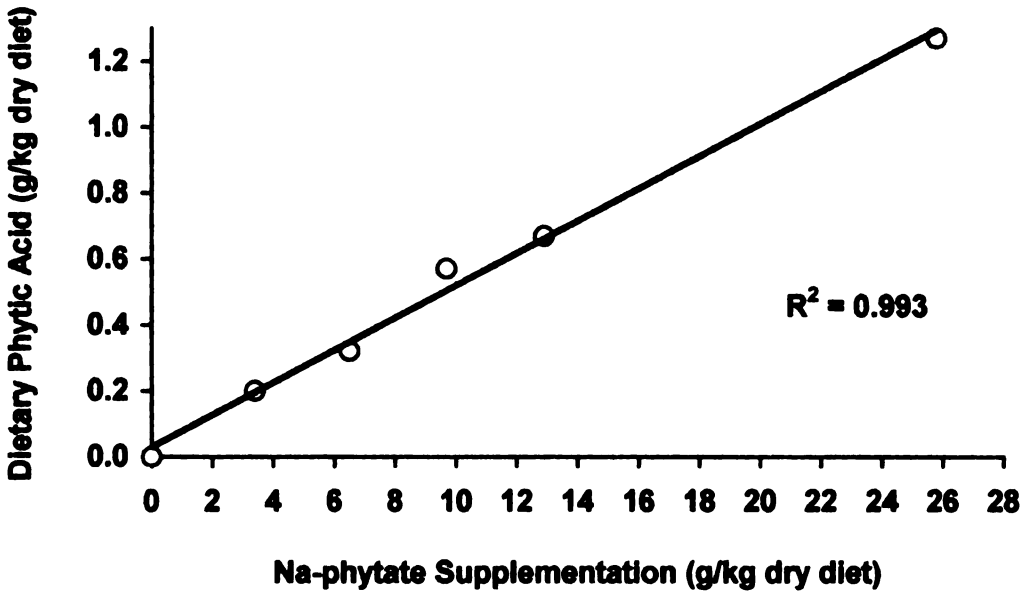

Figure 16. Dietary phytic acid concentrations in fish meal based experimental diets supplemented with phytic acid as Na-phytate.

Table 11. Weight gain, whole body crude protein, whole body lipid, and moisture in juvenile tilapia fed a fish meal based diet supplemented with graded levels of phytic acid as Na-phytate. Also shown is the level of SBM incorporation (% dietary CP) providing the equivalent phytic acid concentration. Values represent the mean of three replicates of pooled samples (n=8). Values within a column with different superscripts are significantly different ($P<0.01$).

Na-Phytate Supplementation (g/kg dry diet)	SBM Equivalent (% dietary CP)	Weight Gain (%)	Crude Protein (% DM)	Lipid (% DM)	Moisture (%)
0	0	519 ^{ab}	52.76	20.26	75.29
3.4	25	460 ^{bc}	52.69	19.90	75.50
6.5	50	490 ^{abc}	53.71	20.38	74.78
9.7	75	505 ^{abc}	54.06	19.38	74.73
12.9	100	560 ^{a}	54.56	20.02	75.73
25.8	200	430 ^{c}	53.67	17.87	75.62

Table 12. Specific growth rate (SGR), feed efficiency (FE), protein efficiency ratio (PER), and apparent net protein utilization (ANPU) in juvenile tilapia fed a fish meal based diet supplemented with graded levels of phytic acid as Na-phytate. Also shown is the level of SBM incorporation (% dietary CP) providing the equivalent phytic acid concentration. Values represent the mean of three replicates of pooled samples (n=8). Values within a column with different superscripts are significantly different (P<0.01).

Na-Phytate Supplementation (g/kg dry diet)	SBM Equivalent (% dietary CP)	SGR (%/day)	FE	PER	ANPU (%)
0	0	2.94 [a]	0.75	2.20	28.86
3.4	25	2.73 [ab]	0.62	2.09	27.05
6.5	50	2.83 [ab]	0.72	2.02	27.88
9.7	75	2.89 [a]	0.75	2.16	29.41
12.9	100	3.01 [a]	0.80	2.17	29.25
25.8	200	2.60 [b]	0.58	1.99	26.23

Experiment 5 – Solvent Extracted SBM Digestibility Trial

Crude protein and individual amino acid digestibility coefficients were determined for tilapia fed diets incorporating graded levels of phytase treated, or untreated SBM. The apparent crude protein digestibility (ACPD) of the fish meal control diet was 81.7 % (Figure 17). The ACPD of the phytase treated SBM diets ranged from 78.9 – 84.6 %, and the untreated SBM diets from 80.9 – 82.5 %. There were no significant differences among the treatments.

It should be noted that the mean for the phytase treated SBM incorporated at 100 % of the CP was based on only two values. Two replicates resulted in coefficients of 221 % and -13 % due to problems in HROM recovery. After testing for outliers, by examining the standardized residuals, they were excluded from the calculation (Montgomery 1991).

The nitrogen value obtained from fish fed the N-free diet in experiment 6 was 2.32±0.72 % N/g DM. This value was used in an attempt to correct the ACPD to true crude protein digestibility. However, the coefficients ranged from 97.7 – 103.4 % indicating this may not be an appropriate procedure.

The apparent digestibility of individual amino acids is summarized in Table 13. There was a high SEM among all the amino acids in the group fed the diet containing phytase treated SBM as the sole source of protein.

The apparent digestibility of Ala was significantly lower in the phytase treated diet incorporating SBM at 100 % CP than the other diets. Additionally, apparent digestibility of Lys in this diet was lower than that of the fish meal control diet.

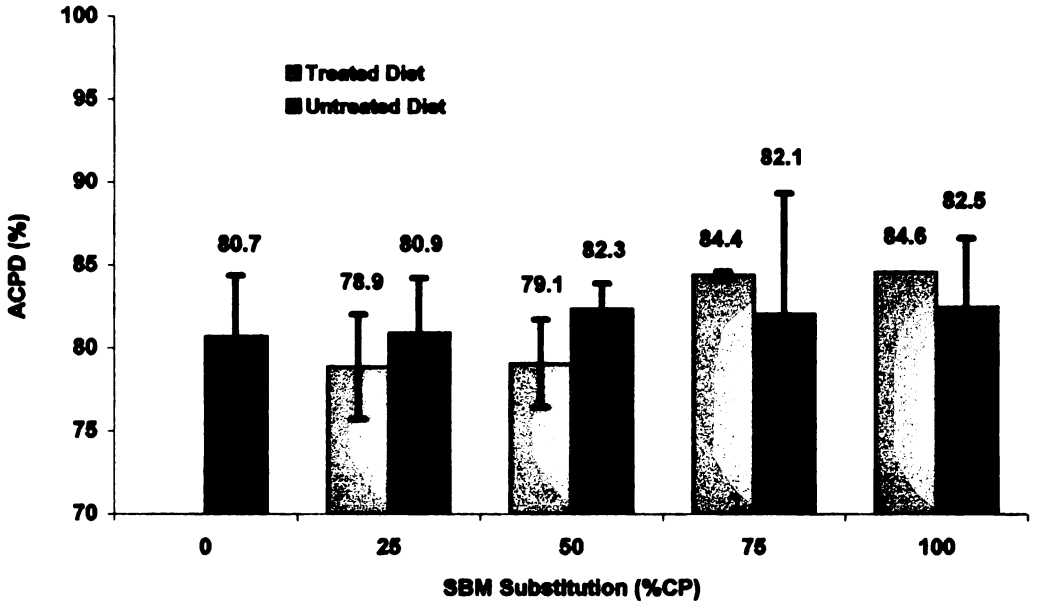

Figure 17. Apparent crude protein digestibility (ACPD) in juvenile tilapia fed diets containing untreated, or phytase treated, solvent extracted SBM substituted into a reference diet at 25, 50, 75, or 100 % of the dietary protein. Values represent the mean of four replicates of pooled samples (n=15), except the two diets with 100 % substitution, where insufficient material limited the analysis to two and three replicates for the treated, and untreated diets, respectively. Error bars represent SEM. No significant differences were detected among the treatments.

Table 13. Apparent amino acid digestibility coefficients in juvenile tilapia fed diets containing untreated, or phytase treated, solvent extracted SBM substituted into a reference diet at 25, 50, 75, or 100 % of the dietary protein. Values represent the mean and (SEM) of four replicates of pooled samples (n=15), except where indicated. Values with different superscripts across a row are significantly different (P<0.05).

Amino Acid	Reference Diet	Phytase 25 %	Phytase 50% [1]	Phytase 75% [1]	Phytase 100% [2]
Alanine	80.6 [a]	77.3 [a]	77.0 [a]	75.4 [a]	48.1 [b]
	(1.94)	(2.89)	(2.99)	(4.24)	(29.6)
Aspartic acid	76.6	73.1	74.3	74.2	50.0
	(2.51)	(3.33)	(3.35)	(4.32)	(27.66)
Arginine	80.9	82.0	82.4	85.2	71.0
	(2.10)	(2.77)	(1.86)	(2.13)	(17.95)
Cysteine	78.8	77.0	77.7	79.3	64.0
	(4.83)	(4.54)	(3.87)	(2.60)	(21.65)
Glutamic acid	84.9	83.0	83.9	85.1	73.7
	(2.43)	(2.46)	(2.60)	(2.61)	(20.25)
Glycine	77.8	77.4	77.1	77.7	53.7
	(2.88)	(2.76)	(3.01)	(3.92)	(26.45)

Table 13 (cont'd).

Amino Acid	Reference Diet	Phytase 25 %	Phytase 50%	Phytase 75%	Phytase 100%
Histidine	77.5	77.7	78.6	79.2	64.1
	(2.49)	(2.73)	(2.35)	(4.01)	(19.93)
Isoleucine	74.8	73.1	74.7	76.9	57.4
	(2.00)	(3.43)	(3.19)	(3.72)	(23.53)
Leucine	78.2	77.0	77.9	78.0	60.3
	(1.58)	(2.64)	(2.46)	(3.90)	(22.35)
Lysine	85.1 [a]	82.8	81.8	79.3	60.6 [b]
	(1.49)	(2.61)	(2.09)	(3.71)	(23.28)
Methionine	79.7	77.0	78.5	78.2	56.8
	(1.57)	(2.77)	(2.23)	(3.73)	(25.07)
Phenylalanine	72.7	73.5	75.0	78.7	63.1
	(2.37)	(3.49)	(3.20)	(3.12)	(20.51)

Table 13 (cont'd).

Amino Acid	Reference Diet	Phytase 25 %	Phytase 50%	Phytase 75%	Phytase 100%
Proline	76.2	76.5	76.5	76.7	59.9
	(3.30)	(3.70)	(3.23)	(4.14)	(22.05)
Serine	71.9	76.3	78.7	79.6	66.0
	(4.29)	(3.35)	(2.61)	(4.75)	(26.45)
Threonine	75.3	76.2	75.5	75.2	56.3
	(2.12)	(3.27)	(2.01)	(6.26)	(25.19)
Tyrosine	65.4	62.2	65.6	69.4	45.6
	(5.05)	(6.46)	(3.34)	(4.32)	(27.66
Valine	76.6	73.1	74.3	74.2	50.0
	(2.51)	(3.33)	(3.35)	(4.32)	(27.66)

Table 13. (cont'd)

Amino Acid	Reference Diet	Untreated[1] 25%	Untreated 50%	Untreated[1] 75%	Untreated[1] 100%
Alanine	80.6	80.8	77.6	82.3	73.2
	(1.94)	(2.84)	(2.46)	(8.39)	(9.13)
Aspartic acid	76.6	78.0	75.7	82.2	74.0
	(2.51)	(3.99)	(2.73)	(8.29)	(8.84)
Arginine	80.9	84.3	84.5	89.0	86.6
	(2.10)	(3.04)	(1.09)	(5.11)	(4.50)
Cysteine	78.8	80.9	77.2	85.1	78.7
	(4.83)	(5.09)	(2.15)	(6.27)	(7.91)
Glutamic acid	84.9	84.6	84.8	89.2	86.1
	(2.43)	(2.57)	(1.62)	(5.16)	(5.39)
Glycine	77.8	78.2	75.8	83.6	76.5
	(2.88)	(3.46)	(2.19)	(7.51)	(7.97)

Table 13 (cont'd).

Amino Acid	Reference Diet	Untreated 25%	Untreated 50%	Untreated 75%	Untreated 100%
Histidine	77.5	82.3	80.7	84.6	81.2
	(2.49)	(3.29)	(1.28)	(7.55)	(7.02)
Isoleucine	74.8	77.2	76.3	82.2	75.9
	(2.00)	(3.72)	(2.84)	(8.72)	(8.39)
Leucine	78.2	80.1	78.7	83.2	77.4
	(1.58)	(2.92)	(2.67)	(8.36)	(8.04)
Lysine	85.1	86.7	85.8	89.2	85.0
	(1.49)	(2.59)	(1.40)	(4.79)	(5.07)
Methionine	79.7	80.0	77.2	84.7	82.1
	(1.57)	(4.06)	(2.15)	(7.23)	(6.13)
Phenylalanine	72.7	77.1	76.9	82.5	77.8
	(2.37)	(3.79)	(2.60)	(8.44)	(7.93)

Table 13 (cont'd).

Amino Acid	Reference Diet	Untreated 25%	Untreated 50%	Untreated 75%	Untreated 100%
Proline	76.2	79.3	77.2	84.3	77.2
	(3.30)	(3.69)	(2.01)	(7.07)	(10.56)
Serine	71.9 [b]	78.4	78.3	85.8 [a]	82.4
	(4.29)	(2.86)	(1.38)	(6.57)	(5.39)
Threonine	75.3	80.3	78.7	83.0	79.1
	(2.12)	(1.49)	(1.91)	(8.90)	(6.57)
Tyrosine	65.4	72.3	72.3	75.9	65.9
	(5.05)	(3.98)	(2.73)	(8.29)	(8.84)
Valine	76.6	78.0	75.7	82.2	74.0
	(2.51)	(3.98)	(2.73)	(8.29)	(8.84)

[1] Values calculated on three replicates.

[2] Values calculated on two replicates.

Conversely, apparent digestibility of Ser in the group fed the control diet was lower than that in the group fed the diet containing untreated SBM incorporated at 75 % of the CP.

Results of the orthogonal contrasts between phytase treated and untreated diets at the same rate of SBM incorporation are displayed in Table 14. The only significant differences detected were between the groups fed the two diets containing SBM at 100 % of the CP. Alanine, aspartic acid, arginine, glycine , histidine, lysine, methionine, threonine, and valine digestibilities were all lower in the group receiving the phytase treated diet.

Experiment 6 – Phytic Acid Supplemented Fish Meal Digestibility Trial

Crude protein and individual amino acid digestibility coefficients were determined for tilapia fed fish meal based diets incorporating graded levels of phytic acid, as Na-phytate. The ACPD of the phytic acid supplemented diets ranged from 75.8 % for the diet supplemented with 25.8 g Na-phytate/kg dry diet to 87.5 % for the diet supplemented with 12.9 g Na-phytate/kg dry diet (Figure 18). There were no significant differences detected between the supplemented diets and the control diet, nor among the supplemented diets. The relatively low coefficient and large standard deviation for the treatment containing Na-phytate at 25.8 g/kg dry diet was due to one replicate with an exceptionally low digestibility coefficient.

The nitrogen value obtained from fish fed the N-free diet was 2.32±0.72 % N/g DM. This value was used to correct the ACPD to true crude protein digestibility. The coefficients ranged from 95.6 – 100.0 %. ANOVA indicated a significant difference between the two blocks in N recovered in the feces of the fish fed the N-free diet

Table 14. Orthogonal contrasts between the means of apparent amino acid digestibility coefficients in tilapia fed diets containing untreated, or phytase treated, solvent extracted SBM substituted into a reference diet at 25, 50, 75, or 100 % of the dietary protein. Significant differences are shown with resultant P values.

	SBM Substitution (%)			
Amino Acid	25 Vs 25	50 Vs 50	75 Vs 75	100 Vs 100
Indispensable				
Arginine	—	—	—	0.0260
Histidine	—	—	—	0.0500
Isoleucine	—	—	—	—
Leucine	—	—	—	—
Lysine	—	—	—	0.0054
Methionine	—	—	—	0.0112
Phenylalanine	—	—	—	—
Threonine	—	—	—	0.0275
Valine	—	—	—	0.0321
Dispensable				
Alanine	—	—	—	0.0285
Aspartic acid	—	—	—	0.0321
Cysteine	—	—	—	—
Glutamic acid	—	—	—	—
Glycine	—	—	—	0.0315
Proline	—	—	—	—
Serine	—	—	—	—
Tyrosine	—	—	—	—

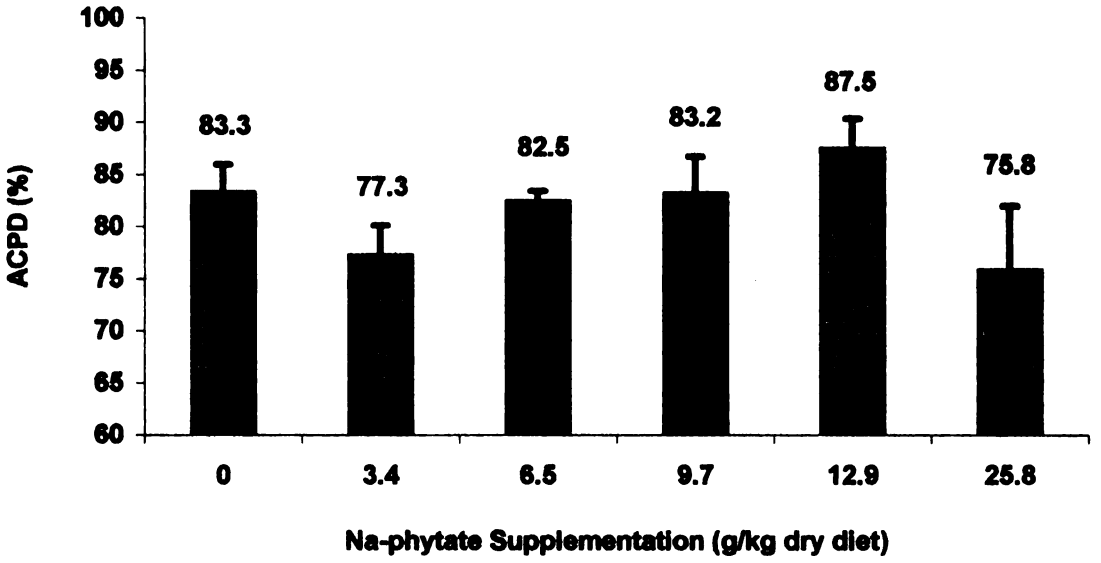

Figure 18. Apparent crude protein digestibility (ACPD) in juvenile tilapia fed fish meal based diets supplemented with graded levels of phytic acid as Na-phytate. Values and error bars represent the mean and SEM of four replicates of pooled samples (n=15).

($P<0.002$). In one block, the mean fecal N value was 2.96 ± 0.15 % N/g DM, and the other 1.68 ± 0.25 % N/g DM, indicating feces from one block may have been subject to contamination more than the other.

The apparent digestibility of individual amino acids is summarized in Table 15. Significant differences were detected among all the amino acid except Ala and Asp. The apparent digestibilities of Gly and Ser were significantly higher in the group fed the diet supplemented with Na-phytate at 12.9 g/kg dry diet than all the other treatments. Apparent digestibility of Ile was higher among this group than those receiving diets with Na-phytate at 3.4, 6.5, and 25.8 g/kg dry diet. Proline apparent digestibility was also higher in this group than those receiving the diet supplemented 25.8 g/kg dry diet. Other than the above exceptions, the apparent digestibility of amino acids was only higher in the group fed the diet supplemented with Na-phytate at 12.9 g/kg dry diet than the groups receiving diets with Na-phytate at 3.4 and 25.8 g/kg dry diet.

Experiment 7 – Tilapia Gastrointestinal Tract pH Profile

GI tracts in tilapia were sampled for pH following a 10 day preliminary period during which the fish were fed a standard commercial trout feed containing 41.0% CP, 12.0% lipid, and 4.0% fiber. Values for pH were recorded at 10 locations in the stomach, and 12 locations in the intestine. Measurements were made on the surface of recovered digesta from the stomach (Appendix 4); on the surface of the gastric mucosa (Appendix 5); and intestinal mucosa (Appendix 6) over an eight hour period following feeding. The pH values of the mucosa along the intestine were similar to those of the feed; however, this was not true of the gastric mucosa and feed recovered in the stomach (Figure 19).

Table 15. Apparent amino acid digestibility coefficients in juvenile tilapia fed fish meal based diets supplemented with graded levels of phytic acid as Na-phytate. Values represent the mean and (SEM) of four replicates of pooled samples (n=15). Values with different superscripts across a row are significantly different (P<0.05).

		Na-phytate Supplementation (g/kg dry diet)				
Amino Acid	Reference Diet	3.4	6.5	9.7	12.9	25.8
Alanine	81.7	75.1	79.8	80.2	86.6	74.8
	(3.09)	(4.38)	(2.32)	(3.98)	(3.74)	(5.56)
Aspartic acid	89.4	81.5	85.1	85.1	90.4	87.6
	(2.15)	(3.70)	(1.33)	(3.04)	(2.34)	(1.96)
Arginine	84.9 ^{ab}	78.4 ^{b}	83.0 ^{ab}	83.1 ^{ab}	89.1 ^{a}	79.3 ^{b}
	(1.74)	(1.30)	(0.68)	(2.23)	(1.96)	(2.87)
Cysteine	83.6 ^{ab}	74.9 ^{b}	81.4 ^{ab}	81.4 ^{ab}	87.9 ^{a}	79.9 ^{ab}
	(1.88)	(4.28)	(0.97)	(3.19)	(2.45)	(1.99)
Glutamic acid	86.3 ^{ab}	78.9 ^{b}	84.0 ^{ab}	83.9 ^{ab}	89.4 ^{a}	84.0 ^{ab}
	(1.91)	(3.14)	(0.88)	(2.81)	(2.39)	(1.44)
Glycine	81.9 ^{b}	78.1 ^{b}	81.0 ^{b}	81.1 ^{b}	90.1 ^{a}	77.8 ^{b}
	(2.46)	(3.46)	(1.39)	(2.90)	(4.00)	(3.20)

Table 15 (cont'd).

Amino Acid	Reference Diet	Na-phytate Supplementation (g/kg dry diet)				
		3.4	6.5	9.7	12.9	25.8
Histidine	81.0 ^{ab}	71.8 ^{b}	77.0 ^{ab}	79.1 ^{ab}	85.4 ^{a}	72.0 ^{b}
	(1.95)	(3.53)	(1.49)	(3.19)	(3.25)	(5.15)
Isoleucine	78.1 ^{ab}	68.8 ^{b}	74.3 ^{b}	76.1 ^{ab}	84.2 ^{a}	69.8 ^{b}
	(1.69)	(3.74)	(1.51)	(3.66)	(3.39)	(5.08)
Leucine	81.7 ^{ab}	73.8 ^{b}	78.4 ^{ab}	80.0 ^{ab}	86.4 ^{a}	75.0 ^{b}
	(1.76)	(3.18)	(1.41)	(3.24)	(3.03)	(4.17)
Lysine	90.6 ^{ab}	86.0 ^{b}	88.7 ^{ab}	88.9 ^{ab}	93.1 ^{a}	86.0 ^{b}
	(0.98)	(0.85)	(0.43)	(1.67)	(1.29)	(1.94)
Methionine	82.0 ^{ab}	72.3 ^{b}	78.7 ^{ab}	78.7 ^{ab}	86.7 ^{a}	73.6 ^{b}
	(1.42)	(4.36)	(1.82)	(3.36)	(2.79)	(4.86)
Phenylalanine	77.6 ^{ab}	67.7 ^{b}	73.5 ^{ab}	75.4 ^{ab}	83.4 ^{a}	68.8 ^{b}
	(1.65)	(3.90)	(1.65)	(3.84)	(3.65)	(5.77)

Table 15 (cont'd).

Amino Acid	Reference Diet	Na-phytate Supplementation (g/kg dry diet)				
		3.4	6.5	9.7	12.9	25.8
Proline	78.9 [ab]	74.2 [ab]	77.6 [ab]	77.6 [ab]	85.6 [a]	72.0 [b]
	(3.18)	(3.61)	(2.09)	(4.27)	(3.63)	(6.42)
Serine	80.1 [b]	73. [b]	78.1 [b]	79.4 [b]	86.1 [a]	7.0 [b]
	(1.83)	(2.67)	(0.46)	(2.80)	(2.42)	(2.37)
Threonine	80.6 [ab]	73.6 [b]	77.9 [ab]	79.8 [ab]	86.0 [a]	76.4 [b]
	(2.68)	(2.95)	(0.64)	(3.42)	(2.62)	(3.09)
Tyrosine	68.6 [ab]	54.7 [b]	63.9 [ab]	67.0 [ab]	76.6 [a]	58.1 [b]
	(1.39)	(5.67)	(2.42)	(4.29)	(4.89)	(5.97)
Valine	79.6 [ab]	71.2 [b]	76.4 [ab]	77.9 [ab]	85.0 [a]	71.5 [b]
	(1.92)	(3.79)	(1.51)	(3.49)	(2.99)	(4.76)

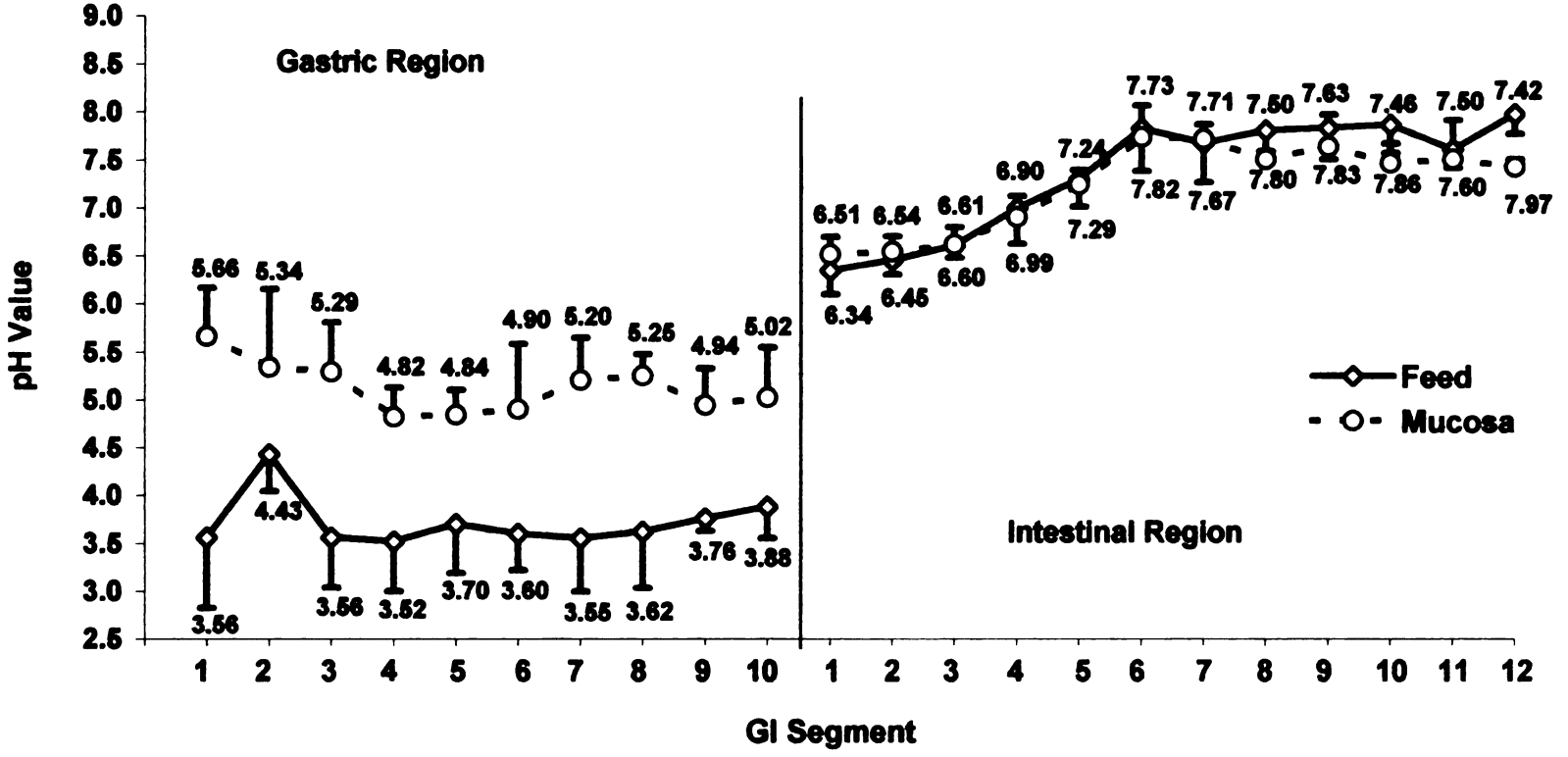

Figure 19. Mean pH values recorded at 10 locations in the stomach (gastric region), and 12 locations in the intestine (intestinal region) two hours following an early morning feeding. Measurements were made on the surface of the mucosa and on the surface of recovered digesta (feed). Error bars represent the standard deviation of 5 samples, except gastric segment 1 and 2 which represent 2, and 3 samples, respectively.

The mean pH at each of the 10 sites measured on the feed was significantly lower than the means of the mucosal pH measurements at 2, 4, and 8 hours postprandially ($P<0.001$).

At 0.5 hr following feeding there were no regional differences in pH values measured on the surface of the digesta. In subsequent samplings, regional differences did exist. Generally, the differences observed were between one or more sites in the fundic region, and the site labeled site 9 (Figure 1).

Statistical analysis indicated pH of food recovered from the esophageal, and pyloric regions, at 2 and 4 hours following feeding were significantly higher than in the gastric region ($P<0.05$). The differences were most pronounced relative to the fundic region. Range and mean pH values of the stomach digesta and gastric mucosa are reported in Table 16. There was a significant decrease in pH of the digesta between 1 and 2 hours following feeding ($P<0.05$). The pH remained low until it began to increase again at 6 hours following feeding, after which it again decreased. The pH of the gastric mucosa was significantly lower as quickly as 0.5 hours following feeding (Table 16). The decrease in pH of the mucosa closely paralleled that of the digesta; however, the decrease was not as pronounced as seen in the digesta.

After measuring pH on the surface of the feed, digesta was collected and analyzed for pH following homogenization. There was little change in the pH of the homogenized digesta samples. After one hour of digestion, pH measurements taken at the surface of the digesta were considerably lower than the homogenized samples (Figure 20).

Table 16. Range and mean pH values from feed in the stomach and gastric mucosa in *O. niloticus* before feeding, and 0.5, 1, 2, 4, 6, and 8 hours post-prandially. Values in parentheses represent the standard deviation of eight values collected from five fish at each sampling period. Values within a column with different superscripts are significantly different ($p<0.0001$).

Post-prandial Time (hrs)	Range of Feed Samples	Mean of Feed Samples	Range of Mucosa Samples	Mean of Mucosa Samples
Before feeding	N/A	N/A	5.60-7.28	6.66 (0.13) [a]
0.5	4.62-6.33	5.36 (0.17) [a]	5.22-7.22	6.12 (0.16) [b]
1.0	3.96-7.30	5.22 (0.45) [a]	5.66-8.33	6.92 (0.19) [a]
2.0	2.75-4.68	3.65 (0.13) [c]	4.37-5.85	5.03 (0.19) [d]
4.0	2.84-4.42	3.52 (0.34) [c]	4.11-6.31	4.94 (0.15) [d]
6.0	2.35-5.29	4.22 (0.32) [b]	4.25-6.71	5.59 (0.31) [c]
8.0	2.72-4.54	3.62 (0.19) [c]	3.19-6.28	4.77 (0.21) [d]

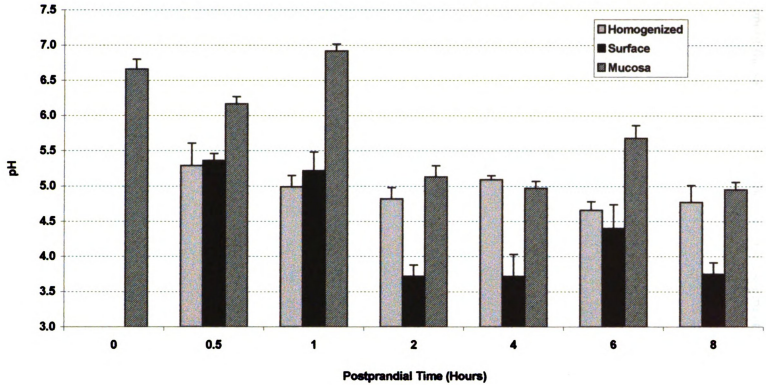

Figure 20. Mean pH values measured on the gastric mucosa, surface of digesta collected from the stomach, and homogenized digesta collected from the stomach before feeding, and 0.5, 1, 2, 4, 6, and 8 hours postprandially. Error bars represent the SEM of five fish collected at each sampling period.

The mean pH values measured in each of the 12 segments in the intestine are presented in Appendix 6. The pH values in the intestine remained relatively consistent throughout the sampling period, both within a segment, and with time.

DISCUSSION

Biological availability of nutrients in fish is dependent on a number of factors. Some factors affecting availability to a given species can be categorized as biological (requirements, size, age, and physiological state), dietary (quality and quantity of protein, energy, and processing of components), environmental (temperature, DO, water quality, and photoperiod), and management (stress, feed intake, and feeding frequency).

Fish culture has historically emphasized maximizing intake and growth, which may not be the most effective approach for fish production (Seymour, 1989; Kaushik 1990). This approach often leads to uneaten feed and lower conversion efficiency, which reduces water quality. This is particularly problematic in recirculating systems where low water exchange occurs. Reduced water quality adversely effects the system, and diminishes organism health and performance (Spotte 1970; Goddard 1995; Saddiqui and Al-Harbi 1999).

The general maxim in regards to feeding strategies for tilapia has been "little and often" (Jauncey and Ross 1982). This principle is grounded in early work done on captured wild fish. Based on feeding behavior, physiology, and GI morphology of wild tilapia, it was reported they require many, frequent meals to achieve greatest efficiency (Moriarity 1973; Moriarity and Moriarity 1973a,b; Balarin and Hatton 1979; Kubaryk 1980; Caulton 1982). In general, fish that eat small particles on a continuous basis have small stomachs (Smith 1989). However, tilapia stomachs have the ability to distend to a large size and function as a storage unit (Fish 1951).

Misconceptions from this early work still persist in regards to the structure, function, and relevancy of the tilapia stomach (Avault 1996). However, a review of the literature indicates there is a sufficient collection of evidence to refute these misconceptions (Al-Hussaini and Kholy 1953; Fish 1960; Moriarity 1973; Kapoor et al. 1975; Bowen 1976; Bowen 1982; Smith 1989; Boujard and Leatherland 1992; Yamada et al 1993).

Wild *O. niloticus* preferentially graze on blue-green algae and bacteria (Bowen 1982). Filtering of algae is energetically costly. Wild tilapia exhibit a higher specific dynamic activity associated with the seeking and processing of food relative to farm raised fish. Therefore wild fish must consume more food to cover this energetic cost (Gerking 1994).

Blue green algae and bacteria contain about 50 % CP; however, assimilation efficiencies of this material is low in tilapia. Assimilation efficiencies range from 50 % to <1 %, with values near 15 % common (Bowen 1982).

Fish reared in intensive production systems have different requirements than those in the wild. In such systems, natural food is limited. All nutrients must be exogenously supplied in the form of high nutrient dense pellets.

Omnivorous species, such as tilapia, are readily trained to eat these nutrient dense diets. The higher quality and consistency of pelleted diets should obviate the need for frequent feedings. Consequently, there is a need for optimal feeding regimens to accommodate this capacity to process formulated diets. The feeding regimen should include an optimum ration, delivered at a rate and frequency that maximizes efficiency.

Greatest growth rate is realized at the maximum ration, but the optimal ration, defined by the highest utilization efficiency, is at a submaximal level. From a practical and economic standpoint, satiation feeding is the best method for both the fish, and the production unit (Tidwell et al. 1991). With satiation feeding, the optimal ration will be defined by the feeding frequency that yields the greatest utilization efficiency. To determine this frequency, tilapia were fed to satiation 1, 2, 3, or 5 times day $^{-1}$ to evaluate total consumption and utilization efficiency.

Mean daily intake among fish fed 3 and 5 meals day $^{-1}$ was similar to the suggested feeding rate for the size of fish and temperature used in this experiment (Jauncey and Ross 1982; NRC 1993). The MDI approached an asymptote at 3 feedings day $^{-1}$, which was verified by broken-line analysis (3.18 feedings day $^{-1}$). A similar response was observed at 4 feedings day $^{-1}$ in 5 g Nile tilapia fed to satiation 1, 2, 4, or 8 times day $^{-1}$ (Kubaryk 1980). An asymptotic response has also been described for sea bass fingerlings (Tsevis et al. 1992), rainbow trout (Grayton and Beamish 1977; Bergot 1979), channel catfish (Andrews and Page 1975), African catfish (Singh and Srivastava 1984), European eels (Seymour 1989), rockfish (Kono and Nose 1971), and filefish, puffer fish, and yellow tail (Ishiwata 1969 a;b). The response is temperature dependent, with lower temperatures resulting in lower optimal frequencies (Seymour 1989).

Fish fed once a day consumed more feed during the morning feeding than fish fed more than once a day (Figure 5). This was also demonstrated in striped bass (Powell 1972), channel catfish (Andrews and Page 1975), African catfish (Singh and Srivastava 1984), and in filefish, puffer fish and yellowtail (Ishiwata 1969 a,b). Similarly, increasing intervals between feedings led to increased meal intake in winter flounder

(Tyler and Dunn 1976), sockeye salmon (Brett 1971), and rainbow trout (Grove et al. 1978). Fish eat available food depending on stomach fullness, and at intervals determined by the rate of gastric emptying (Holmgren et al. 1983).

Increased feeding frequencies can decrease aggressive social behavior in some species, resulting in increased growth rate and reduced size variation (Jobling 1994). This has been demonstrated in rainbow trout (Grayton and Beamish 1977; Holm et al. 1990), and eels (Seymour 1989). However, there is a limit to the frequency of feeding beyond which an increase in growth is negligible (Ishiwata 1969; Bergot 1979; Tsevis et al. 1992). In the hybrid tilapia (*O. mossambicus* x *O. niloticus*), growth was not significantly different with increasing frequency beyond twice a day (Siraj et al. 1988).

Growth over the four week period in the current study was slightly higher in fish fed 3 meals day $^{-1}$, although not statistically different from the other groups fed more than once a day (Figure 6). Consumption was also not statistically different. These results are similar to those observed in rainbow trout (Grayton and Beamish 1977), sea bass (Tsevis et al. 1992), and winter flounder (Tyler and Dunn 1976), where growth closely paralleled intake.

Kubaryk (1980), also working with Nile tilapia found significant differences in growth among all groups except those fed the two highest frequencies, 4 and 8 times day $^{-1}$. The diets used in Kubaryk's study were similar to those used in our study. However, the nutrient density was diluted by adding an additional 33.6 % water before feeding (Kubaryk 1980). Total consumption was significantly higher in fish fed 4 times day $^{-1}$ than fish fed twice day $^{-1}$. However, it was not reported if consumption was on an as fed basis, or on a dry matter basis, making direct comparisons difficult.

Although fish eat to meet their energy requirements, there is a physical limit to the amount of bulk that can be consumed (Boujard and Leatherland 1992). Kubaryk (1980) suggested bulk limitations may have limited consumption in fish fed the lower frequencies.

Additionally, the fish evaluated by Kubaryk (1980) were smaller (4.8 g) than the fish used in our study (34.5 g). Smaller fish have a higher requirement for protein and energy. Fish will increase their feeding frequency to maintain a constant energy intake when diets are diluted (Jobling 1980). Fish fed at the lower frequencies may not have been able to obtain sufficient protein and energy to maximize growth.

Kubaryk (1980) evaluated fish fed at 2 and 4 feedings day $^{-1}$. If 3 feedings day $^{-1}$ had been evaluated it may have been the optimal feeding frequency. This assumption is supported by plotting the data from their study; both consumption and growth plots indicated an asymptote would have been reached at 3 feedings day $^{-1}$.

The fish fed 3 meals day $^{-1}$ in the current study contained the highest level of GE. This was due to higher whole body lipid levels since these fish also contained the lowest whole body CP. No differences were detected in proximate components in rainbow trout fed different feeding frequencies (Bergot 1979). However, the analysis of proximate components was performed on carcass instead of whole body. Feeding frequency differences did exist in the liver and viscera; therefore, a whole body analysis may have resulted in significant differences.

Grayton and Beamish (1977) also working with rainbow trout evaluated whole body composition. The patterns in moisture and lipid were similar to those observed in our study. The differences they detected were not statistically significant. The

researchers suggested feeding frequency may not have a direct effect on body composition (Grayton and Beamish 1977). However, rainbow trout generally grow slower than tilapia, and the inability to detect significant differences may have been due to the short term of their study.

Specific growth rate and FE were lower in fish fed once day $^{-1}$ than the other feeding frequencies evaluated in this study. This was also observed in red tilapia (Siraj et al. 1988) and eels (Seymour 1989). Conversely, SGR and FE were not significantly different among rainbow trout fed one meal day $^{-1}$ to satiation, or multiple meals day $^{-1}$ to satiation (Grayton and Beamish 1977). Unlike tilapia and eels, rainbow trout consumed as much in one feeding as in multiple feedings and this was reflected in growth and efficiency.

Energy retention in fish fed 3 meals day $^{-1}$ was high, and significantly greater than in fish fed 5 meals day $^{-1}$. There was a drop in efficiency parameters in fish fed 5 meals day $^{-1}$ compared to those fed 2 or 3 meals day $^{-1}$. A decreasing trend in efficiency with increasing feeding frequency beyond optimal was also observed in catfish (Singh and Srivastava 1984), and rainbow trout (Grayton and Beamish 1977). In comparison, a plateau in efficiency, but not a decrease, was observed in red tilapia (Siraj et al. 1988) and sea bass (Tsevis et al. 1992) fed feeding frequencies deemed to be beyond optimal.

Ofojekwu and Ejike (1984) proposed an optimal ration and feeding regimen should take into account a combination of the interplay between feed conversion ratio (FCR) and SGR. Feed efficiency is the inverse of FCR and can be used for determining the optimal feeding frequency in a similar manner. Evaluating these parameters for fish fed in the present study suggests the optimal feeding frequency for both parameters is

either three or four feedings day $^{-1}$ (Figure 21). This is similar to the general recommendation of 4 – 5 times day $^{-1}$ for fingerlings, and 2 – 3 times day $^{-1}$ for adult (> 100 g) tilapia (Jauncey and Ross 1982; NRC 1993), and 4 times day $^{-1}$ for Nile tilapia (Kubaryk 1980). However, this is more than the 2 times day $^{-1}$ recommended for red tilapia (Siraj et al. 1988) suggesting there may be species differences among the tilapia.

The determination of an optimum feeding regimen should be evaluated from two aspects; 1) physiology of the species, and 2) economics of the aquaculture production unit (Tsevis et al. 1992). The economics of the production unit would suggest 3 feedings day $^{-1}$ is superior due to the cost of labor. Although significant differences could not be detected, the efficiency parameters may still have biological significance. In a recirculating system where nutrients accumulate over time, ER and ANPU should warrant as much consideration as growth.

Caution must be exercised in interpreting the results of this study. The data suggest 3 feedings day $^{-1}$ is optimal for *O. niloticus*, but it should be noted the feedings were given at 4 - 5 hour intervals during the course of the day. The interval between feedings may be a more important determinant than the total number of feedings. Tsevis et al. (1992) suggested the interval between feedings was responsible for differences observed in sea bass consumption, growth, and performance. A management strategy utilizing a longer daily feeding period, particularly during the summer, may benefit from additional feedings if spaced at 4 hour intervals. The optimal interval between feedings will vary with factors influencing the return of appetite (Gwyther and Grove 1981), and is related to the capacity of the stomach and the rate of digestion (Brett 1971; Seymour 1989).

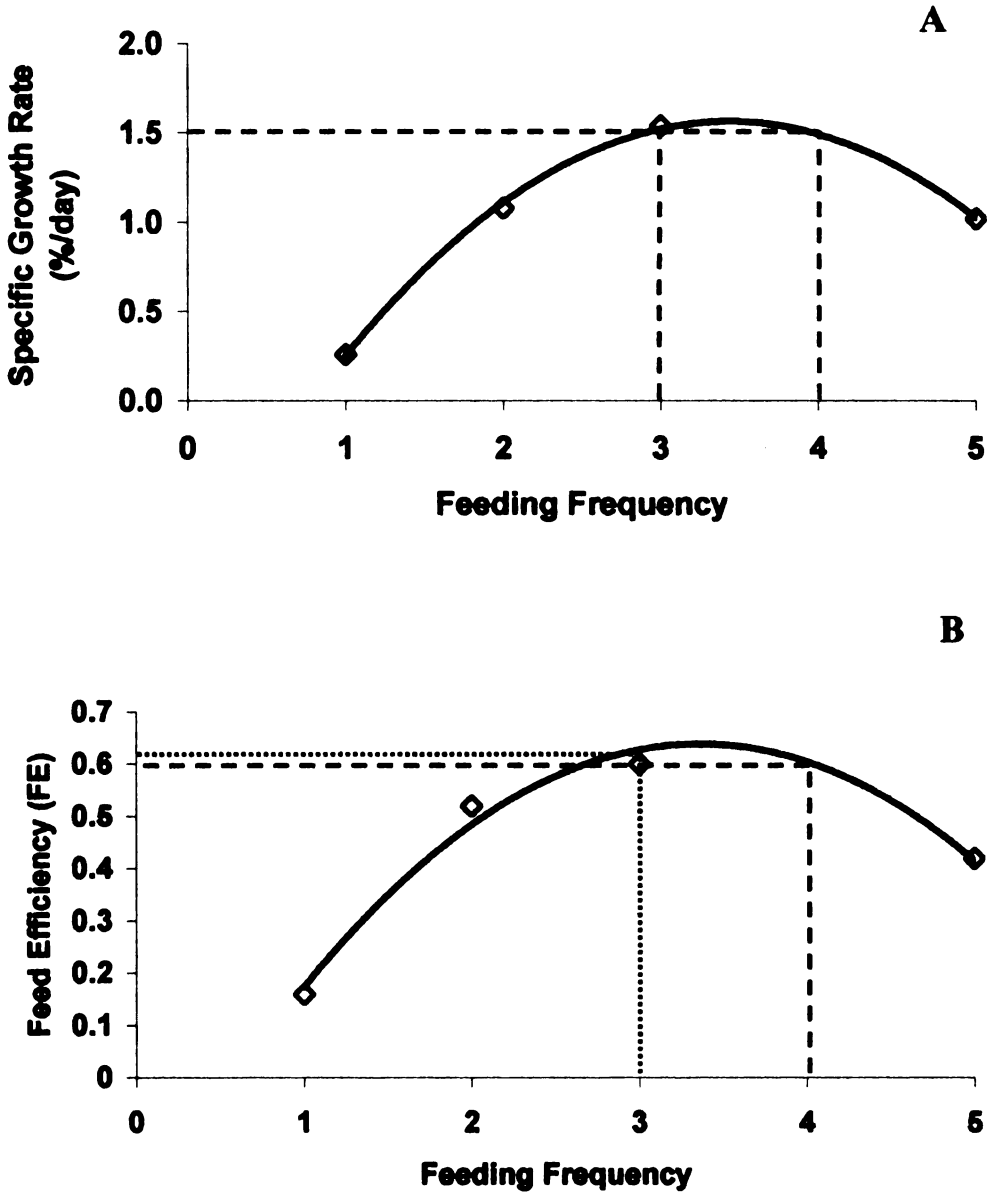

Figure 21. (**A**) Specific growth rate (SGR), and (**B**) feed efficiency (FE) as a function of feeding frequency in *O. niloticus* fed to satiation 1, 2, 3, or 5 times day $^{-1}$. The relationship for SGR is described as SGR = - $0.2209x^2 + 1.5187x - 1.0476$ ($R^2 = 0.999$), and for FE as FE = - $0.0832x^2 + 0.5595x - 0.3027$ ($R^2 = 0.979$).

Understanding rate of digestion and its relationship to GER is important for determining the return of appetite. Making food available as soon as appetite has returned can maximize intake, and increase efficiency. Gastric evacuation rate is a function of temperature, fish weight, meal size, dietary energy level and composition, and feeding frequency (Windell et al. 1969; Grove et al. 1978; Flowerdew and Grove 1979; Grove and Crawford 1980; Jobling 1980; Hofer and Schiemer 1981; Holmgren et al. 1983). Demonstrating a consistent relationship between stomach fullness and appetite will allow an optimal feeding interval to be predicted from factors that control GER.

A number of mathematical models have been developed to describe GER, time for total gastric evacuation (GET), and prediction of consumption. There are two principal groups of models; volume dependent and surface-area dependent. The two are similar in describing the relationship between food left in the stomach and time. Both models describe a curvilinear relationship, but differ in that they are based on different assumptions.

Volume dependent models (VDM) are based on volume of feed ingested as it effects distension of the stomach, and modifies the rate at which food is evacuated (Jobling 1981). Volume dependent models describe a relationship where the larger the original volume, the greater is the initial rate of emptying. Gastric evacuation curves are linearized by square root transformation of stomach residuum and plotted against postprandial time. The rationale is that distension of the stomach initiates peristaltic contraction, and the circumferential tension is proportional to the radius, therefore being proportional to the square root of the volume of the residuum (Jobling 1981).

Surface-area dependent models (SDM) are based on the assumption that the surface area of the food influences digestion and evacuation. Gastric evacuation curves are linearized by an exponential transformation of stomach residuum and plotted against postprandial time. The rationale being digestive enzymes attack the outer surface of the food, therefore the rate of digestion is proportional to the particle surface area (Jobling 1981). The rate of evacuation is predicted to be higher for a meal consisting of small food particles than large particles, for a given size meal.

Plotting gastric evacuation curves for the data obtained in this study indicate curvilinear relationships (Figure 7). The data from these gastric evacuation curves were subjected to both square root transformation, and natural log transformation. The transformed data were plotted against postprandial time, and the two models subjected to least squares estimates of the residuals. Both feeding frequencies were best described by the exponential function $V_T = V_0 e^{-b(x)}$. The slopes described by the term b represent the instantaneous rate of gastric evacuation (Figure 22). The instantaneous rate for the two feeding frequencies in this study were nearly identical at 0.153 (3 meals day $^{-1}$) and 0.149 (5 meals day $^{-1}$), and were not different from each other.

The accuracy of the model is dependent on two assumptions. The first assumption was the Fe_2O_3 marker was evacuated from the stomach at the same rate as the rest of the meal. The second assumption was 95 % evacuation is the practical limit to which GER and GET can be applied in a culture setting. Since there is no end-point on a semilogarithmic plot, a practical limit must be applied (Grove and Crawford 1980). The information of value to a culturist is not what point the stomach contains zero residuum,

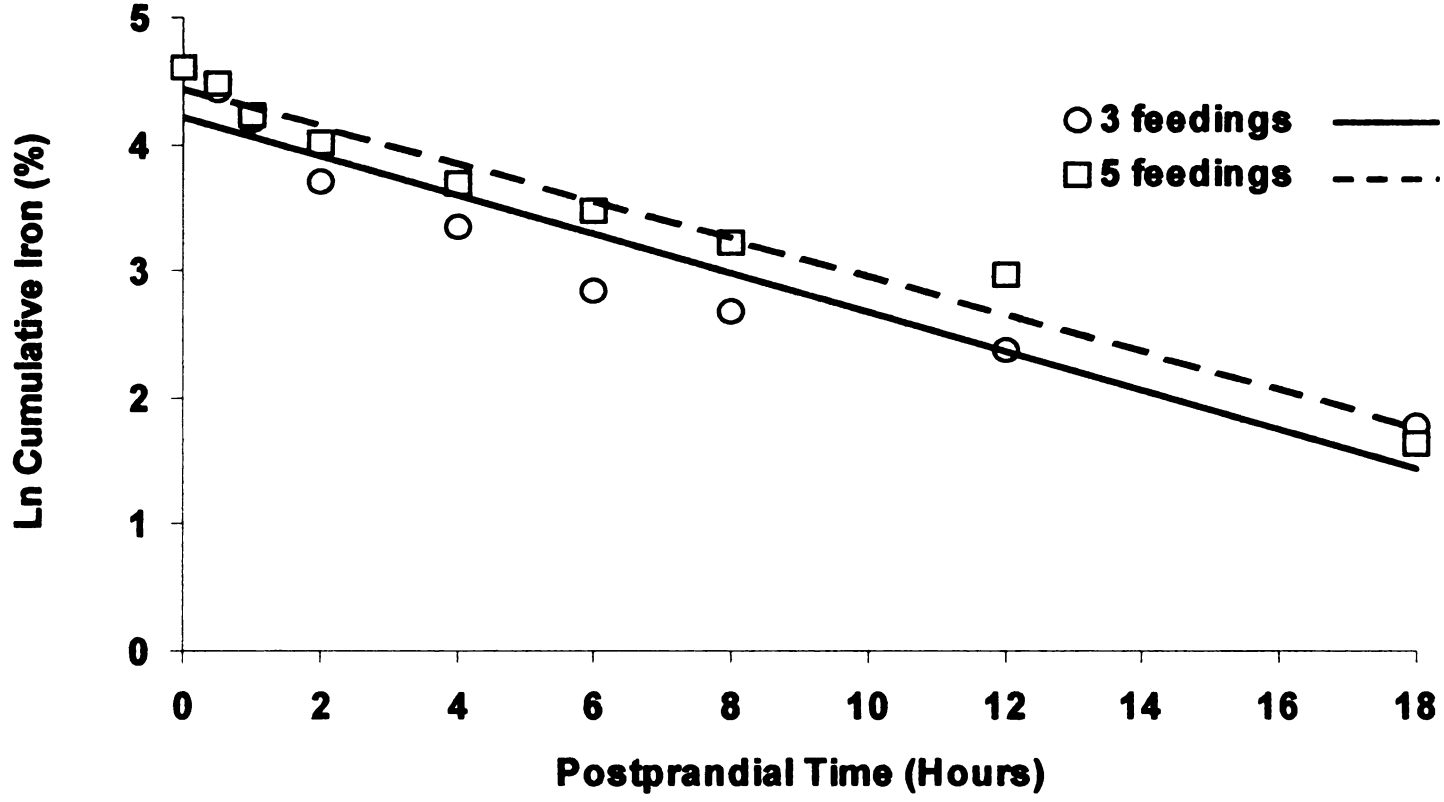

Figure 22. Semilogarithmic plot of cumulative iron collected from *O. niloticus* fed an experimental diet containing Fe_2O_3 as an external marker at time 0, against postprandial time. Fish were fed either 3 feedings day $^{-1}$ to satiation (8:00, 12:00, and 17:00), or 5 feedings day $^{-1}$ to satiation (8:00, 10:00, 12:00, 15:00, and 17:00). Cumulative iron is relative to total iron collected 24 hrs following feeding. The lines describing the relationships are $y = -0.153x + 4.205$, R^2=0.90 (3 feedings day $^{-1}$); and $y = -0.149x + 4.443$, R^2=0.97 (5 feedings day $^{-1}$). The slopes 0.153 and 0.149 represent the instantaneous rates of evacuation.

but at what point appetite returns. Therefore, 95 % evacuation seems a reasonable point to estimate the practical time at which the gastric residuum is brought to zero.

Fish were the same size (weight), and the variables diet (quality and energy), temperature, amount to satiation, and particle size were constant between the two treatments. The only factor that was altered was the frequency at which the two treatments were fed. The instantaneous rate of evacuation would be expected to be the same since consumption at first feeding was the same.

The two curves are offset by the difference in the constant V_0, which represents the maximum volume at time 0. The model suggests fish fed 5 feedings day $^{-1}$ had a higher maximum volume. However, maximum stomach volume is proportional to weight, and when filled, the stomach stretches isometrically (Flowerdew and Grove 1979; Jobling 1980; Holmgren et al. 1983). Since fish between the two treatments were the same weight, maximum volume would be predicted to be the same. The likely explanation for this incongruity is that fish fed 5 feedings day $^{-1}$ were fed a subsequent meal 2 hours following the first meal. This corresponds to where the two gastric evacuation curves begin to deviate. The net effect was to fill the stomach again and shift the gastric evacuation curve.

The curvilinear relationship would predict a faster evacuation of the gastric contents with multiple feedings. The rate of evacuation will be faster with greater food volume in the stomach. This was observed in dab (Fletcher et al. 1984), and catfish (Andrews and Page 1975). However, this was the opposite observed in bluegill (El-Shamy 1976), as it was in our study. The rate of evacuation appeared to slow for the treatment receiving 5 meals day $^{-1}$ (Figure 7).

In some species, food ingested leaves the stomach on a first in – first out basis (Fletcher et al. 1984). This does not appear to be the case with tilapia. Tilapia possess the ability to by-pass the fundic region of the stomach; food passing directly from the esophagus to the pylorus and into the intestine (Moriarity 1973). Ingested food retained in the stomach passes down the ventral side and accumulates in the fundic region. At this point it comes in contact with acid-secreting cells localized to the ventral face of the mucosa (Moriarity 1973). Ingested food by-passing the stomach passes to the intestine without the benefit of initial hydrolysis and mixing.

Assuming gastric evacuation is incomplete two hours postprandially, it was conceivable some of the newly ingested food given to fish receiving 5 feedings day $^{-1}$ would pass to the intestine without the benefit of initial hydrolysis. In this context, two scenarios can be envisioned. The first scenario would be, food passing directly to the intestine undergoes inefficient digestion resulting in lower utilization efficiency at the same level of consumption. This was the indication in the feeding frequency study where fish fed 3 meals day $^{-1}$ consumed as much as those fed 5 meals day $^{-1}$, but were more efficient in converting nutrients. Gwyther and Grove (1981) determined there is a significant positive correlation between feeding frequency and meal size with stomach emptying time. Gastric evacuation rate increases with more frequent feedings leading to decreased digestion efficiency (Brett et al 1969; Powell 1972; Tsevis et al. 1992). There are a number of reports of less efficient digestion and utilization in species fed at short intervals (Tsevis et al. 1992).

The second scenario would be, feed from the initial feeding is unable to pass to the intestine because of the newly ingested feed. In this case it would appear as though

the feed were being evacuated more slowly because the initial feed is unable to pass to the intestine. The feed ingested two hours postprandially is not following the first in – first out dictum. It would follow then, that a greater portion of ingested Fe_2O_3 would remain in the stomach. This was the indication, with a greater percent of ingested iron leaving the stomach and appearing at the terminus in the group fed 3 meals day $^{-1}$, following the feeding at two hours postprandially. Additionally, fish fed 5 meals day $^{-1}$ were observed to have residual Fe_2O_3 in the stomach at 24 hours, where those fed 3 meals day $^{-1}$ did not.

Grove et al. (1978) estimated that it requires 15 hours for rainbow trout to evacuate a 1 % BW meal. According to the evacuation curve used, it was estimated 80 – 90 % evacuation would require 6 hours, which they felt corresponded to the return of appetite (Grove et al. 1978). The evacuation curves constructed from the data collected in our study would predict 8 hours are required to attain 80 % evacuation.

Fish should be fed when appetite has returned, and not before (Grove et al. 1978). Fish receiving meals at 2, 3, 4, or 5 hour intervals are predicted to have evacuated 27, 36, 64, and 70 % of their initial meal to satiation, respectively. Conversely, during their ensuing meal they consumed the equivalent of 52, 58, 73, and 69 % of their original meal, respectively, to once again reach satiation (Table 17). Fish being fed at intervals of 4 – 5 hours appear to be consuming as much as they have evacuated. This would imply fish receiving meals at 2 – 3 hour intervals are evacuating more quickly than predicted, or feed is being fed before it can be efficiently utilized.

Seymour (1989) suggested the optimal feeding frequency in eels is one in which the feeding interval corresponds to the volume and rate of emptying of the stomach.

Table 17. Hours between feedings, predicted gastric evacuation (%) from evacuation curves, and consumption at the ensuing meal as a percent of satiation.

3 Feedings

Hours Between Meals	Predicted Gastric Evacuation(%)	Consumption (% Satiation)
4	64	73
5	70	69

5 Feedings

Hours Between Meals	Predicted Gastric Evacuation(%)	Consumption (% Satiation)
2	27	53
2	27	49
3	36	58
2	27	55

Assuming the optimum interval between feedings is the point at which evacuation of the previous meal is matched by consumption, then the optimum interval between feedings for *O. niloticus* appears to be 4 – 5 hours, depending on the energy and composition of the diet. This is slightly longer than the previously reported food passage rate of 2.5 – 3.0 hrs at 30°C in fish feeding on phytoplankton (Hargreaves et al. 1988).

It was determined from the growth and efficiency parameters evaluated in the first preliminary trial that 3 feedings day $^{-1}$ leads to optimum growth and efficiency in tilapia when fed over a 12 hour day. Additionally, the 4 – 5 hour interval implied by this feeding strategy corresponds to return of appetite in tilapia. Therefore, this feeding management strategy was employed during all subsequent studies evaluating the ANF phytic acid.

There are a number of ANFs in SBM reported to decrease growth and efficiency in fish. These include proteinase inhibitors (Krogdahl et al. 1994; Olli et al. 1994), antigens (Kaushik et al. 1995; Rumsey et al. 1995), alcohol soluble components (Arnesen et al. 1989; Olli and Krogdahl 1995), lectin and agglutinin (Hendricks et al. 1990), oligosaccharides (Rumsey et al. 1995), and phytic acid (Spinelli et al. 1983).

Effects of phytic acid on mineral availability in fish are well known. They are documented in rainbow trout (Spinelli et al. 1983; Cain and Garling 1995; Riche and Brown 1996; Ramseyer et al. 1999), channel catfish (Satoh et al. 1989), carp (Hossain and Jauncey 1993), and tilapia (McClain and Gatlin 1988). Decreased protein digestibility of diets supplemented with salts of phytic acid led to depressed growth and poor performance in rainbow trout (Spinelli et al. 1983), Chinook salmon (Richardson et al. 1985), and carp (Hossain and Jauncey 1993). Cain and Garling (1995) found rainbow

trout fed diets pretreated with phytase, to hydrolyze phytic acid, exhibited superior growth relative to diets without pretreatment. Increased performance was attributed to improved protein quality, as has been demonstrated in terrestrial animals (Atwal et al. 1980; Satterlee and Abdul-Kadir 1983; Mroz et al. 1994; Martin et al. 1998; Sebastian et al. 1997). In this investigation, tilapia were fed graded levels of phytase treated SBM, or untreated SBM, substituted into a FM based diet to determine the effects of phytic acid on growth, performance, and digestibility of CP and individual amino acids.

The overall growth and SGR during this study was slightly better than those reported for similar size tilapia, fed similar diets (Wee and Shu 1989). Weight gain among fish fed diets containing SBM exhibited a distinct trend. There was a lower percent gain with increasing SBM inclusion, regardless of treatment. Fish fed diets substituting phytase treated SBM, or untreated SBM, at 25 % of the CP, resulted in higher growth than the FM control, although the differences were not significant. The same outcome was observed in red drum (Reigh and Ellis1992), and Mozambique tilapia (Jackson et al. 1982).

A small inclusion of plant protein in tilapia diets results in increased growth and efficiency. A 25 % incorporation of the legume *Phaseolus aureus* (DeSilva and Gunasekera 1989) resulted in increased growth. However, decreased performance occurred at the 50 % incorporation rate. The researchers concluded *Phaseolus aureus* could be substituted up to 37 % of the dry diet without deleterious effects. Substitution of copra, groundnut, SBM, and rapeseed, at 25 % of the CP increased weight gain and performance of Mozambique tilapia over a FM diet (Jackson et al. 1982). Increased

growth and performance was attributed to improved IAA patterns with plant protein incorporation at 25 % of the CP (Dabrowski et al. 1989).

There was a linear decrease in weight gain, relative to the control, with inclusion of SBM beyond 25 % of the CP. Correlation coefficients for this trend were 0.94 and 0.74 for the untreated, and phytase treated diets, respectively. However, weight gain relative to the control diet was not significantly depressed until SBM was substituted at 100 % CP.

A large depression in growth occurred in fish fed the phytase treated diet incorporating SBM at 50 % of the CP relative to those fed phytase treated SBM at 25 % and 75 % substitution. It is unclear why this occurred. However, Jackson et al. (1982) reported similar findings at the same level of substitution suggesting these observations may have biological relevance and are not spurious.

Significant differences in growth and performance were observed in tilapia fed diets of varying CP (15 – 36 %), with graded levels of SBM up to 100 % substitution for FM (Davis and Stickney 1978). The differences occurred at the lower protein levels, but not the higher protein levels, and only among the diets with 100 % CP as SBM. However, the diets used in their study contained considerably more energy and Met than those used in the current study. Methionine levels in the diets used by Davis and Stickney (1978) were supplemented to match the level in their 36 % CP FM diet. It is possible at the lower CP levels some other IAA, such as Lys, became limiting.

In contrast, tilapia fed a low protein diet (24 % CP) substituting 33, 67, and 100 % of the FM with SBM only showed significant differences relative to the FM control diet when the diet contained SBM as 100 % of the CP (Shiau et al. 1989). It was suggested

the decreased performance was due to a deficiency in IAA requirements other than Met (Shiau et al. 1989). The differences in growth, FE, and PER occurred with, or without Met supplementation (Shiau et al. 1989). The researchers concluded SBM could replace up to 67 % of the FM in a 24 % CP diet. This level of substitution was equivalent to 37 % of the dry diet, which was similar to the 50 % CP substitution in the current study.

Davies et al. (1989) reported they could substitute SBM or soy protein concentrate (SPC) up to 75 % of the FM in their tilapia diets (50 % dietary CP) without any detriment to growth or performance. They observed better performance with SPC and attributed it to higher energy retention. The same has been reported for rainbow trout (Tacon et al. 1983). Incorporation of SPC in place of SBM was also reported to reduce antigenic effects associated with the globular proteins glycinin, and beta-conglycinin (Rumsey et al. 1995).

The growth data was used to fit two different dose-response curves (Jobling 1994) to estimate maximum level of incorporation of phytase treated and untreated SBM into diets for tilapia. The first curve was fitted using the two slope broken-line method (Robbins 1986). This model suggests SBM should be restricted to 31 % and 14 % of the CP for untreated, and phytase treated SBM, respectively (Figure 23). The second curve was fitted using a quadratic equation, where 95 % of the maximum response details maximum rate of incorporation (Lanari et al. 1998). This model suggests maximum rates of 38 % and 17 % of the CP for untreated and phytase treated SBM, respectively (Figure 24).

The values from these models represented 20 – 25 % of the diet on a DM basis for untreated SBM, which was similar to suggested limits for tilapia (Jackson et al. 1982;

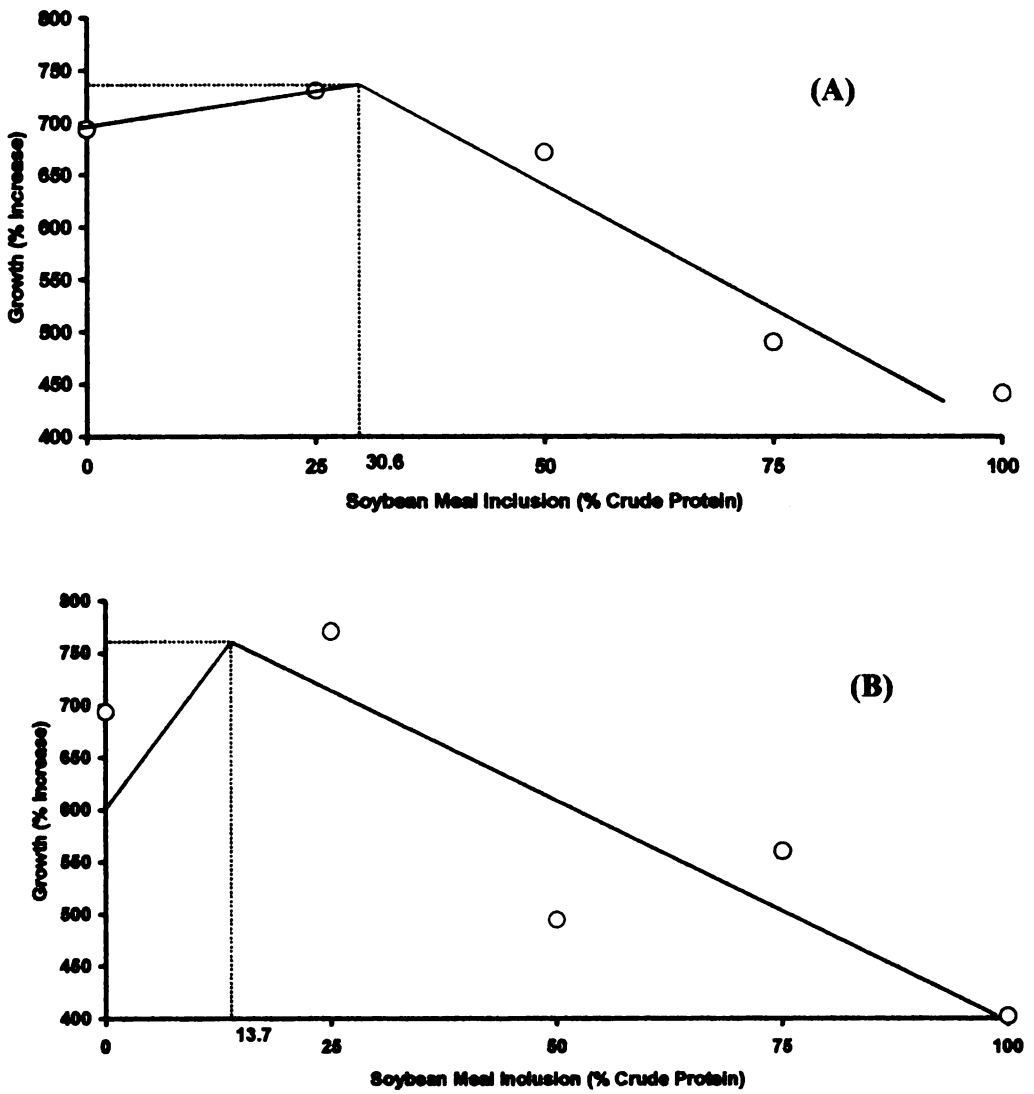

Figure 23. Growth data (% increase) of juvenile tilapia fed graded levels of (A) untreated solvent extracted SBM, and (B) phytase treated solvent extracted SBM, as a percent of the crude protein. Data were fitted with a two-slope broken line model utilizing a non-linear procedure as described in methods. Dotted lines indicate model parameter estimates. Diminishing returns and lower growth are predicted with greater than 30.6 % of the CP as untreated solvent extracted SBM (A), and 13.7 % of the CP as phytase treated solvent extracted SBM (B).

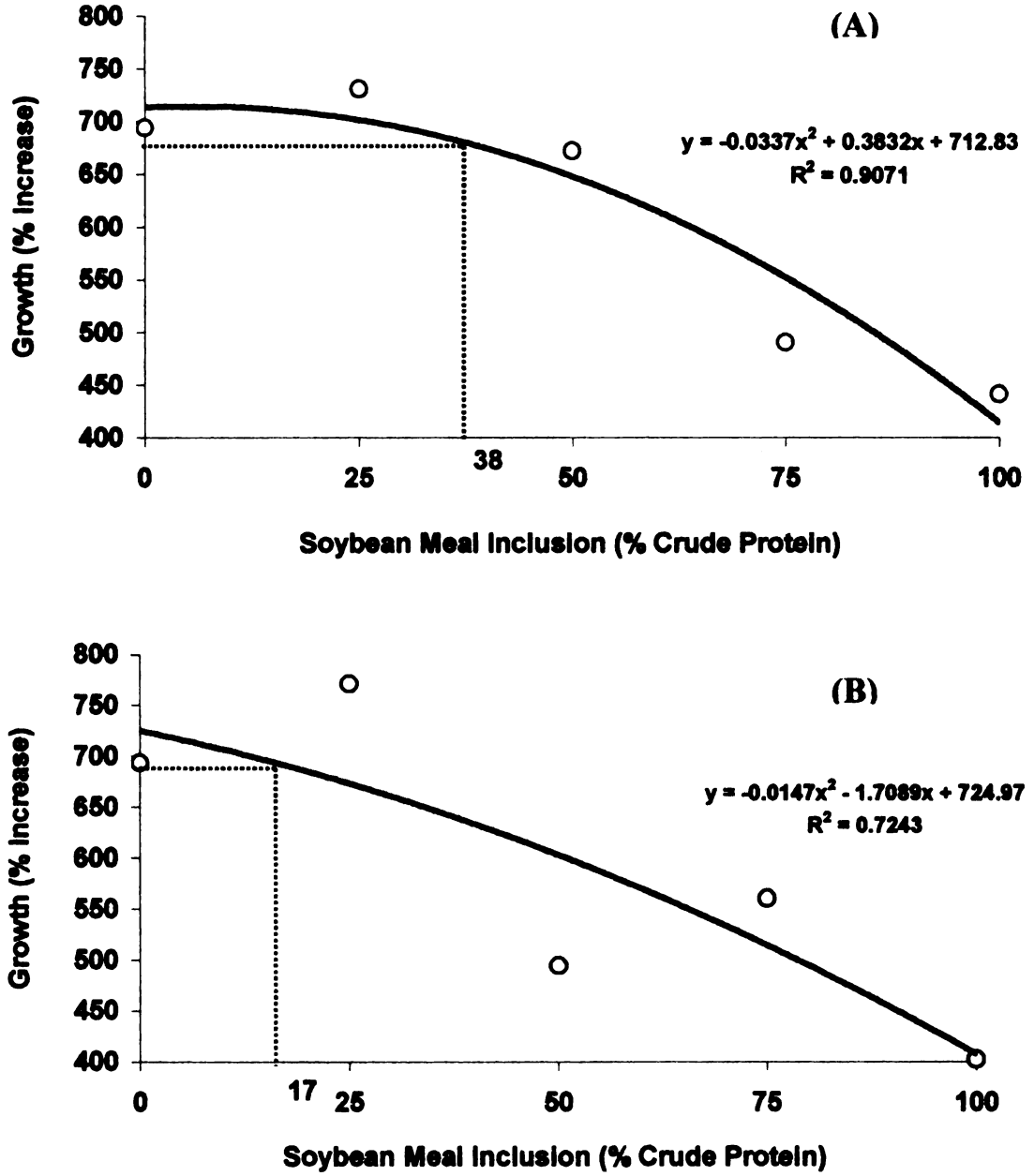

Figure 24. Growth data (% increase) of juvenile tilapia fed graded levels of (A) untreated solvent extracted SBM, and (B) phytase treated solvent extracted SBM, as a percent of the crude protein. Data were fitted with a quadratic model. Dotted lines indicate model parameter estimates. Diminishing returns and lower growth are predicted with greater than 38 % of the CP as untreated solvent extracted SBM (A), and 17 % of the CP as phytase treated solvent extracted SBM (B).

Shiau et al. 1990), and in agreement with the industry standard limit of 25 % of the dry diet (John Stanley, Star Milling Co., personal communication). In comparison, the models suggested phytase treated SBM should be restricted to no more than 10 % of the diet on a DM basis.

The whole body proximate composition analysis indicated the values for CP, lipid, and moisture were the same as reported for similar size tilapia fed defatted SBM at 52 % of the dry diet (Wee and Shu 1989). In smaller tilapia fed graded levels of SBM, CP and moisture were similar, but lipid levels higher, relative to the current study (Davies et al. 1989).

The dietary effects on proximate composition showed a clear trend in increasing CP with increasing levels of untreated SBM. There were no dietary effects on CP or on whole body lipid levels in fish fed the phytase treated SBM. This is similar to Davis and Stickney (1978) who observed no dietary effects on proximate composition of *Tilapia aurea* fed graded levels of SBM. In contrast, others reported no effect on CP (Wee and Shu 1989; Davies et al. 1989). However, contradictory dietary effects on whole body lipid levels were reported (Wee and Shu 1989; Davies et al. 1989).

Both phytase treated, and untreated diets, showed the same trend in SGR and efficiency as observed for growth. There was a slight increase in performance at 25 % SBM substitution relative to the control diet, followed by a steady decline. Significant differences were not detected among fish fed the untreated SBM diets until the level of incorporation surpassed 75% of the CP as SBM. This is similar to the findings of others (Davis and Stickney 1978; Shiau et al. 1989; Davies et al. 1989). In contrast, significant

differences were detected in all efficiency parameters, except FE, in fish fed the phytase treated diets once the level of substitution surpassed 25 % of the CP as SBM.

Specific growth rate, FE, PER, and ANPU for all SBM diets, except those at 100 % of the CP, were higher in this experiment than previously reported for SBM diets fed to Nile tilapia (Wee and Shu 1989), and Mozambique tilapia (Davies et al. 1989), but slightly lower than reported for red tilapia (DeSilva et al. 1991). In the study with Nile tilapia, the highest level of SBM incorporated with low TIA activity comprised 52 % of the dry diet (Wee and Shu 1989). The highest level in the present study was 60 % of the dry diet. At these levels, the results between the two were comparable. Nile tilapia are sensitive to soybean trypsin inhibitors. However, the TI analysis indicated TIA was below levels detrimental to tilapia (Wee and Shu 1989; Shiau et al. 1990).

The FE was similar, but PER slightly lower than reported for similar size tilapia fed SBM substituted at 30 % of the CP (Shiau et al.1987; Shiau et al. 1990). This is not surprising as the researchers were feeding a suboptimal CP diet, and PER increases when fish are fed lower levels of protein. Additionally, the efficiency parameters reported here are the same as those reported for 2.9 g tilapia fed diets incorporating graded levels of the legume *Phaseolus aureus* (DeSilva and Gunasekera 1989).

Fish fed the untreated SBM diets exhibited slightly better FE and PER than those fed phytase treated diets, but the differences were only significant at 50 % of the CP. The ANPU was slightly higher with phytase treated SBM at 25 % of the CP. At higher levels of substitution the ANPU from untreated diets was significantly higher.

The phytase treated SBM diets, and untreated SBM diets, resulted in ANPU reduction with increasing levels of substitution. However, only fish receiving untreated

SBM at 100 % of the CP was significantly reduced relative to the FM control diet. In contrast, the phytase treated SBM resulted in significantly reduced ANPU relative to the FM control beyond 50 % of the CP. This would indicate better bioavailability of IAA or energy from the untreated SBM beyond 25 % of the CP. The inference from ANPU is phytase treated SBM should be restricted to less than 17 % of the diet on a dry matter basis. This is in agreement with the values predicted from the growth models.

Increasing ANPU is important for economic success of any aquaculture venture, but is critical in recirculating systems. As a direct measure of dietary protein retention, it is an implied measure of IAA bioavailability. When confronted with excess, or improperly balanced AA, fish readily degrade the unusable AA resulting in NH_3/NH_4^+ excretion (Walton 1985; Cai et al. 1996). A major limiting constraint associated with recirculating systems is the accumulation of nutrients, particularly nitrogenous products.

Phytic acid reduces digestion and availability of amino acids for uptake (Cheryan 1980; Reddy et al. 1989). Diets supplemented with salts of phytic acid were believed to depress growth and decrease protein digestibility in rainbow trout (Spinelli et al. 1983), Chinook salmon (Richardson et al. 1985), and carp (Hossain and Jauncey 1993). Additionally, elimination of phytic acid from swine diets significantly increased total tract digestibility of CP, all IAA , and ileal digestibilty of Met and Arg (Mroz et al. 1994). Increased protein digestibility, higher biological value, and increased N retention have been demonstrated with diets containing lower phytic acid (Satterlee and Abdul-Kadir 1983; Mroz et al. 1994). Therefore, a digestibility study was conducted to determine if removal of phytic acid would increase the digestibility of CP and individual AA from SBM fed to tilapia.

Apparent crude protein digestibility among the diets containing SBM ranged from 78.9 – 84.6 %. No significant differences were detected, as was observed in hybrid striped bass (Papatryphon et al. 1999). The values reported here are similar to those reported for tilapia fed diets with SBM substitution up to 67 % of the CP (Shiau et al. 1989). Beyond 67 % of the CP, ACPD dropped to 70 % with Met supplementation, and 60 % without Met supplementation (Shiau et al 1989). In two other studies, the ACPD were the same or slightly higher than in this study, depending on dietary CP level (Shiau et al. 1987; Shiau et al. 1990).

In the present study, Ala digestibility was lower in the phytase treated SBM substituted at 100 % of the CP than the reference, or other phytase treated SBM diets. The reason for differences in Ala digestibility is unclear. A survey of the literature did not uncover a suitable explanation for differences in Ala digestibility.

Digestibility of Lys in the reference diet was also greater than in the phytase treated SBM diet substituting SBM at 100 % of the CP. Despite low digestibility coefficients for the phytase treated diet with SBM at 100 % of the CP, no other differences in AA digestibility were detected among the phytase treated SBM diets, or the reference diet. This was likely a result of the large SEM for the diet with SBM at 100 % of the CP. An insufficient amount of feces from fish fed the diet with SBM at 100 % of the CP limited statistical analysis to two samples. Therefore, it is difficult to draw conclusions about this treatment as the biological relevance may be confounded by sampling error.

The only difference in AA digestibility among the diets incorporating graded levels of untreated SBM was a higher digestibility of Ser in the diet at 75 % than the

reference diet. As with Ala in the phytase treated diets, the reason for the difference in digestibility is unclear

Amino acid digestibility coefficients for the untreated SBM diets were similar to values reported by Sadiku and Jauncey (1995) for tilapia (*O. niloticus*) fed graded levels of SBM in a SBM:poultry meat meal blend diet (Table 18). Amino acid digestibilities were similar except for Met, which were lower in our study. However, it is possible the Met values were underestimated in our study due to loss upon hydrolysis for analysis.

Values reported by Sadiku and Jauncey (1995) became more similar with higher rates of SBM incorporation. This was likely due to the poor digestibility of poultry meat meal (Cho 1991; Alexis et al. 1985; Sadiku and Jauncey 1995). Dilution of the poultry meat meal with increasing SBM incorporation increased the digestibility of the diet.

All diets were formulated to contain sufficient IAA to meet the known requirements of tilapia (*O. niloticus*). Analysis of dietary IAA indicated all diets contained sufficient levels, except Met in the two diets containing 75 % of the CP as SBM, and Met in the diet containing untreated SBM at 50 % of the CP. Cysteine can contribute up to at least 50 % of the Met requirement in tilapia (Jackson and Capper 1982). Therefore the Met requirement is more appropriately termed a requirement for TSAA. If at least 20 % of dietary Cys could be used to meet the TSAA requirement, then the diets met all the known requirements for tilapia.

Incorporating sufficient IAA into the diet does not ensure an adequate supply to meet the needs of the animal. Incompletely digested AA are lost to the environment in feces, and uptake of digested AA in excess of immediate needs are catabolized and

Table 18. Comparison of apparent digestibility coefficients for indispensable and dispensable amino acids (AA) in tilapia (*O. niloticus*) fed graded levels of SBM (25, 50, and 75 % of the crude protein) substituted into a fish meal based diet [1], or a poultry meat based diet [2].

	SBM 25 % Crude Protein		SBM 50% Crude Protein		SBM 75 % Crude Protein	
	Fish Meal	Poultry Meat	Fish Meal	Poultry Meat	Fish Meal	Poultry Meat
Indispensable AA						
ARG	84.3	76.2	84.5	83.1	89.0	82.9
HIS	82.3	74.6	80.7	82.7	84.6	84.6
ILE	77.2	72.9	76.3	80.0	82.2	81.0
LEU	80.1	77.1	78.7	79.9	83.2	83.3
LYS	86.7	77.9	85.8	84.1	89.2	85.7
MET	80.0	89.4	77.2	91.5	84.7	93.2
PHE	77.1	74.6	76.9	79.9	82.5	82.1
THR	80.3	71.5	78.7	77.5	83.0	80.6
VAL	78.0	72.3	75.7	76.7	82.2	76.7
Dispensable AA						
ALA	80.8	76.7	77.6	77.1	82.3	78.2
ASP	78.0	73.2	75.7	80.1	82.0	74.1
CYS	80.9	83.4	77.2	82.2	85.1	85.1
GLU	84.6	78.6	84.8	82.8	89.2	83.8
GLY	78.2	80.0	75.8	82.1	83.6	79.0
PRO	79.3	78.8	77.2	83.2	84.3	84.3
SER	8.4	72.6	78.3	79.2	85.8	84.1
TYR	72.3	74.4	72.3	77.8	75.9	80.3

[1] Values from this study, experiment 6.

[2] Values from Sadiku and Jauncey (1995).

unavailable for protein synthesis (Walton 1985). Dependence of protein synthesis upon dietary protein supply in fish is greater than in mammals (Fauconneau 1985). Apparent digestibility coefficients were applied to dietary IAA concentrations to determine if available IAA met requirements (Table 19).

The availability of all IAA was higher from the two SBM diets at 25 % of the CP than from the reference control diet. This is likely the reason for the slightly better growth and conversion efficiency observed in fish fed diets containing 25 % of the CP as SBM. Available Phe in the reference diet was 17 % below the requirement, and 25 % lower than in the two SBM diets. Therefore, in addition to the overall IAA availability it is possible Phe became the limiting amino acid relative to the SBM diets at 25 % of the CP.

Available TSAA and Thr were below reported dietary requirements in all diets. However, as stated previously, the TSAA values may be underestimated due to potential loss of Cys and Met upon hydrolysis for analysis. A deficiency of available Thr with a high level of SBM incorporation were also noted in rainbow trout (Davies and Morris 1997)

The availability of IAA from diets containing phytase treated SBM was similar to that of the untreated SBM, with a few exceptions. The availability of Lys was lower in phytase treated SBM diets than the untreated SBM diets at each level of SBM substitution. The availability of Met and Thr were lower in phytase treated SBM diets than the untreated SBM diets once the level of SBM substitution surpassed 50 % of the CP. This trend appeared to be similar for most of the IAA.

Table 19. Requirements and available dietary indispensable amino acids (IAA) for tilapia fed graded levels of untreated, or phytase treated, SBM as a percent of dietary crude protein.

			Untreated SBM (% Dietary CP)			
IAA	Requirement [1]	Control	25	50	75	100
Arg	1.18	1.55	1.89	1.87	2.21	2.30
His	0.48	0.49	0.60	0.58	0.66	0.69
Ile	0.87	1.00	1.15	1.09	1.24	1.22
Leu	0.95	1.91	2.14	2.02	2.21	2.16
Lys	1.43	1.67	1.92	1.70	1.71	1.67
Met	0.75	0.67	0.70	0.55	0.61	0.61
TSAA	0.90	0.81	0.87	0.70	0.80	0.81
Phe	1.05	0.87	1.10	1.11	1.30	1.37
Thr	1.05	0.85	0.96	0.89	1.00	0.93
Val	0.78	1.24	1.34	1.21	1.36	1.26

		Phytase Treated SBM (% Dietary CP)			
IAA	Requirement	25	50	75	100
Arg	1.18	1.83	1.86	2.03	1.81
His	0.48	0.57	0.60	0.62	0.56
Ile	0.87	1.12	1.16	1.19	0.98
Leu	0.95	2.14	2.15	2.11	1.73
Lys	1.43	1.76	1.55	1.47	1.14
Met	0.75	0.66	0.61	0.55	0.42
TSAA	0.90	0.81	0.77	0.73	0.58
Phe	1.05	1.08	1.13	1.29	1.15
Thr	1.05	0.92	0.88	0.89	0.66
Val	0.78	1.29	1.30	1.25	0.89

[1] Requirements taken from NRC (1993).

A correlation analysis was run between weight gain and individual available amino acids from the SBM diets fed to tilapia during the eight week growth trial. The same analysis was performed on the efficiency parameters SGR, FE, PER, and ANPU. All AA exhibiting a significant correlation ($P<0.10$) are reported along with their Pearson correlation coefficient for the relationship (Table 20). Two trends are readily apparent for both growth, and efficiency parameters.

In fish fed the untreated SBM diets, there was a significant negative correlation between Arg, His, and Phe with weight gain, SGR, and PER. Increasing SBM substitution led to higher dietary levels of Arg, His, and Phe, with resulting depression in growth. Although the Pearson correlation coefficients indicate a strong relationship, their statistical relevance were marginal ($0.05 < P < 0.10$). The possible relationship observed with His is unclear. Feeding His and Thr well beyond their requirements in chum salmon had no adverse effect on growth or conversion (Akiyama et al. 1985). Although suggestive of a relationship, correlation analysis does not necessarily indicate a cause and effect.

A possible explanation for the relationship observed with Arg is an Arg/Lys antagonism. It has been demonstrated in fish that Arg and Lys compete for the same intestinal transporters, as they do in mammals (Ash 1985), but an antagonistic relationship has yet to be convincingly demonstrated in fish (Wilson 1989; NRC 1993). Similarly, there is evidence for competition between Phe and Met for transport into the enterocyte (Ash 1985). Higher levels of Phe with increasing SBM incorporation may have exacerbated any Met deficiency.

Table 20. Correlations between weight gain and efficiency parameters, and available individual amino acids in phytase treated SBM, and untreated SBM, experimental diets fed to tilapia. Parameters include specific growth rate (SGR), feed efficiency (FE), protein efficiency ratio (PER), and apparent net protein utilization (ANPU). Experimental diets incorporated SBM at 0, 25, 50, 75, and 100 % of the dietary crude protein. Shown are IAA and associated Pearson correlation coefficients for all relationships with ($P<0.10$).

	Treatment	IAA	Pearson Correlation Coefficient	P - value
Weight Gain	Untreated	ARG	-0.867	0.057
(% Increase)	Untreated	HIS	-0.805	0.098
	Untreated	PHE	-0.852	0.067
	Phytase	LYS	0.908	0.033
	Phytase	MET	0.853	0.066
	Phytase	TSAA	0.836	0.078
SGR	Untreated	ARG	-0.863	0.060
(% /day)	Untreated	HIS	-0.806	0.099
	Untreated	PHE	-0.849	0.069
	Phytase	LYS	0.930	0.022
	Phytase	MET	0.879	0.050
	Phytase	TSAA	0.871	0.055
FE	Phytase	LYS	0.974	0.005
	Phytase	MET	0.948	0.014
	Phytase	TSAA	0.946	0.015
	Phytase	THR	0.840	0.075
	Phytase	VAL	0.820	0.089
PER	Untreated	ARG	-0.851	0.068
	Untreated	HIS	-0.819	0.090
	Untreated	PHE	-0.840	0.075
	Phytase	LYS	0.975	0.005
	Phytase	MET	0.964	0.008
	Phytase	TSAA	0.953	0.012
	Phytase	THR	0.809	0.097
ANPU	Phytase	LYS	0.985	0.002
(%)	Phytase	MET	0.962	0.009
	Phytase	TSAA	0.947	0.015
	Phytase	THR	0.811	0.096

In fish fed the phytase treated SBM diets, there was a strong correlation between Lys, Met, and TSAA and weight gain. Similar correlations were observed between Lys, Met, and TSAA, and all efficiency parameters (Table 20). Additionally, there was a statistically marginal effect of Thr on FE, PER, and ANPU, and of Val on FE. Davies and Morris (1997) found depressed ANPU in rainbow trout, which they attributed to deficiencies in Met, Lys, and Thr.

Although correlations with available TSAA were significant, the effects were due to Met, as the correlation coefficients decreased and P values increased after including Cys in the relationship. The high correlations observed with available Lys and Met suggest these two IAA are responsible for depressed weight gain and poor performance of fish fed increasing levels of phytase treated SBM.

A plot of dietary and available Met in relation to dietary CP, and as a function of SBM substitution, indicates dietary and available Met decreased with increasing substitution of phytase treated SBM. This occurred even as dietary CP increased (Figure 25 (A)). At the 100 % substitution level, availability decreased substantially in relation to dietary Met and CP. Similar observations were made in rainbow trout (Dabrowski et al. 1989).

In contrast, dietary and available Met in the untreated SBM diets paralleled each other and were constant following crystalline Met supplementation (Figure 25 (B)). These plots would suggest a factor associated with the phytase treated SBM was responsible for the observed decrease in Met availability and performance in fish fed diets incorporating phytase treated SBM at 75 % and 100 % of the CP.

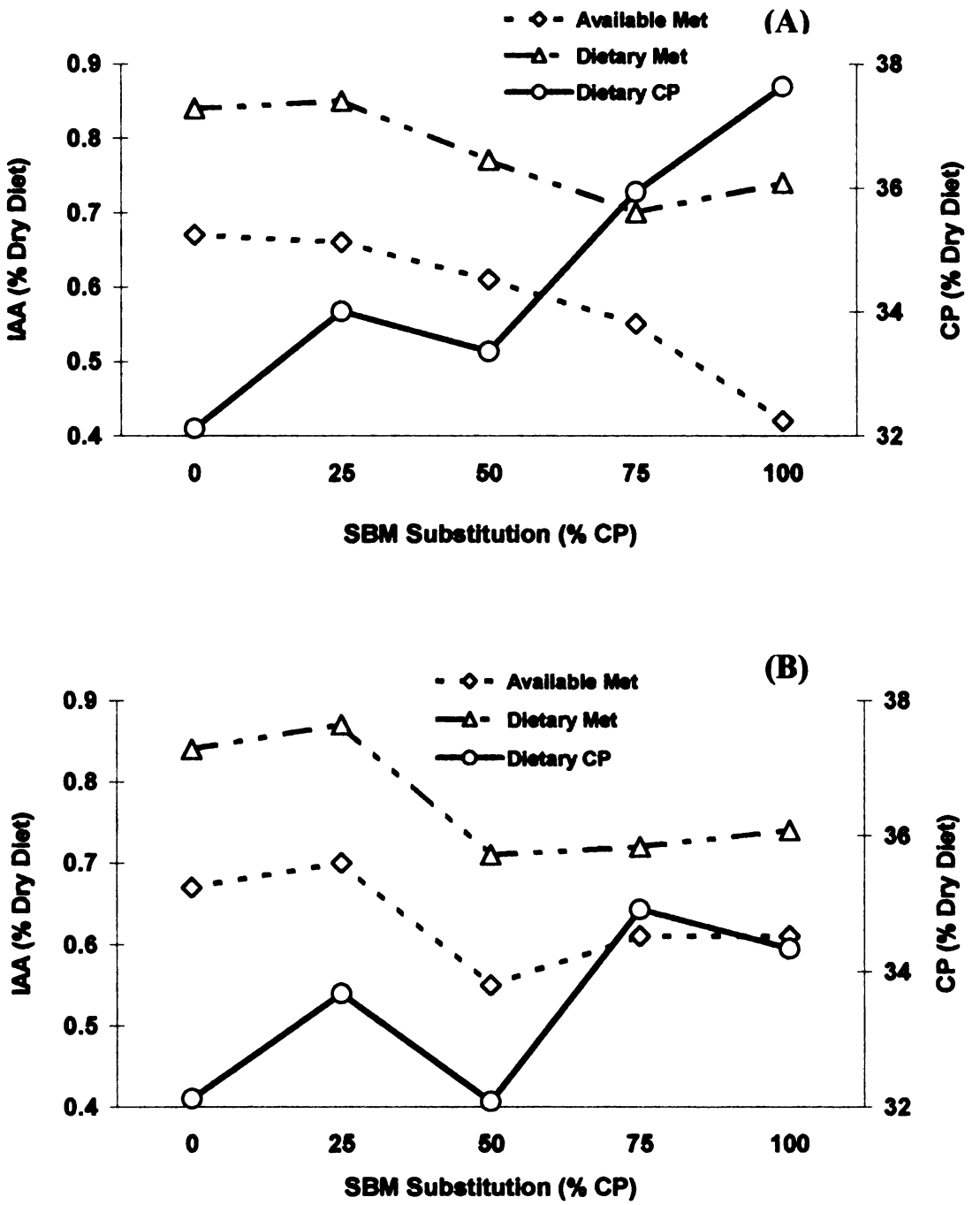

Figure 25. Dietary Met and available Met in relation to dietary CP. Diets contained graded levels of phytase treated SBM (A), or untreated SBM (B) as a percent of CP in the diet.

A plot of dietary and available Lys in relation to dietary CP, and as a function of SBM substitution, indicates dietary and available Lys follow an identical trend to Met (Figure 26). However, whereas Met availability began to decrease relative to dietary Met at 100 % substitution, the Lys availability began to decrease relative to dietary Lys when the level of substitution surpassed 25 % of the CP. This pattern in decreased Lys availability was the same pattern observed for weight gain, SGR, PER, and ANPU. Although a Lys/Arg antagonism has not been conclusively demonstrated in fish, there is insufficient evidence to rule out the possibility that high levels of Arg did not exacerbate the Lys deficiency observed in phytase treated SBM diets.

Soybean meal has a favorable IAA profile for replacing FM in formulated diets for tilapia, although it has a relatively low chemical score (Tacon and Jackson 1985). At high rates of incorporation the diets become deficient in one or more IAA. The first two IAA considered limiting for tilapia, and most fish species in general, are Met and Lys, although Thr is also mentioned. In a number of studies, SBM diets have been supplemented with IAA to compensate for this deficiency. Reports of success with this approach vary (Rumsey and Ketola 1975; Viola et al. 1981;Walton et al. 1982; Viola et al. 1983; Shiau et al. 1989; El-Sayed 1989; El-Dahhar and El-Shazly 1993; Davis et al. 1995; Ng et al. 1996; Davies and Morris 1997; Coyle et al. 2000).

Tilapia fed diets with SBM at 100 % of the CP supplemented with Lys and Met did not show an increase in growth (Viola and Arieli 1983). Additionally, tilapia fed diets containing 33, 67, and 100 % of the CP as SBM supplemented with Met exhibited no differences in growth except at the 100 % inclusion level. At this level there was a

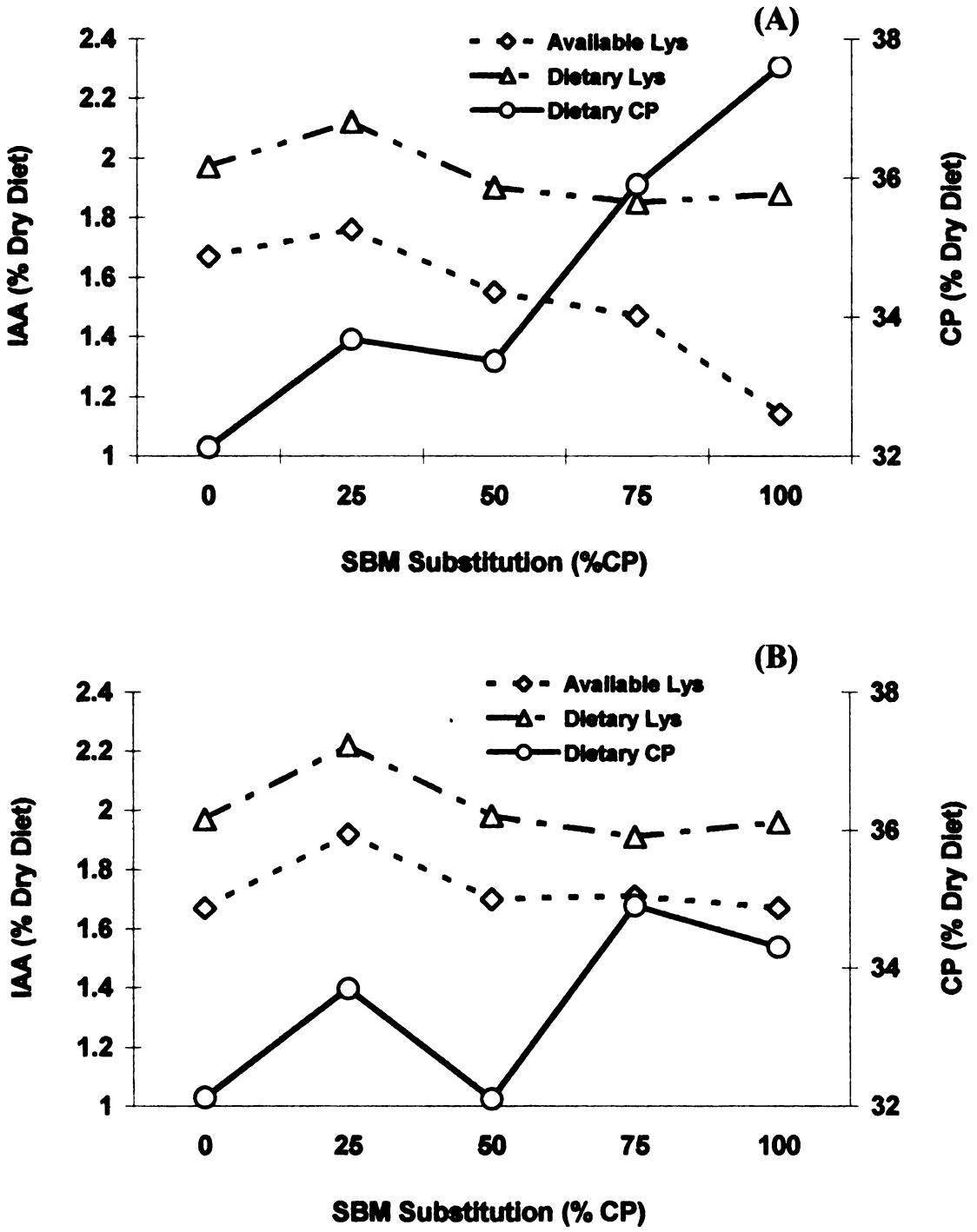

Figure 26. Dietary Lys and available Lys in relation to dietary CP. Diets contained graded levels of phytase treated SBM (A), or untreated SBM (B) as a percent of CP in the diet.

significant increase over the unsupplemented diet, but still significantly lower than the other diets with or without supplementation (Shiau et al 1989).

When the experimental diets were formulated, it was apparent dietary Met was below requirements for tilapia. Crystalline L-methionine was incorporated in the diets containing 50 % or more of he CP as SBM to meet the requirement. Although dietary Met met the requirement, on an available basis, the level of Met was below the requirement. The crystalline Met appeared to be available to tilapia in the diets containing untreated SBM (Figure 27). As Met in the intact protein decreased with increasing levels of SBM substitution, available Met as a percent of the dietary requirement improved slightly, suggesting crystalline Met was utilized.

Conversely, Met supplementation in the diets containing phytase treated SBM appeared to be completely unavailable to tilapia (Figure 27). Diets containing phytase treated SBM resulted in severely depressed available Met despite Met supplementation. However, a closer examination of the available Met values from these diets indicates the supplemental Met may be as effective as the intact protein at supplying Met. The measured total available Met was similar to the dietary Met supplied as intact protein, appearing as though crystalline Met is unavailable (Table 21). Applying the apparent digestibility coefficient to both the Met supplied in the intact protein, and the supplementation, results in nearly identical values for calculated and measured available Met (Table 21).

There is evidence that crystalline amino acids are readily leached from diets (Wilson 1989). The potential for leaching would be high from the experimental diets used in this study. The large surface area to volume ratio implied by the feed size

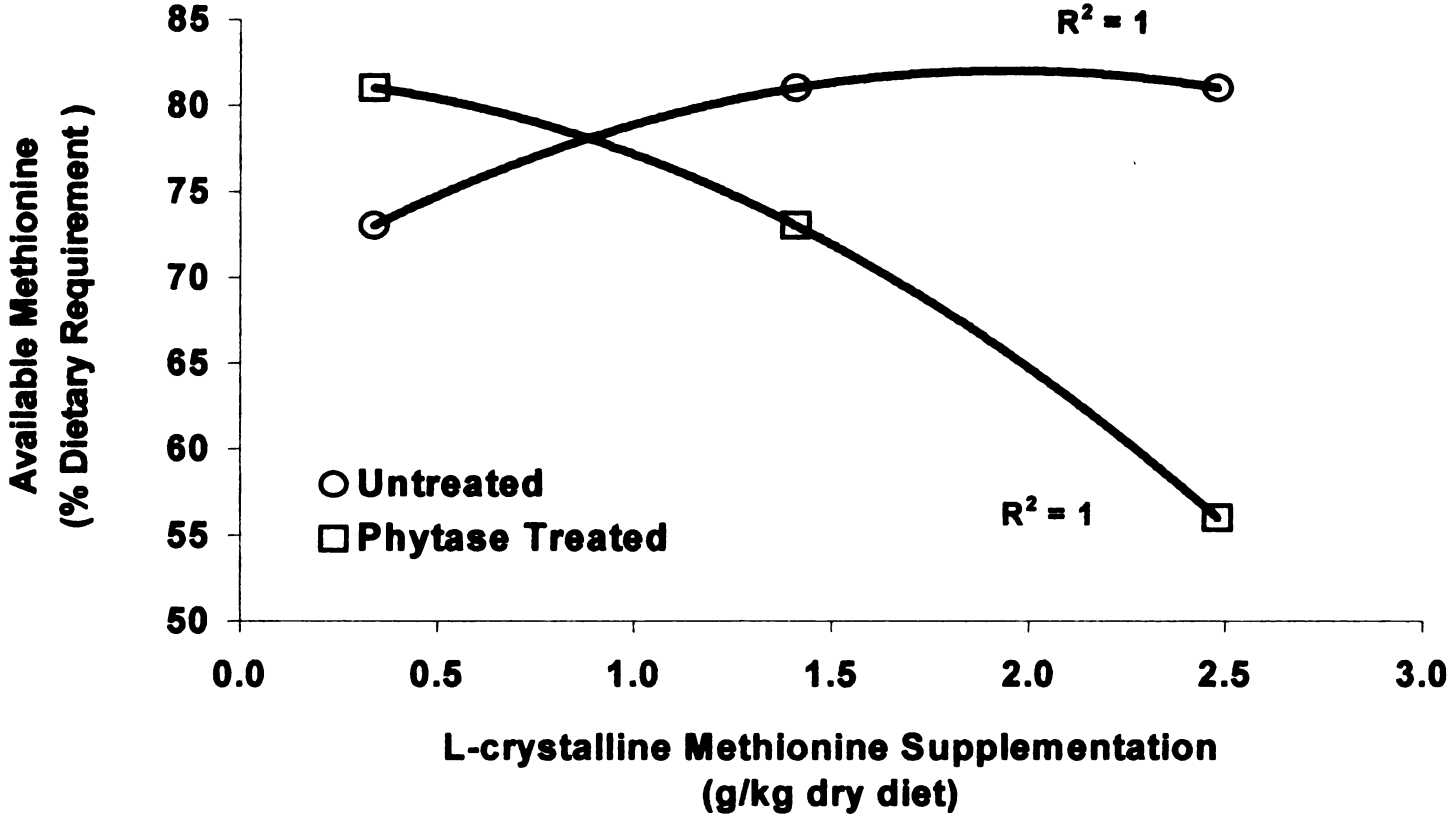

Figure 27. Available methionine (% dietary requirement) as a function of L-crystalline methionine dietary supplementation. Methionine was supplemented into phytase treated SBM, and untreated SBM diets incorporating SBM at 50, 75, and 100 % of the dietary crude protein to meet the dietary methionine requirement for tilapia. Curves were fitted by quadratic regression.

Table 21. Dietary methionine supplied by intact protein and L-crystalline methionine supplementation in diets incorporating graded levels of phytase treated SBM as a percent of dietary crude protein. Also shown are the apparent digestibility coefficients for the diets, the calculated availability of methionine from intact protein and the supplement, and measured available methionine.

SBM (% CP)	Met [1] Intact Protein (g/kg dry diet)	Met Supplement (g/ kg dry diet)	Apparent Digestibility Coefficient	Available Met Intact Protein (g/kg dry diet)	Available Met Supplement (g/kg dry diet)	Total Available Met Calculated (g/k g dry diet)	Total Available Met Measured (g/kg dry diet)
0	0.96	0	79.7	0.77	0	0.77	0.67
25	0.84	0	77.0	0.65	0	0.65	0.66
50	0.71	0.04	78.5	0.56	0.03	0.58	0.61
75	0.58	0.17	78.2	0.45	0.13	0.58	0.55
100	0.45	0.30	56.8	0.26	0.17	0.43	0.42

[1] Methionine values for Herring meal and SBM taken from NRC (1993).

required by 1.3 g fish, coupled with feeding behavior of tilapia make leaching a strong possibility. It can not be determined if available Met was solely from the intact protein or partitioned between the two sources. This is a needed area for future investigation.

It appears crystalline L-Met supplementation was effective in improving growth, performance, and digestibility of the untreated SBM diets used in this study, relative to the phytase treated SBM diets. This is contrary to other findings in tilapia (Shiau et al. 1989; Robinson et al. 1984); however, effectiveness of supplementation may be dependent on dietary CP levels (Shiau et al. 1987). Suitability of crystalline amino acid supplementation has been demonstrated in hybrid striped bass (Griffin et al. 1992), red drum (Brown et al. 1988), rainbow trout (Davies and Morris 1997), Atlantic salmon (Olli et al. 1995), carp (Nose et al. 1974), *Tilapia zilli* (El-Sayed 1989), and channel catfish (Wilson et al. 1977).

Care should be taken in interpreting the results of Met and TSAA availability. It is possible the feed and fecal values of both Cys and Met were underestimated. Cysteine and Met have been shown to be unstable under the conditions used to hydrolyze the feed and fecal samples. Typically, to protect them from destruction, Cys and Met are converted to the more stable derivatives cysteic acid and methionine sulfone before hydrolysis (Waters 1989). A lack of sufficient material precluded taking these preventative measures before hydrolysis. Although it is possible some of the Met and Cys were destroyed before analysis, measured dietary Met was virtually the same as calculated from the diet formulation, suggesting losses may have been minimal.

A number of hypotheses lend themselves to an explanation for reduced availability of AA and energy from the diets containing phytase treated SBM. Principal

among these is the feeding behavior of tilapia. Tilapia have been described as preferential grazers as opposed to meal eaters, such as trout or salmon that swallow food whole. Tilapia are equipped with palatine teeth and pharyngeal gill rakers allowing them to grind feed and filter material before swallowing (Jauncey and Ross 1982; Bowen 1982). Feeding tilapia repeatedly pick up food items and expel them numerous times before consumption (Hanley 1985; personal observations). This process effectively creates exceedingly small particles with high surface to volume ratio to facilitate digestion. In addition, tilapia will take hours to finish a meal while the feed sits on the bottom. This feeding activity enhances the potential for leaching of soluble components. Phytic acid acts to decrease solubility of nutrients (Reddy et al. 1989) and its removal may lead to a loss of nutrient availability.

The behavioral response of the tilapia to the diet substituting phytase treated SBM at 100 % of the CP may also indicate dietary deficiencies or imbalances. The tilapia displayed avoidance to the diet. The behavior was originally attributed to poor palatability. However, this behavior may also have been a response to an AA deficiency, or imbalanced amino acids, as has been exhibited in terrestrial animals (Gietzen 1993).

Phytic acid may also serve in a protective capacity. Phytic acid binds to the globular proteins glycinin, and β-conglycinin (Reddy et al. 1989), both of which have been found to be ANFs in fish (Kaushik et al. 1995; Rumsey et al. 1995). Additionally, phytic acid may protect sensitive AA, such as Lys, from degradation during processing or pelleting.

Finally, the phytase treatment process itself may be responsible for altering availability of AA, energy, or some other essential nutrient. Tilapia appear to adapt to the

increased levels of SBM substitution with time, possibly by inducing increased activity of amylase (Anderson et al. 1991). In ducks, phytase addition to the diet decreased amylase activity (Martin et al. 1998). Tilapia amylase activity is high for fish species (Nagase 1964) allowing them to make better use of carbohydrates than other species (Anderson et al. 1991). The potential reduction of amylase activity may have decreased available energy. Unfortunately, the limitation of small fecal samples precluded the analysis of digestible energy.

The results from the SBM growth and digestibility trials would suggest phytase was successful in hydrolyzing the phytic acid associated with SBM. Incorporating phytate pretreated SBM beyond 25 % of the CP led to a depression in weight gain and decreased efficiency. In comparison, incorporation of untreated SBM into the reference diet did not decrease weight gain, or diminish efficiency, until the level of substitution surpassed 75 % of the CP. This would suggest phytic acid may play a role in sustaining protein integrity and availability of AA in tilapia. Less conclusive is whether phytic acid is beneficial to amino acid availability, or the absence of phytic acid causes a decrease in amino acid availability. If phytic acid effects growth and efficiency, the results should be reproducible in a dose-response manner using a purified, or semi-purified form. This approach has been used successfully in determining the effects of phytic acid on mineral availability (Spinelli et al. 1983; Satoh et al. 1989; Hossain and Jauncey 1993). To evaluate the effect phytic acid may have on growth, efficiency, and amino acid digestibility, tilapia were fed a fish meal based diet with graded levels of phytic acid as Na-phytate.

Fish fed the fish meal diets supplemented with Na-phytate exhibited differences in growth and SGR. Only the treatment receiving the highest level of supplementation grew less than the control treatment (without supplementation). No significant differences were detected in FE, PER, ANPU, or whole body proximate composition, regardless of rate of supplementation. Weight gain and all efficiency parameters were lower in this trial than in the SBM growth trial. This applied to the reference control diet as well, which was the same diet for both trials, suggesting the differences were independent of dietary effects.

The data suggest a superficial trend toward increased growth with increasing levels of phytic acid supplementation. However, dietary CP in these diets ranged from 31 – 37 % of the dry diet, and shows an almost identical trend to that of growth (Figure 28). A correlation analysis indicated a strong relationship between growth and all available IAA. Pearson Correlation coefficients ranged from 0.90 for Thr to 0.95 for His, with an overall mean of 0.94 ($P<0.01$). This would suggest growth was independent of dietary phytic acid, lending support to the conclusion increased growth was due to dietary CP, or the DE:P ratio.

The CP digestibilities of these diets showed no significant differences when dietary Na-phytate ranged from 0 – 25.8 g/kg of the dry diet. As was noted for the growth response, there appeared to be a superficial trend of increasing ACPD with increasing phytic acid supplementation. A multiple regression analysis on ACPD of the Na-supplemented FM diets indicated ACPD was correlated to level of CP and independent of dietary phytic acid.

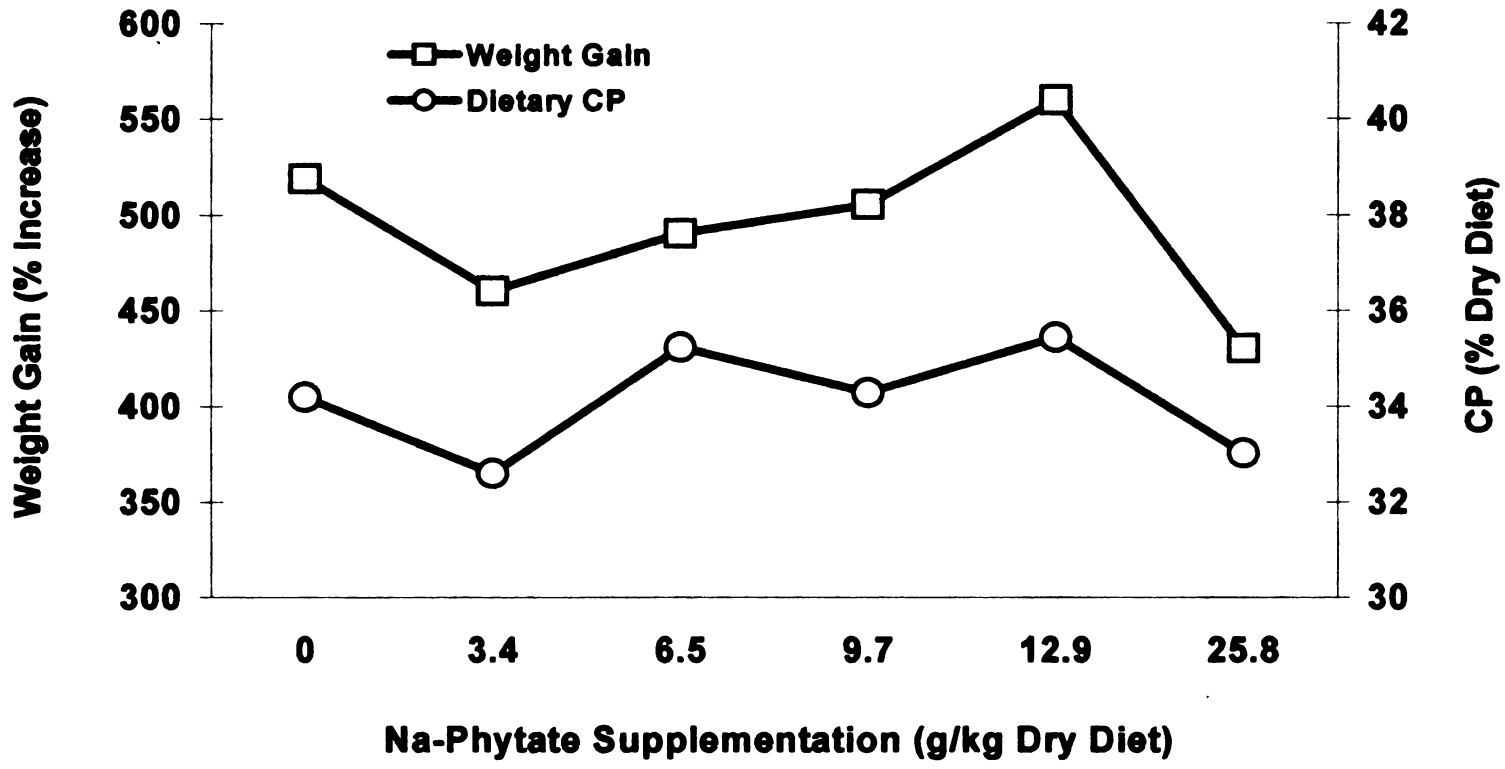

Figure 28. Weight gain in relation to dietary crude protein (CP), and as a function of dietary phytic acid supplementation as Na-phytate (g/kg dry diet).

Apparent digestibility of individual amino acids in the Na-supplemented FM diets exhibited a parallel trend to that of growth and ACPD. Other than a few exceptions, the AA digestibility coefficients followed the same pattern for each IAA. The diet containing 12.9 g Na- phytate/g dry diet had the highest digestibility coefficient for all AA. However, in general they were only significantly different from the two diets containing 3.4 and 25.8 g Na-phytate/kg dry diet. No significant differences were observed for Ala and Asp among any of the diets. Additionally, Gly and Ser were significantly higher in the diet containing Na-phytate at 12.9 g /kg dry diet than the other diets.

All diets were formulated to meet or exceed the known IAA requirements for tilapia. The only difference in preparation was the level of incorporation of Na-phytate at the expense of cellulose. Analysis indicated dietary levels of all IAA were sufficient to meet the requirements for tilapia. Apparent digestibility coefficients were applied to dietary IAA concentrations to determine if available IAA met requirements (Table 22). The apparent availability was low for TSAA, Phe, and Thr in the diets containing Na-phytate at 3.4, 6.5, 25.8 g/kg dry diet. Not surprisingly these were the slowest growing treatments. The data from the phytic acid supplemented diets suggest there was neither a beneficial, or detrimental effect due to the supplementation in terms of growth, efficiency, body composition, or digestibility.

Tilapia exhibit plasticity of the GI tract providing variability and adaptability. Moriarity (1973) observed highly acidic stomachs in wild Nile tilapia, with pH values as low as 1.0-2.0. These values are similar to values reported for other wild captured tilapia species (Bowen 1982). Low gastric pH in wild tilapia assists digestion by hydrolysis of

Table 22. Requirements and available dietary indispensable amino acids (IAA) in tilapia fed fish meal based diets supplemented with graded levels of phytic acid as Na-phytate.

IAA	Requirement [1]	Na-phytate Supplementation (g/kg dry diet)					
		0	3.4	6.5	9.7	12.9	25.8
Arg	1.18	1.85	1.65	1.75	1.82	2.11	1.65
His	0.48	0.60	0.50	0.53	0.59	0.67	0.50
Ile	0.87	1.21	1.02	1.10	1.16	1.43	1.03
Leu	0.95	2.34	2.04	2.14	2.30	2.67	2.04
Lys	1.43	2.18	1.89	2.03	2.10	2.46	1.92
Met	0.75	0.86	0.71	0.79	0.80	0.97	0.74
TSAA	0.90	1.03	0.85	0.95	0.96	1.16	0.89
Phe	1.05	1.09	0.91	0.99	1.05	1.28	0.93
Phe + Tyr	1.55	1.81	1.49	1.64	1.78	2.16	1.55
Thr	1.05	1.05	0.96	0.98	1.09	1.24	0.96
Val	0.78	1.48	1.28	1.35	1.45	1.71	1.27

[1] Requirements taken from NRC (1993).

phytoplankton and bacterial cell walls, and in mineral decomposition of periphytic detrital aggregate (Moriarity 1973; Bowen 1976; Bowen 1982).

Maximal inhibition of acidic proteases by phytic acid occurs near pH 2.0 (Camus and Laporte 1976; Vaintraub 1991). Gastric pH reported by Moriarity (1973) suggest an environment favorable for enzyme inhibition. However, maximum activity of a protease isolated from *O. niloticus* gastric mucosa was observed at pH 3.5, with minimal activity reported at pH values outside the range of 2.0 - 5.5 (Yamada et al. 1993). In the pH range reported for wild tilapia, and favorable for phytic acid inhibition, proteolytic activity would likely be severely diminished.

Measurements of pH were taken at 10 defined sites on the surface of digesta removed from the gastric region at 0.5, 1, 2, 4, 6, and 8 hours postprandially. The lowest pH recorded on the digesta was 2.35, 6 hours postprandially. Values as low as 2.5 – 2.75 were recorded on sites in contact with the acid-secreting cells localized to the ventral face of the mucosa. However, the mean values of the 10 sampling sites ranged from 5.36 shortly following feeding, to 3.52 at 4 hours postprandially. The pH values following the feeding of a high nutrient dense diet were closer to the optimal pH for enzyme activity than were pH values from wild fish.

The range and mean pH values measured on the gastric mucosa were higher than measured on the digesta, providing further evidence for acid secretory cells described by Fish (1960) and Kapoor et al. (1975). Although pH on the surface of the feed decreased with time, the pH values of the homogenized digesta remained relatively constant over time. This implied that gastric mixing of the digesta was minimal when fish were fed pelleted diets. The small abundance of phytoplankton, coupled with its small size,

provides a large surface area for acidic degradation, mixing, and proteolytic activity observed by Moriarity (1973).

The small surface area to volume ratio of feed pellets, short gastric retention time, and minimal mixing, potentially decreases the efficiency of gastric hydrolysis and digestion. This interpretation is further supported by the inability of the volume dependent model of GER to adequately describe GER in tilapia fed pelleted diets.

The pH values in the intestine remained relatively consistent throughout the sampling period, both within a segment and with time. The pH values increased in intestinal segments posterior to segment 1, which is the site receiving the bile contents. The mean pH values measured in these posterior segments ranged from near 7.0 – 8.3. The values were similar (pH 6.8 – 8.8) to those reported for wild tilapia (Fish 1960; Nagase 1964; Moriarity 1973). These values are optimal for protein-cation-phytate complex formation (Cheryan 1980; Reddy et al. 1989). In addition to demonstrating tilapia manifest a suitable environment for protein-phytic acid complex formation, the data gathered can be used to mimic *in vivo* pH conditions in *in vitro* enzyme assays.

SUMMARY AND CONCLUSIONS

Fish reared in intensive production systems have different requirements than those in the wild. In such systems natural food is limited. All nutrients must be exogenously supplied in the form of high nutrient dense pellets. Consequently there is a need for optimal feeding regimens to accommodate this capacity to process formulated diets.

An optimal ration and feeding regimen should take into account a combination of the interplay between FCR and SGR. Evaluating these parameters for fish fed in this study suggests the optimal feeding frequency for both parameters is either three or four feedings day $^{-1}$. The determination of an optimum feeding regimen should be evaluated from two aspects; 1) physiology of the species, and 2) economics of the aquaculture production unit (Tsevis et al. 1992). The economics of the production unit would suggest 3 feedings day $^{-1}$ is superior due to the cost of labor.

Understanding rate of digestion and its relationship to GER is important for determining the return of appetite. Making food available as soon as appetite has returned can maximize intake, and increase efficiency. Demonstrating a consistent relationship between stomach fullness and appetite will allow an optimal feeding interval to be predicted from factors that control GER. Therefore, GER and return of appetite were evaluated in fish fed to satiation 3, and 5 times day $^{-1}$.

Gastric evacuation curves indicated curvilinear relationships. Both feeding frequencies were best described by the exponential function $V_T = V_0 e^{-b(x)}$. The slopes described by the term b represent the instantaneous rate of gastric evacuation. The instantaneous rate for the two feeding frequencies in this study were nearly identical.

Optimal feeding frequency corresponds to the volume and rate of emptying of the stomach. If the optimum interval between feedings is the point at which evacuation of the previous meal is matched by consumption, then the optimum interval for *O. niloticus* is 4 – 5 hours, depending on the energy and composition of the diet.

Decreased protein digestibility of diets supplemented with salts of phytic acid led to depressed growth and poor performance in rainbow trout, and carp. In this investigation, tilapia were fed graded levels of phytase treated SBM, or untreated SBM, substituted into a FM based diet to determine the effects of phytic acid on growth, performance, and digestibility of CP and individual amino acids.

The growth data was used to fit two different dose-response curves to estimate maximum level of incorporation of phytase treated and untreated SBM into diets for tilapia. The two models suggested similar levels of restriction, 31 – 38 % and 14 – 17 % of the CP for untreated, and phytase treated SBM, respectively. The values for the untreated SBM represented 20 – 25 % of the diet on a DM basis, which was similar to suggested limits for tilapia, and in agreement with the industry standard limit of 25 % of the dry diet. In comparison, the models suggested phytase treated SBM should be restricted to no more than 10 % of the diet on a DM basis.

Increased protein digestibility, higher biological value, and increased N retention have been demonstrated in terrestrial animals with diets containing lower phytic acid. Therefore, a digestibility study was conducted to determine if removal of phytic acid would increase the digestibility of CP and individual AA from SBM fed to tilapia.

Apparent crude protein digestibility among the diets containing SBM ranged from 78.9 – 84.6 %, and no significant differences were detected. Apparent digestibility

coefficients were applied to dietary IAA concentrations to determine if available IAA met requirements.

In fish fed the untreated SBM diets, there was a significant negative correlation between Arg, His, and Phe with weight gain, SGR, and PER. Increasing SBM substitution led to higher dietary levels of Arg, His, and Phe, with resulting depression in growth. Although the Pearson correlation coefficients indicated a strong relationship, their statistical relevance were marginal ($0.05 < P < 0.10$). Although suggestive of a relationship, correlation analysis does not necessarily indicate a cause and effect.

In fish fed the phytase treated SBM diets, there was a strong correlation between Lys, and Met, with weight gain. Similar correlations were observed between Lys, Met, and TSAA, and all efficiency parameters. Additionally, there was a statistically marginal effect of Thr on FE, PER, and ANPU, and of Val on FE.

Crystalline L-methionine was incorporated in the diets containing 50 % or more of he CP as SBM to meet the requirement. Although dietary Met met the requirement, the calculated available Met was below the requirement. The L-crystalline Met appeared to be available to tilapia in the diets containing untreated SBM and was effective in improving growth, performance, and digestibility of the untreated SBM diets, relative to the phytase treated SBM diets used in this study.

Conversely, evidence suggested Met supplementation in the diets containing phytase treated SBM appeared to be unavailable to tilapia. A closer examination of the available Met values indicated the evidence may be misleading. It could not be determined if available Met was solely from the intact protein or partitioned between the two sources. This is a needed area for future investigation.

A number of hypotheses were presented to explain the reduced availability of AA and energy from the diets containing phytase treated SBM. Principal among these was that the feeding behavior of tilapia enhances the potential for leaching of soluble components. Additionally, phytic acid may serve in a protective capacity by reducing the effects of other ANFs, or protecting sensitive AA, such as Lys, from degradation during processing or pelleting. Finally, the phytase treatment process itself may be responsible for altering availability of AA, energy, or some other essential nutrient.

Incorporating phytate pretreated SBM beyond 25 % of the CP led to a depression in weight gain and decreased efficiency. In comparison, incorporation of untreated SBM into the reference diet did not decrease weight gain, or diminish efficiency until the level of substitution surpassed 75 % of the CP. This would suggest phytic acid may play a role in sustaining protein integrity and availability of AA in tilapia.

If phytic acid effects growth and efficiency, the results should be reproducible in a dose-response manner using a purified, or semi-purified form. To evaluate the effect phytic acid may have on growth, efficiency, and amino acid digestibility, tilapia were fed a fish meal based diet supplemented with graded levels of phytic acid as Na-phytate.

No significant differences were detected in FE, PER, ANPU, or whole body proximate composition, regardless of rate of supplementation. The CP digestibilities of these diets showed no significant differences. A multiple regression analysis on ACPD of the Na-supplemented FM diets indicated ACPD was independent of dietary phytic acid. Apparent digestibility of individual amino acids in the Na-supplemented FM diets was similar to ACPD. Other than a few exceptions, the AA digestibility coefficients followed the same pattern for each IAA. The data from the phytic acid supplemented

diets suggest there was neither a beneficial, or detrimental effect due to the supplementation in terms of growth, efficiency, body composition, or digestibility.

Wild Nile tilapia have been reported to have gastric pH values as low as 1.0-2.0. Low gastric pH in wild tilapia assists digestion by hydrolysis of phytoplankton and bacterial cell walls, and in mineral decomposition of periphytic detrital aggregate. Maximal inhibition of acidic proteases by phytic acid occurs near pH 2.0 suggesting an environment favorable for enzyme inhibition. However, maximum activity of a protease isolated from *O. niloticus* gastric mucosa was observed at pH 3.5, with minimal activity reported at pH values outside the range of 2.0 - 5.5. Measurements of pH were made in the tilapia GI tract, following feeding, to determine if pH in fish fed nutrient dense diets were similar to those in the wild.

Values as low as 2.5 – 2.75 were recorded on sites in contact with the acid-secreting cells localized to the ventral face of the mucosa. However, the mean values ranged from 5.36 shortly following feeding, to 3.52 at 4 hours postprandially. Values following the feeding of a high nutrient dense diet were closer to the optimal pH for enzyme activity than were pH values from wild fish. The resultant pH values provided evidence that gastric mixing of the digesta was minimal.

The pH values in the intestine remained relatively consistent throughout the sampling period, both within a segment and with time. The mean pH values measured in the posterior segments of the intestine ranged from near 7.0 – 8.3. These values are optimal for protein-cation-phytate complex formation in the intestine.

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APPENDICES

APPENDIX 1

RECIRCULATING SYSTEM DIAGRAM

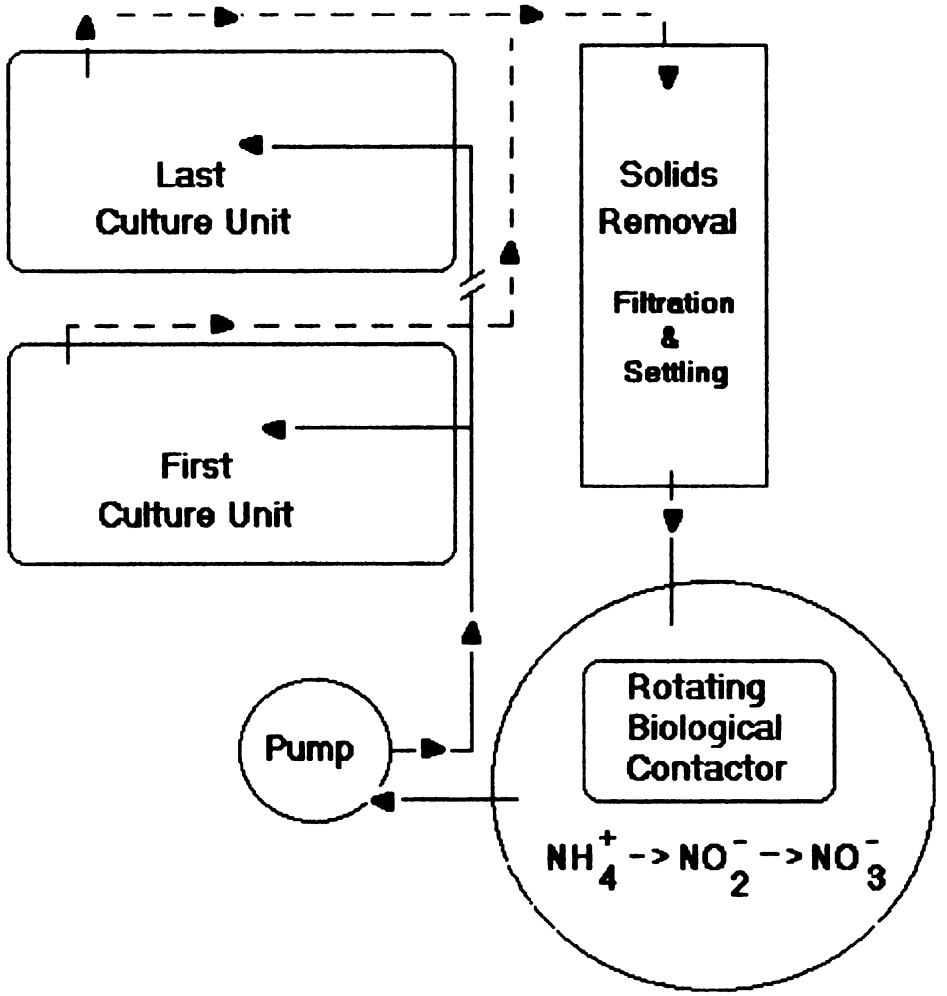

Figure 29. Conceptual flow diagram of the recirculating system used in the experiments.

APPENDIX 2

Processing Steps for Solvent Extracted Soybean meal obtained from Zeeland Farm Services, Zeeland, MI.

Preparation:

1. Conditioning – heat to 150 F
2. Dry soybeans – heat to 150 - 190 F
3. Crack beans in half
4. Dehull beans by aspiration
5. Crack beans to ¼ and crack beans again to 1/8
6. Dehull and recondition by heating to 150 F
7. Flaking – down to 15/1000 of an inch

Extraction:

1. Extract with hexane solvent
2. Desolventize
3. Toast
4. Dry
5. Cool

APPENDIX 3

Table 23. Predictive equations and R^2, (n=12) for linear regression and non-linear regression models applied to growth and efficiency parameters in juvenile tilapia fed diets containing untreated, or phytase treated, solvent extracted SBM substituted into a reference diet at 25, 50, 75, or 100 % of the dietary protein. Parameters include specific growth rate (SGR), feed efficiency (FE), protein efficiency ratio (PER), and apparent net protein utilization (ANPU).

Parameter	Model	Predictive Equation	R^2
Weight Gain			
Phytase Treated	Linear	$y = -4.16x + 817.2$	0.621
	Quadratic	$y = 0.047x^2 - 10.07x + 965$	0.661
Untreated	Linear	$y = -4.20x + 846.5$	0.623
	Quadratic	$y = 0.004x^2 - 4.71 + 859$	0.624
SGR			
Phytase Treated	Linear	$y = -0.0113x + 4.02$	0.600
	Quadratic	$y = 7x10^{-5}x^2 - 0.02x + 4.24$	0.611
Untreated	Linear	$y = -0.0114x + 4.10$	0.610
	Quadratic	$y = -1x10^{-5}x^2 - 0.01x + 4.06$	0.610
Feed Efficiency			
Phytase Treated	Linear	$y = -0.0003x + 0.872$	0.709
	Quadratic	$y = -4x10^{-6}x^2 - 0.002x + 0.859$	0.710
Untreated	Linear	$y = -0.003x + 0.957$	0.715
	Quadratic	$y = -5x10^{-5}x^2 + 0.003x + 0.813$	0.795
PER			
Phytase Treated	Linear	$y = -0.0114x + 2.78$	0.824
	Quadratic	$y = -2x10^{-5}x^2 - 0.0085x + 2.71$	0.825
Untreated	Linear	$y = -0.0093x + 2.84$	0.727
	Quadratic	$y = -1x10^{-3}x^2 + 0.0086x + 2.40$	0.813
ANPU			
Phytase Treated	Linear	$y = -0.166x + 42.14$	0.857
	Quadratic	$y = -0.0002x^2 - 0.189x + 42.74$	0.857
Untreated	Linear	$y = -0.117x + 43.06$	0.624
	Quadratic	$y = -0.0026x^2 + 0.208x + 34.90$	0.779

APPENDIX 4

Table 24. Mean pH values (± SE) on surface of feed collected at the esophagus, pyloric sphincter, and eight sites within excised stomachs as described in text. Numbers within parentheses represent number of samples collected. Samples were collected before feeding and 0.5, 1, 2, 4, 6, and 8 hours following an initial feeding. The two groups labeled 6 and 8 hours were sampled 2 and 4 hours following a second meal, respectively.

	Esophagus	Pylorus	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10
Before Feeding	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
0.5 Hour	N/A	N/A	5.51±0.24 (5)	5.23±0.25 (5)	5.65±0.60 (5)	5.48±0.55 (5)	5.23±0.28 (5)	5.32±0.32 (5)	5.28±0.37 (5)	5.18±0.54 (5)
1 Hour	N/A	N/A	5.32±0.32 (4)	5.12±0.40 (4)	5.40±1.21 (4)	4.96±0.28 (4)	6.06±0.99 (4)	5.01±0.17 (4)	4.51±0.45 (4)	5.41±0.72 (4)
2 Hours	3.56±0.74 (2)	4.43±0.39 (3)	3.56±0.52 (5)	3.52±0.52 (5)	3.70±0.52 (5)	3.60±0.39 (5)	3.55±0.56 (5)	3.62±0.59 (5)	3.76±0.14 (5)	3.88±0.33 (5)
4 Hours	4.77±2.47 (2)	4.27±1.11 (2)	3.30±0.33 (5)	3.00±0.20 (5)	3.81±0.17 (5)	3.50±0.20 (5)	3.45±0.35 (5)	3.26±0.27 (5)	3.99±0.34 (5)	3.87±0.43 (5)
6 Hours	N/A	N/A	N/A	4.39±0.23 (5)	4.43±0.36 (5)	4.09±0.65 (5)	4.00±0.72 (5)	3.94±0.95 (5)	3.75±0.80 (5)	4.72±0.22 (5)
8 Hours	4.34±1.16 (5)	4.20±0.43 (5)	3.74±0.47 (5)	3.71±0.11 (5)	3.41±0.41 (5)	3.40±0.42 (5)	3.79±0.37 (5)	3.84±0.41 (5)	3.37±0.26 (5)	3.65±0.28 (5)

APPENDIX 5

Table 25. Mean pH values (± SE) of gastric mucosa collected at the esophagus, pyloric sphincter, and eight sites within excised stomachs as described in text. Numbers within parentheses represent number of samples collected. Samples were collected before feeding and 0.5, 1, 2, 4, 6, and 8 hours following an initial feeding. The two groups labeled 6 and 8 hours were sampled 2 and 4 hours following a second meal, respectively.

	Esophagus	Pylorus	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10
Before Feeding	6.81±0.39 (5)	6.81±0.47 (5)	6.51±0.84 (5)	6.77±0.52 (5)	6.58±0.42 (5)	6.80±0.26 (5)	6.78±0.28 (5)	6.45±0.51 (5)	6.63±0.51 (5)	6.70±0.28 (5)
0.5 Hour	6.27±0.52 (5)	6.40±0.62 (4)	6.27±0.31 (5)	6.25±0.25 (5)	6.03±0.28 (5)	6.05±0.08 (5)	6.03±0.17 (5)	5.90±0.25 (5)	6.08±0.22 (5)	6.37±0.55 (5)
1 Hour	7.01±0.64 (4)	6.79±0.76 (4)	7.10±0.22 (4)	7.02±0.67 (4)	7.07±0.88 (4)	7.11±0.52 (4)	6.74±0.47 (4)	6.66±0.22 (4)	6.99±0.62 (4)	6.67±0.50 (4)
2 Hours	5.66±0.51 (5)	5.34±0.81 (5)	5.29±0.51 (5)	4.82±0.31 (5)	4.84±0.26 (5)	4.90±0.68 (5)	5.20±0.44 (5)	5.25±0.22 (5)	4.94±0.38 (5)	5.02±0.52 (5)
4 Hours	4.99±0.92 (5)	5.21±0.66 (5)	4.77±0.92 (5)	4.83±0.33 (5)	4.90±0.49 (5)	4.87±0.48 (5)	4.97±0.50 (5)	5.13±0.50 (5)	4.87±0.75 (5)	5.18±0.64 (5)
6 Hours	5.91±0.65 (5)	6.19±0.35 (5)	5.85±0.64 (5)	6.07±0.49 (5)	5.36±0.59 (5)	5.40±0.66 (5)	5.11±0.55 (5)	5.50±0.27 (5)	5.64±0.34 (5)	5.80±0.25 (5)
8 Hours	5.77±0.76 (5)	5.61±0.94 (5)	4.68±0.26 (5)	4.93±0.60 (5)	4.77±0.85 (5)	4.92±0.61 (5)	4.30±0.85 (5)	4.72±0.60 (5)	4.85±0.74 (5)	4.96±0.61 (5)

APPENDIX 6

Table 26. Mean pH values (± SE) of intestinal mucosa measured at three sites in the most anterior segment, and nine other sites as described in Figure 2. Measurements were made before feeding, and 0.5, 1, 2, 4, 6, and 8 hours following an initial feeding. Values represent 5 measurements in each segment.

	Before Feeding	0.5 Hours Post-Feed	1 Hour Post-Feed	2 Hours Post-Feed	4 Hours Post-Feed	6 Hours Post-Feed	8 Hours Post-Feed
Segment 1							
Anterior	6.77±0.16	6.44±0.10	7.38±0.97	6.51±0.18	6.74±0.52	6.60±0.29	6.64±0.13
Middle	6.93±0.32	6.77±0.20	6.98±0.36	6.54±0.16	6.82±0.51	6.71±0.32	6.54±0.21
Posterior	6.90±0.34	6.97±0.33	7.26±0.20	6.61±0.18	6.92±0.77	6.72±0.23	6.67±0.16
Segment 2	7.19±0.46	6.93±0.31	7.75±0.69	6.90±0.22	6.89±0.41	6.86±0.23	6.82±0.29
Segment 3	7.17±0.41	7.11±0.18	7.67±0.44	7.24±0.15	7.23±0.48	7.00±0.30	7.26±0.36
Segment 4	7.24±0.31	7.11±0.34	7.75±0.59	7.73±0.33	7.47±0.35	6.92±0.45	7.44±0.38
Segment 5	7.34±0.09	7.04±0.28	7.96±0.82	7.71±0.16	7.99±0.20	7.14±0.50	7.82±0.28
Segment 6	7.72±0.49	6.78±0.30	7.55±0.92	7.50±0.08	7.77±0.49	7.55±0.47	7.45±0.63
Segment 7	7.28±0.25	6.86±0.45	7.29±0.80	7.63±0.34	7.53±0.26	6.98±0.21	7.39±0.25
Segment 8	7.40±0.23	6.96±0.13	7.70±0.73	7.46±0.20	7.23±0.18	6.91±0.30	7.35±0.35
Segment 9	7.19±0.21	6.77±0.18	7.99±0.85	7.50±0.41	7.54±0.31	7.15±0.40	7.65±0.35
Segment 10	7.19±0.39	6.72±0.58	8.29±0.81	7.42±0.08	7.42±0.30	7.20±0.22	7.21±0.23