

**EFFECT OF REPLACING FISH MEAL WITH SIMPLE OR
COMPLEX MIXTURES OF VEGETABLE INGREDIENTS
IN DIETS FED TO NILE TILAPIA
(*OREOCHROMIS NILOTICUS*)**

A Thesis Submitted to the
College of Graduate Studies and Research
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in the Department of Animal and Poultry Science
University of Saskatchewan
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ABSTRACT

The effect of fractionating flax, peas and canola on the digestibility of these ingredients in Nile tilapia was determined. Dehulling of flax, and processing peas and canola to pea protein concentrate and canola protein concentrate, resulted in significant increases in the energy and dry matter digestibility's of these products ($P < 0.05$). The protein digestibility of flax was significantly improved by dehulling but there were no differences between the protein digestibilities of peas, canola and their protein concentrates. The ability of one of the most digestible ingredients from peas, flax and canola determined the digestibility trial, to replace fish meal in tilapia diets was examined in an eight week growth trial. The diet formulations were arranged in a 2 x 4 factorial design with 2 types of plant protein mixtures used to replace fish meal (simple: soybean meal and corn gluten meal or complex: soybean meal, corn gluten meal, dehulled flax, pea protein concentrate and canola protein concentrate) and 4 levels of protein originating from fish meal (100%, 67%, 33% and 0%). Diets contained equal concentrations of digestible protein (380 g kg^{-1}) and digestible energy (17.6 MJ kg^{-1}). Fifty six tanks containing 10 male Nile tilapia each were used in this experiment. Fish were fed to apparent satiation twice daily for a total of 56 days and growth and feed intake was measured for the entire experimental period. On day 56 of the experiment, one fish per tank was euthanized and a 1 cm segment of small intestine was prepared for measurement of villus length. The average daily gains (0.97 g d^{-1}), specific growth rates ($1.79 \% \text{ d}^{-1}$) and feed efficiencies (3.28 g d^{-1}) of fish fed diets with 0% fish meal were significantly lower than fish fed diets with the 33.3, 66.6 or 100% fish meal levels. Fish fed the complex diets had significantly higher average daily gains (2.29 g d^{-1}), specific

growth rates ($3.40\% \text{ d}^{-1}$), feed: gain ratios (1.46 g d^{-1}) and protein efficiency ratios ($1.59 \text{ gain protein intake}^{-1}$) than those fed the simple diets. Villus length decreased with decreasing levels of fish meal and increased with increased diet complexity but the effects were not significant. The results indicated that the use of a complex mixture of plant ingredients may allow for a greater replacement of fish meal in diets fed to Nile tilapia.

DEDICATION

This thesis is dedicated to ...

...my grandfather who cherished the simple life: family, farming
and fishing.

ACKNOWLEDGEMENTS

It is with great appreciation that I thank my supervisor, Dr. Murray Drew for his guidance, academic advice, and encouragement throughout my course. I would also like to acknowledge the time and constructive criticism put in by my committee members, Dr. Dave Christensen, Dr. Phil Thacker and Dr. Fiona Buchanan.

I would like to thank the Canada-Agro Innovation Fund and EWOS Ltd. for there funding of this project. As well, I would also like to thank the following for there contributions of feed ingredients: Cargill, MCN Bioproducts, Oleet Processing, Parrheim Foods and Western Grain Ltd.

I would like to acknowledge Debbie Theissen for showing me the ropes in the Prairie Aquaculture Research Center and teaching me a thing or two about raising fish. It is with your patience and attention to detail that the barn was able to run smoothly (most days!).

It was my privilege to be able to work with Lisa White. She has made the last two years much more manageable and at times even enjoyable. Being quick on her toes she is able to solve unforeseeable problems with ease (fish jumping out of tanks, water leaking from pipes, fish stuck in pumps, heaters not heating, and propane freezing). Her advice, humor and love for good books were greatly welcomed.

A special thanks to Jodi Bourn. A supportive friend who always lends an ear is something that a grad student can not be without! I think a barn-buddy like you will be hard to top.

I would like to thank my parents, Bob and Connie for there patience and understanding. You finally have your daughter back! Without your help Dad, fixing pipes, opening sticky valves, supplying me with tools and ladders and helping me net

those quick fish, I would still be in the barn to this day. I would also like to thank my brother Craig, for his encouragement and help weighing diets- I still can't understand why you never wanted to help me clean tanks.

Lastly, I would like to extend a heartfelt thank you to my husband, Steve for his encouragement, much needed distractions and patience when I had to "quickly stop at the barn". I will never forget the night we spent hours upon hours trying to catch those little fish only to end up soaked.

TABLE OF CONTENTS

PERMISSION TO USE STATEMENT.....	I
ABSTRACT.....	II
DEDICATION.....	IV
ACKNOWLEDGEMENTS	V
LIST OF TABLES	X
LIST OF TABLES	X
LIST OF FIGURES	XII
1.0 INTRODUCTION.....	1
2.1 WORLD FISH SUPPLIES.....	4
2.1.1 STATE OF WORLD FISHERIES.....	4
2.1.2 AQUACULTURE	6
2.1.2.1 Sustainable Aquaculture Practices	7
2.1.2.2 Cultured Species	9
2.1.2.3 Tilapia	12
2.2 TILAPIA	12
2.2.1 PHYLOGENY	12
2.2.2 ORIGIN	13
2.2.3 ENVIRONMENTAL FACTORS	13
2.2.3.1 Temperature	13
2.2.3.2 Salinity	14
2.2.3.3 pH.....	15
2.2.3.4 Dissolved Oxygen.....	15
2.2.4 ECOLOGY OF OREOCHROMIS NILOTICUS	16
2.2.4.1 Feeding.....	16
2.2.4.2 Breeding Stock.....	16
2.2.4.3 Mating	17
2.2.4.4 Parental Care	17
2.2.4.5 Reproduction.....	18
2.2.5 PRODUCTION OF TILAPIA	20
2.2.5.1 Culture Systems	21
2.2.5.2 Economic Importance of Tilapia.....	23
2.2.5.3 Marketing Tilapia.....	23
2.3 NUTRIENT REQUIREMENTS FOR TILAPIA	24
2.3.1 ENERGETICS	24
2.3.2 ENERGY VALUE OF FEED INGREDIENTS	25
2.3.2.1 PROTEIN	25
2.3.2.2 LIPID	31
2.3.2.3 CARBOHYDRATE	32

2.4 PEAS, CANOLA AND FLAX AS INGREDIENTS IN TILAPIA DIETS	34
2.4.1 PEAS.....	34
2.4.1.1 Antinutritional Factors	35
2.4.1.2 Digestibility of Peas in Aquaculture	38
2.4.2 CANOLA	39
2.4.2.1 Antinutritional Factors	39
2.4.2.2 Digestibility of Canola in Aquaculture	42
2.4.3 FLAX	43
2.4.3.1 Antinutritional Factors	43
2.5 PROCESSING AQUATIC FEEDS.....	44
2.5.1 CHEMORECEPTION	45
2.5.1.1 Olfaction	45
2.5.1.2 Gustation	45
2.5.2 FISH FEEDING CHARACTERISTICS	46
2.5.3 PELLET CHARACTERISTICS.....	46
2.5.3.1 Dry vs. Moist Feed.....	46
2.5.3.2 Physical Characteristics of Dry Feed	47
2.5.4 EXTRUSION	48
2.5.4.1 Types of Extruders	48
2.5.4.2 Flavors.....	49
2.5.4.3 Nutrients.....	49
2.5.4.4 Antinutritional Factors	52
2.5.4.5 Buoyancy	53
2.5.4.6 Stability of Feed in Water	54
3.0 EFFECT OF REPLACING FISH MEAL WITH SIMPLE OR COMPLEX MIXTURES OF VEGETABLE INGREDIENTS IN DIETS FED TO NILE TILAPIA (OREOCHROMIS NILOTICUS)	55
3.1 INTRODUCTION.....	55
3.2 MATERIALS AND METHODS	57
3.2.1 Digestibility Trial.....	57
3.2.2 Environmental Conditions and Fish Management.....	59
3.2.3 Laboratory Analyses	60
3.2.4 Statistical Analysis.....	60
3.2.5 Growth Trial.....	63
3.2.6 Environmental Conditions and Fish Management.....	66
3.2.7 Experimental Diet Analysis	66
3.2.8 Statistical Analysis.....	66
3.3 RESULTS.....	68
3.3.1 Digestibility Trial.....	68
3.3.2 Growth Trial.....	70
3.4 DISCUSSION.....	73
4.0 CONCLUSIONS	79
5.0 REFERENCES.....	82

6.0 APPENDICES	105
APPENDIX A: EXTRUSION PROCESS USED TO MANUFACTURE THE FEED USED IN THE TILAPIA GROWTH TRIAL.	105
APPENDIX B: METHODS USED TO PROCESS INGREDIENTS USED IN THIS STUDY.....	115
APPENDIX C: FISH MANAGEMENT FOR DIGESTIBILITY AND GROWTH TRIAL.	116
APPENDIX D: GROWTH TRIAL DATA ARRANGED IN 2 WEEK PERIODS.....	120
APPENDIX E: TILAPIA VILLI MICROGRAPHS (50X).....	124

LIST OF TABLES

Table 2.1 Weight and economic value for the top aquaculture species consumed in 1997.....	10
Table 2.2 Canadian aquaculture production and value for finfish and shellfish in 2000.....	11
Table 2.3 Inclusion rates of dietary protein for tilapia species at varying stages of growth.....	28
Table 2.4 Amino acid requirements for tilapia.....	28
Table 3.1 Composition of reference diet used in digestibility trial.....	58
Table 3.2 Nutrient analysis of test ingredients incorporated into the experimental diets.....	61
Table 3.3 Nutrient analysis of reference diet and experimental diets with 30% of the test ingredient used for the digestibility trial (n=2).....	62
Table 3.4 Growth trial experimental diet formulation for simple and complex diets with varying levels of protein originating from fishmeal (g kg ⁻¹).....	64
Table 3.5 Nutrient analysis of experimental diets used for the growth trial (n=2).....	67
Table 3.6 Apparent digestibility coefficients of experimental diets with test ingredients (%) determined in digestibility trial.....	69
Table 3.7 Amino acid content of the diets fed in the growth trial (g kg ⁻¹ as-fed basis).....	71
Table 3.8 Performance of tilapia based on simple and complex diets with varying levels of fishmeal for days 0-56 of the growth trial.....	72

Table B1 Processing methods used to prepare the pea, canola and flax products used in this study.....	113
Table C1 Fish mortality in the tilapia digestibility trials.....	114
Table C2 Fish mortality in the tilapia growth trial.....	116
Table C3 Stocking density of tilapia with different weights and feeding rates (Rakocy, 1989).....	117
Table D1 Performance of tilapia based on simple and complex diets with varying levels of fishmeal for days 0-14 of the growth trial.....	118
Table D2 Performance of tilapia based on simple and complex diets with varying levels of fishmeal for days 14-28 of the growth trial.....	119
Table D3 Performance of tilapia based on simple and complex diets with varying levels of fishmeal for days 28-42 of the growth trial.....	120
Table D4 Performance of tilapia based on simple and complex diets with varying levels of fishmeal for days 42-56 of the growth trial.....	121

LIST OF FIGURES

Figure 2.1 Energy flow through fish (National Research Council, 1993).....	26
Figure 3.1 Interaction between diet complexity and the level of protein originating from fish meal for specific growth rate (A) and feed:gain ratio (B) of tilapia for days 0-56 in the growth trial. Means with different superscripts are significantly different ($P < 0.05$).....	74
Figure A1 Mixed feed is placed into a hopper bin where it drops onto a conveyor belt at a pre-determined rate.....	103
Figure A2 The mixed diet travels along the conveyor belt to the pre-conditioner and then into the barrel of the extruder.....	104
Figure A3 The barrel consists of 8 different sections with 6 different temperature probes, water injection sites, a twin co-rotating screw and a 6-hole strand die plate. The screw speed was held constant between 200 and 225 rpm and the temperature ranged between 70 - 116° C between the 8 sections of the barrel.....	105
Figure A4 A single screw consists of 38 elements making a total length of 1.5 meters.....	106
Figure A5 Individual elements are placed together specifically to meet the needs of client. In our case the elements consist of left hand and right hand kneading blocks, concave and non-concave elements.....	107
Figure A6 Feed is moved through the barrel being exposed to shearing, high temperatures and pressure averaging 2400 kPa and is then released though the 6 hole strand die.....	108

Figure A7 Drying the extruded product in a continuous fluid bed dryer for approximately 15 minutes at a temperature of 40°C.....	109
Figure A8 Feed dropping out of the continuous fluid bed dryer with a moisture content of approximately 8%.....	110
Figure A9 Adding fish oil following the drying procedure. The diet is placed into a rotating vacuum tumbler along with the fish oil and is mixed for 15 minutes at 11 rpm. Because the feed expanded as it exited the die, it becomes porous allowing for the fish oil to be absorbed during the vacuum process.....	111
Figure A10 Pelleting the diets using a quadro mill.....	112
Figure C1 Anatomical differences between male and female tilapia (Auburn University, 2005).....	116
Figure E1 Tilapia intestinal section from fish fed the 0% Simple diet.....	122
Figure E2 Tilapia intestinal section from fish fed the 0% Complex diet.....	123
Figure E3 Tilapia intestinal section from fish fed the 100% Fishmeal diet.....	124

1.0 Introduction

As the global population continues to grow, so to does the world's need for food, especially for sources of high quality protein (Chamberlain, 1993). With the world population currently over 6 billion, and forecasts exceeding 8 billion by the year 2030, the consumption of seafood at that time is predicted to reach between 150-160 million tonnes (Food and Agriculture Organizations of the United Nations, 2005). However, because of the decline in wild fish catches in a large part due to the unsustainability of industrialized fishing, aquaculture will be called upon to fill this gap.

Between 1984 and 1990, the world aquaculture sector grew at an average annual rate of 16% for finfish and shellfish production (Tacon, 1993). While the growth of the aquaculture industry has slowed down slightly since 1990, it continues to grow at a rate of 5% per year (Chamberlain, 1993). According to Chamberlain (1993), an annual growth of 6.5% is needed to meet the demand for seafood by the year 2025. To continue this level of growth, research and development on farming techniques will be required in order to obtain the most efficient, safe and cost effective methods for producing seafood in the aquaculture industry. A primary concern for rearing finfish in a culture setting is the high cost of feed, and more importantly the heavy reliance on fish meal and fish oil as the primary protein and energy sources in these feeds.

In 1990, approximately 30% of the total fish catch was converted into fish meal and fish oil for use in animal and fish feeds (Tacon, 1993). While the total production of fish meal and fish oil has decreased slightly since 1990, exports have decreased in countries that typically produced large amounts, due to the rapid growth of their

aquaculture industries (Tacon, 1993), which is an indicator of the limited availability of these products.

Fish meal and fish oil have become staples in aquaculture diets due to their many nutritional benefits (Tacon, 1993). Fish meal is very palatable and has exceptional nutritional value including an excellent balance of essential amino acids and essential fatty acids, which closely meet the requirements of most farmed fish, and also provides an excellent source of digestible energy and vitamins (Tacon, 1993). However, fish meal and fish oil are considered essential dietary requirements for virtually all finfish species. While it remains a challenge to replace fish meal and fish oil with suitable plant ingredients in diets fed to carnivorous species, herbivorous fish species seem well suited to the consumption of plant-based diets.

One of the most important herbivorous fish species reared in aquaculture systems is the tilapia (Fisheries of the United States, 2001). Production of tilapia reached over 1.2 million metric tons in 2001 (Fisheries of the United States, 2001) and is produced in nearly every country world in the world. There are three genera of tilapia; *Oreochromis*, *Sarotherodon* and *Tilapia*. The primary genus reared for aquaculture is *Oreochromis* which includes Nile Tilapia (*O. niloticus*), Mozambique Tilapia (*O. mossambicus*), and Blue Tilapia (*O. aureus* and *O. urolepis hornorum*) (Fitzsimmons, 1997). With the continued growth of tilapia production, the need for suitable diets tailored using local ingredients that are produced within each country has become a necessity.

Tilapias are naturally accustomed to eating plant ingredients, and are typically considered strict herbivores once they reach maturity (Keenleyside, 1991). A substantial amount of research is already underway, testing potential protein sources that can replace fish meal in tilapia diets. These plant protein sources include soyabeans,

sunflower, rapeseed, wheat bran, corn gluten meal, cassava leaf meal, barley and alfalfa (Jackson et al., 1982; Ng et al., 1989; Davies et al., 1990; Olvera-Novoa et al. 1990; Wu et al., 1995; El Sayed, 1998; Belal, 1999; Maina et al., 2002; Abelghany, 2004). While many plant protein sources have the potential for use in tilapia diets, there are a number of problems associated with the inclusion of these ingredients. First and foremost is the quality of protein. Generally plant ingredients that are to be used in animal or fish feeds are by products of ingredients that have undergone processing for entry into the human food chain, or are considered poor quality and are therefore used by the aquafeed industry (De Silva and Anderson, 1995). Because of this, the quality of protein or more specifically, the balance of amino acids may be inferior. For example, legumes tend to be high in lysine but limiting in methionine, whereas cereal grains are limiting in lysine (De Silva and Anderson, 1995). A second potential problem is the presence of antinutritional factors in many plant protein sources (De Silva and Anderson, 1995). These naturally occurring compounds found in feedstuffs can have a negative effect on the performance of fish species (De Silva and Anderson, 1995). For example, phytic acid, tannins, protein protease inhibitors, erucic acid, glucosinolates, mucilage and fibre are some of the antinutritional factors found in peas, canola and flax (Castell et al., 1996; Naczek et al., 1994; Grant, 1998). Processing methods such as dehulling, aqueous extraction, and extrusion can reduce the negative effects of these antinutritional factors as well as improving the availability of proteins and carbohydrates in the plant ingredients (De Silva and Anderson, 1995).

Based on these observations, the objectives of this project were to assess the effect of fractionation on the digestibility of experimental diets with single ingredients produced in Western Canada including flax, peas and canola in Nile tilapia

and to determine the effect of replacing fish meal with simple (soyabean and maize gluten meal) or complex (soyabean meal, maize gluten meal, dehulled flax, pea protein concentrate and canola protein concentrate) mixtures of plant proteins on the performance of Nile tilapia.

2.0 Review of Literature

2.1 World Fish Supplies

2.1.1 State of World Fisheries

The advent of steam trawlers gave fishing a new identity known as industrialized fishing (Pauley et al., 2002). Vessels were equipped with diesel engines and power winches following the First World War. Shortly after the Second World War, fishing fleets became outfitted with freezer trawlers, radar and acoustic fish finders (Pauly et al., 2002). With overzealous fishing fleets, new technology and fishing strategies, it was clear that fish stocks would be significantly affected. However, no one could predict how long it would take for the fish stock to become depleted.

The collapse of the Peruvian anchovy fishery in 1971-1972 was the first reported incident to shake the fishing community. Originally explained as an El Niño event, it is now evident that overfishing played an important role in this demise (Pauley et al., 2002). In 1992, the Canadian Grand Banks, a lucrative fishing ground off the coast of Newfoundland were closed to fishermen by the Canadian Department of Fisheries and Oceans due to the collapse of the Atlantic cod stocks (Schiermeier, 2002). The devastation caused by these two events has created a great deal of animosity among scientists, politicians and fisherman (Pauley et al., 2002).

Within a 15 year period, it is estimated that predatory fish stocks of four continental shelves and nine oceanic systems had been depleted by 80% due to industrialized fishing (Myers and Worm, 2003). Analysis of fishing data by Myers and Worm (2003) indicates that with dominant fish populations declining, harvesting of alternative predatory fish species increased. The result of this type of cycle is fewer predatory fish and fishing fleets targeting species lower on the food web (Myers and Worm, 2003).

Models have been developed by fisheries scientists in an attempt to understand how to obtain maximum sustainable yields of fish stocks. These models have led to advisory notices stating that a reduction in the size of fishing fleets and catch quotas must be implemented as well as rules and regulations regarding the use of specific fishing gear and acceptable fishing zones (Schiermeier, 2002; Pauley et al., 2002).

Recently, the validity of these models has been questioned. A number of the scientific models lack a complete understanding of the dynamics of fish populations with respect to predators, prey and habitat. Single population models have proven to be inaccurate because they rely on information pertaining to stock status and stock withdrawal, which will always be error prone (Pauley et al., 2002). Single population models do not consider the effect of predator and prey populations on the complexity of the marine food web. As fishing fleets continue to exhaust the ocean of fish stocks, they continuously fish at lower trophic levels leading to an ecosystem susceptible to extensive damage (Schiermeier, 2002).

In spite of the research that has been conducted thus far, it is evident that an ideal solution has yet to be found for the declining fish stocks. Until scientists, fishers and

politicians agree on one strategy and join in the effort, world fish stocks will continue to be in jeopardy.

With an expected human population of 8.5 billion by the year 2025, world fisheries will be unable to fulfill the increasing demand for seafood. Seafood consumption is estimated to reach 162 million tones based on a global per capita seafood consumption of 19 kg per year (Chamberlain, 1993). Currently one quarter of all fish consumed by humans is produced by the aquaculture sector (Naylor et al., 2000). Clearly only aquaculture has the potential to fill in the gap between supply and demand caused by the decline in world fisheries wild catches and human population growth.

2.1.2 Aquaculture

Aquaculture production has continued to grow explosively in the last several decades. Aquaculture production, as a percentage of total fisheries landings by weight increased from 5.3% in 1970 to 32.3% in 2000 (Food and Agriculture Organizations of the United Nations, 2002). Aquaculture has maintained an average annual growth rate of 8.9% since 1970 compared with growth rates of terrestrial farmed meat production systems of 2.8% and capture fisheries of 1.4% (Food and Agriculture Organizations of the United Nations, 2002).

Environments and communities can benefit from aquaculture in a number of ways (Frankin and Hershner, 2003) including an increase in the food supply, an increase in employment, an increase in food value, protection of aquatic biodiversity through restocking, a reduction in need for wild catches and an improvement of fish habitats.

With all the benefits aquaculture has to offer, it also has the potential to be very destructive. The production of carnivorous species and shrimp may result in habitat destruction, contamination of waters through waste disposal, introduction of exotic species, pathogens, and the removal of large amounts of fish meal and fish oil to meet their feed requirements (Naylor et al., 2000). If aquaculture is to aid in the recovery of world fish stocks through intense production, it must exchange the “fishing down and farming up” strategy for a more sustainable approach (Watson and Pauly, 2001).

2.1.2.1 Sustainable Aquaculture Practices

Naylor et al. (2000) compiled a list of goals for the aquaculture industry to strive towards in order to develop an industry that will survive and continue to grow for years to come. Topping their list is farming down the food web and reducing the use of fish meal and fish oil. The food web is made up of four trophic levels with the first level, producers, harvesting energy directly from the sun. The second level is the primary consumers or herbivores followed by the third level, secondary consumers or omnivores, and finally the fourth level, tertiary consumers or carnivores. With each increase in trophic level, the amount of energy transferred between organisms is decreased (Naylor et al., 2000).

Carnivorous fish, such as salmon and trout, require large amounts of fish meal in their diets in order to satisfy dietary protein requirements (Naylor et al., 2000).

Typically, one kilogram of fish raised in an intensive aquaculture setting requires 1.9 kilograms of wild fish byproducts (Naylor et al., 2000). Farming low trophic level fish (primary consumers) with herbivorous diets will reduce the amount of fish meal being used because of the ability of these fish to utilize plant based proteins. As well, plant

protein sources may be more affordable as the price of fish meal continues to rise with an increase in demand.

Integrated production systems, otherwise known as polyculture, house a number of fish species together in order to effectively utilize all aspects of food and water resources (Naylor et al., 2000). China typically produces silver carp, grass carp, common carp and bighead carp together. These fish are phytoplankton filter feeders, herbivorous macrophyte feeders, omnivorous detritus bottom feeders and zooplankton filter feeders respectively. This system is very practical and its use can reduce costs, increase productivity and remove effluents from the water (Naylor et al., 2000).

The final goal to achieve sustainability in the aquaculture industry listed by Naylor et al. (2000) is to promote environmentally sound production and resource management. Initiatives must be put in place to regulate development in mangrove swamps or coastal wetlands, implement biosafety procedures for moving stock, water quality assurance and proper waste disposal, and finally implementing fines to systems with high numbers of escapes from pens.

A joint effort from the public and private sector is necessary to reach sustainability in the aquaculture industry. Government regulations put in place to control the destruction of coastal habitats and reckless management with private industry members whose goal is to eliminate pollution, reduce their dependence on fish meal and fish oil, and limit the number of non-native fish introductions will pave the way towards a prosperous and sustainable industry (Naylor et al., 2000).

In 2002, Canada contributed 0.4% of the total world aquaculture production of 40 million tonnes (Food and Agriculture Organizations of the United Nations, 2004) and brought in a revenue of \$640 million. The aquaculture industry employs 14,000

Canadians year round with the majority of the production and processing occurring in rural and coastal communities (Department of Fisheries and Oceans, 2003). Canada has recognized the impact that this industry can have both socially and economically and is moving forward with regulations and policy frameworks that will enable the Canadian industry to compete on a global scale (Department of Fisheries and Oceans, 2003). The Department of Fisheries and Oceans has listed a number of elements that must be established in order to create a sustainable industry including assuring quality of life and a safe environment for generations to come, protecting the interest of all resource users when decisions are made, using environmentally responsible practices, complying with Aboriginal and treaty rights, promoting research and development and encouraging stakeholders, individuals and communities to participate in decision-making.

2.1.2.2 Cultured Species

Today, there are more than 220 species of finfish and shellfish used in aquaculture (Naylor et al., 2000). Asia tops the list of global aquaculture production, with China accounting for two thirds of Asia's production, followed by Japan, South Korea, and the Philippines (Hanfman, 1989). Table 2.1 shows the top nine aquaculture species consumed globally. Silver carp, grass carp and common carp are among the top aquaculture species consumed. However, giant tiger prawns bring in higher revenues with considerably lower production (Hanfman, 1989). Global production (wild catch and farmed) of tilapia is ranked the fifth major species produced (Hanfman, 1989). Aquaculture finfish and shellfish production for Canada is shown in Table 2.2. Salmon production is number one in both total production and production value (Food and Agriculture Organizations of the United Nations, 1999).

Table 2.1 Weight and economic value for the top aquaculture species consumed in 1997.

	(000 tonnes)	(\$ million CAN)	\$ / tonne
Silver Carp	3,146	3,623	1.15
Pacific Cupped Oyster	2,968	3,930	1.32
Grass Carp	2,662	3,036	1.14
Common Carp	2,237	3,365	1.50
Tilapia	742	1,099	1.48
Atlantic Salmon	639	2,625	4.11
Giant Tiger Prawn	490	4,349	8.86
Milkfish	393	866	2.20
Channel Catfish	238	462	1.94

Source: Food and Agriculture Organizations of the United Nations, 1998

Table 2.2 Canadian aquaculture production and value for finfish and shellfish in 2000.

Species	tonnes	(\$ CAN / thousand)	Ave. \$ / kg
Salmon	78,495	495,555	6.29
Mussels	21,287	27,213	1.28
Oysters	10,024	16,915	1.69
Trout	6,407	31,460	4.91
Steelhead	5,523	24,889	4.49
Clams	1,000	5,900	5.90
Other Finfish	694	6,770	9.72
Other Shellfish	359	1,775	4.93
Scallops	59	362	6.12

Source: Statistics Canada, 2002

2.1.2.3 Tilapia

Tilapias are ranked as one of the most widely produced food fishes in the world with ten species being successfully cultured (Stickney, 1986). *Tilapia aurea*, *Tilapia mossambica* and *Tilapia nilotica* are among the top culture species of tilapia with hybrids of these species being produced in a number of regions (Strickeny, 1986). Because of their rapid growth, tolerance to high stocking densities and poor water quality, high reproductive rates, and susceptible to few diseases, tilapia are an excellent candidate for aquaculture production (Chamberlain, 1993; Stickney, 1986).

2.2 Tilapia

2.2.1 Phylogeny

Tilapia, found in the family Cichlidae, is a common name given to three species (*Oreochromis*, *Sarotherodon* and *Tilapia*) of the genus *Tilapia sensu lato*. The three species are grouped primarily by reproductive behaviour. Substrate spawners such as the genus *Tilapia* differ from *Sarotherodon*, a paternal or biparental mouthbrooder, and *Oreochromis* a maternal mouthbrooder (Pouyaud and Agnese, 1995). During mating, there is virtually no sexual dimorphism in *Tilapia* and *Sarotherodon* (Pouyaud and Agnese, 1995). However *Oreochromis* males tend to be larger in size than the female and display very distinct mating colours (Pouyaud and Agnese, 1995). *Tilapia* and *Sarotherodon* practice monogamy while *Oreochromis* are polygamous and polyandrous (Pouyaud and Agnese, 1995). Further differences are seen when the eggs hatch into larvae. Prior to hatching, *Tilapia* place larvae into pits dug by both parents (Trewavas, 1983). This differs from the larvae of *Sarotherodon* and *Oreochromis* which hatch in the maternal or paternal mouth (Trewavas, 1983).

Tilapiine species have different distinct forms of parental behaviour. However, they do have a number of characteristics in common. For instance, tilapiine species are generally classified as herbivorous in nature (Trewavas, 1982). Because of this type of diet, their intestines are coiled within the abdomen and may be as long as fourteen times the length of their body as compared with carnivores whose intestinal length is only 0.2 - 2.5 times their body length (Food and Agriculture Organizations of the United Nations, 1980). Their mouths are lined with bicuspid, tricuspid and pharyngeal teeth which are necessary for shredding plant material (Trewavas, 1982).

2.2.2 Origin

Africa, with the exception of Madagascar, including the Jordan Valley and the surrounding coastal rivers, is the birthplace of over eighty tilapiine species (Philippart and Ruwet, 1980; Trewavas, 1983). Within Africa, colonization of extremely different habitats by tilapia, such as rivers with and without rapids, permanent and temporary, tropical and subtropical waterways have been observed. Furthermore, the tilapiine have been found to inhabit lakes that vary in depth, alkalinity or salinity, hot springs, volcanic craters, open and closed estuaries, lagoons, and marine habitats (Philippart and Ruwet, 1980).

2.2.3 Environmental Factors

2.2.3.1 Temperature

Temperature is the main factor that dictates whether or not any species of tilapia will survive in any given body of water (Philippart and Ruwet, 1980). Lethal limits, upper and lower temperatures that are fatal for all individuals, vary for each species

(Philippart and Ruwet, 1980). Three categories of temperature tolerance have been discussed by Philippart and Ruwet (1980). *Oreochromis niloticus* is described as having a high tolerance for a range of temperatures, with a lower lethal limit of 8°C and an upper limit of 42°C. *Tilapia rendalli* have a lower tolerance to high or low temperatures (11°C to 41°C) whereas *Sarotherodon melanocheilus* has a smaller window of acceptable temperatures ranges (18°C - 33°C). Temperature tolerance not only depends on each species, but it also depends on the size of the individual. Young fish, which are smaller in size than adults, are typically more tolerant of high and low temperatures (Philippart and Ruwet, 1980).

Even though tilapias have the ability to withstand extreme temperatures, both high and low, growth and reproduction will become impaired. Below 20°C, tilapias will show a marked reduction in feed intake and normal growth will stop completely below 16°C. Temperatures below 16°C will render tilapia inactive. Similarly, reproduction will cease to occur at temperatures below 22°C (Chervinksi, 1980).

2.2.3.2 Salinity

Most tilapiine species are considered euryhaline, in other words, they are capable of withstanding a wide range in salinity (Philippart and Ruwet, 1980). *Oreochromis niloticus* is considered less euryhaline than most species, but it can survive in bodies of water with salt concentrations equal to 30% of ocean water (Philippart and Ruwet, 1980).

While tilapias that are euryhaline may be beneficial for producers, underlying problems related to salinity and growth can occur. All tilapias are both osmotic and ionic regulators due to their external medium (Jobling, 1994). When tilapia that are

adapted to freshwater, are exposed to higher saline concentrations, the metabolic cost of regulating the flow of ions and water into and out of the body can be extreme. With more energy required for osmotic and ionic regulation, feed intake and growth become impaired (Jobling, 1994). Energy demand for osmoregulation may exceed energy availability resulting in negative growth, with body reserves being mobilized (Payne et al., 1987). Not only is there a decrease in feed intake and growth, Payne et al (1987) reported an increase in water consumption with *Oreochromis spilurus spilurus* due to increasing salt concentrations, leading to hydration of tissues and ultimately a poor quality product.

2.2.3.3 pH

Research concerning pH values for tilapiine species has resulted in a wide range of values being reported as acceptable. The most favorable pH has been determined to be as close to neutral as possible in order to maintain optimum growth and health of the fish (Phillipart and Ruwet 1980; Chervinski, 1980). *Oreochromis mossambicus* were capable of withstanding acidified water (pH of 4) for 37 days without any impairment of physiological conditions including glucose, cortisol, haemoglobin, sodium and chloride levels (van Ginneken et al., 1997). In addition, there was no change in feed consumption or growth.

2.2.3.4 Dissolved Oxygen

Tilapias are very tolerant of dissolved oxygen and can tolerate levels as low as 1 part per million which is considerably lower most fish species which require 7 - 8 parts

per million (Chervinske, 1980). When oxygen drops below this level, they may utilize atmospheric oxygen which is demonstrated through the survival of tilapias found in small mud puddles at harvest time (Chervinski, 1980). Tilapias have developed a method to obtain oxygen by gulping at the water-air interface, which allows oxygenated water to pass over their gills (Stickney, 1986). Although tilapias are very hardy and can survive extremely low levels of oxygen, growth and feed intake will decrease with limited oxygen supply (Jobling, 1994).

2.2.4 Ecology of *Oreochromis niloticus*

2.2.4.1 Feeding

Fry of the *Oreochromis niloticus* species, as well as other species of tilapia, need more protein than do the adults of the species (Trewavas, 1983). Fry are considered to be omnivores, feeding on aquatic and terrestrial insects, and aquatic larvae (Trewavas, 1983). As they grow, they begin to eat more and more phytoplankton until it becomes their primary source of food. By 5 cm, this species is almost strictly herbivorous (Trewavas, 1983).

2.2.4.2 Breeding Stock

Tilapias are asynchronous spawners (Tave, 1987), with sexual maturity having a direct correlation with body length (Trewavas, 1987). This can be a challenge under culture conditions when selecting for growth traits. Early maturing lines of tilapia (*O. niloticus*) demonstrated the relationship between growth, age and size at maturity as they grew faster and larger when compared with a late maturing line (Uraiwan, 1987).

In most cases, the males are larger and more colorful in order to attract the female's attention (El-Zarka, 1970 as cited in Trewavas, 1983 pp. 187). However, this is not the case for *Oreochromis niloticus*. The males and females are similar in size at spawning and both display a red hue on the belly and lower flanks. The males red hue is usually brighter than the females (El-Zarka, 1970 as cited in Trewavas, 1983).

2.2.4.3 Mating

Oreochromis niloticus species are maternal mouthbrooders with no pair bonding. The male selects an appropriate mating site to dig a nest, one meter in diameter and one half a meter deep (Boulenger, 1908 as cited by Trewavas, 1983). With a little luck, the male attracts a ripe female who lays eggs in the nest. She then picks up the eggs while the male is fertilizing them. The female takes the fertilized eggs in her mouth and finds a safe zone. This series of events lasts only minutes. *Oreochromis niloticus* may also be polyandrous and polygamous, with females allotting eggs for more than one male, and males fertilizing eggs for more than one female (Keenleyside, 1991).

2.2.4.4 Parental Care

Parental care is a rare feature in most fish, but is a successful reproductive strategy in fish of the Cichlidae family (Keenleyside, 1991). The importance of parental care is to increase fitness by supporting growth and development of the young and providing protection from predators (Keenleyside, 1991). Parental care is provided predominantly by the maternal side in *Oreochromis niloticus* species (Keenleyside, 1991). The female will carry her brood until they develop into free swimming fry. She may release the brood from time to time so that she can forage.

Egg churning, moving the eggs around in the mouth in a circular motion, is seen in all mouthbrooders (Keenleyside, 1991). It allows for metabolic waste from the eggs to be removed. As the brood develops into wrigglers (non-free swimming young) and finally into fry, their oxygen needs rise and is also accounted for by churning (Keenleyside, 1991).

2.2.4.5 Reproduction

Regardless of the species of tilapia, the most important factors in determining their breeding season are environmental changes including the change in temperature, the amount of rainfall, and the lunar cycle. The breeding season for *Oreochromis niloticus* in its natural habitat is dependent on latitude (Trewavas, 1983). For example, the increase in water temperature in April allows for the start of spawning in the Nile delta (Trewavas, 1983). The spawning period can last until August with a peak in fertility in May (Trewavas, 1983). In some cases, the breeding season can go all year round as seen in equatorial waters.

A study of the effect that photoperiod and temperature had on *Oreochromis aureus*, *Oreochromis niloticus*, and *Oreochromis mossambicus* showed that *Oreochromis aureus* were strongly influenced by long days (>12 hours of light) and inhibited by short days regardless of favorable temperatures (Baroiller, 1997). *Oreochromis niloticus* was inhibited by short days. However, minor increases in temperature stimulated spawning activity (Baroiller, 1997).

Tilapia production occurs primarily in the tropics with year round breeding seasons. However, problems arise with mixed sex populations (Keenleyside, 1991). If

tilapias are placed in a confined space, with mixed sex populations that are allowed to breed freely, the result is an early maturing and highly prolific stock that will lead to overpopulation with undersized fish (Keenleyside, 1991). This approach from a management perspective is inefficient, which is why most producers collect the fry or fingerlings. In order to regulate population size, fry can be easily netted because they swim in schools for several days once they leave the mouthbrooder and then placed in alternative culture systems (Stickney, 1986).

In intensive culture systems some producers strip the females of their eggs by using shaker tables (Stickney, 1986). The basic idea behind this method is that the eggs are removed from the mouthbrooder through a shaking motion. The eggs are then incubated in conical shaped incubation chambers with updraft water flow (Stickney, 1986). This method is labor intensive and reports indicate that it may lead to poor survival (Stickney, 1986).

Once the fry or eggs have been collected, it is ideal to have an all male population for controlling reproduction (Keenleyside, 1991). This can be achieved by one of three management strategies. The easiest is manual sexing of the fry. This method is very time consuming, results in half the stock being discarded and has a high source of error (Keenleyside, 1991). The second method involves producing all male F1 hybrid progeny through cross breeding tilapias of different species. Crossing *Oreochromis niloticus* with *Oreochromis aureus*, *Oreochromis mossambicus*, or *Oreochromis urolepis hornorum* will result in a high percentage of the progeny being male. However, if the parental lines are not kept separate or backcrossing of progeny to parents occurs, the all male effect will be lost (Keenleyside, 1991).

The final method for producing all male progeny is sex reversal of females through hormone treatment (Keenleyside, 1991). This method has been difficult to implement in intensive culture systems but is currently being used in a number of Taiwanese hatcheries (Keenleyside, 1991).

2.2.5 Production of Tilapia

Tilapia production has recently expanded to virtually every country (Courtenay, 1997). The Caribbean islands were among the first nations to have introduced tilapia. The introduction occurred around 1940 (Courtenay, 1997). The primary objectives with the introduction of tilapias were to reduce mosquito populations, provide food for human consumption and be sold as ornamental fish (Courtenay, 1997). In 1954, Auburn University in Alabama, received tilapia, marking the first introduction of tilapia in North America (Courtenay, 1997).

Due to the introduction of tilapia species for biological control and food resources, escapes from culture facilities as well as releases by hobbyists, feral populations can be found in every country that tilapias have been imported to (Courtenay, 1997). Currently, there are few regulations governing the importation of non-indigenous fish species. The Department of Fisheries and Oceans has submitted a draft form regarding the National Policy on Introductions and Transfers of Aquatic Organisms with the intention of reducing risk of diseases and parasites as well as altering existing populations and ecosystems (Courtenay, 1997).

2.2.5.1 Culture Systems

Tilapia can be raised under a number of different culture systems including cages, ponds, raceways, closed and semi-closed indoor recirculating systems. While each culture system is unique and has many advantages and disadvantages, they all share the common goal of efficient production.

Cages are used in circumstances where fish can not be controlled without human interaction (Stickney, 1986). Lakes, streams, and reservoirs often require fish to be enclosed with wire fabric or nylon netting which is attached to a rigid frame that may be suspended to a floating platform or flotation devices (Stickney, 1986). Generally, good water quality is achieved in cage systems which allow for higher stocking rates. The start up costs, maintenance, and replacement costs of cages can be quite high (Stickney, 1986). As well, it is difficult to control diseases, and production may be lost due to net damage.

Ponds are used by small culturists and researchers because they are easy to maintain and have low costs associated with them (Stickney, 1986). Culture ponds are rectangular or square in shape, man made, do not receive runoff from near by water bodies and are filled with well water or water pumped from a stream (Stickney, 1986). Production levels are directly related to pond design including side slopes, drain structure and inflow lines, which are needed to ensure adequate water levels at all times, provide proper drainage and prevent vegetation growth within the pond (Stickney, 1986).

The production of tilapia in raceways, an enclosed channel system, is very efficient. Raceways can accommodate extremely high densities of fish which can be

maintained due to the high rates of water flow (from gravity) resulting in exceptional water quality (Stickney, 1986).

2.2.5.1.1 Recirculating Systems

Closed systems, also known as recirculating systems, allow water to circulate within a number of culture tanks passed through a settling column to remove solids, and a biofilter to detoxify the ammonia produced by fish (Stickney, 1986). Recirculating systems can also be a semi-closed system where small amounts of water are removed and replaced with fresh water. Closed or semi-closed systems are beneficial for production of tilapia species in countries that do not have warm year-round climates or have a shortage of fresh water or available land (Losordo, 1997).

Recirculating systems designs are based on cost-effective water treatment. An effective recirculation aquaculture system must provide for the effective removal of waste solids, fine and dissolved solids, oxidization of ammonia and nitrite nitrogen, addition of oxygen as well as removal of carbon dioxide (Losordo, 1997).

Culturing tilapia in recirculating systems has a number of disadvantages. Complete diets must be formulated and a feeding system implemented using either hand or a mechanical feeder. Depending on the type of system, pollution of surrounding water or land with nutrients and organic matter may arise from water and waste discharge (Rakocy, 1989). Pumps, aerators, filters, CO₂ and heaters can be costly and need constant upkeep (Rakocy, 1989). Finally, with high stocking densities, fish are subjected to increased stress which may result in disease outbreaks (Rakocy, 1989).

2.2.5.2 Economic Importance of Tilapia

In 2000, according to the Food and Agriculture Organizations of the United Nations, the world aquaculture and commercial catch of tilapia was 1,943,389 tonnes. Commercial catch accounted for 677,609 tonnes while aquaculture produced 1,265,780 tonnes (FAO, 2002).

China is currently the largest producer of tilapia with 157,233 tonnes/year followed by the Philippines, Indonesia, Thailand and Egypt (Food and Agriculture Organizations of the United Nations, 1994). Canada produces approximately 100 tonnes of tilapia per year (Fitzsimmons, 2000) mainly in recirculation systems in Ontario and Alberta. Most tilapia in Canada are sold as live or unfrozen fish for the restaurant trade (Fitzsimmons, 2000). U.S. production of tilapia is a \$65 million industry with tilapia consumption reaching over 10,000 tonnes in 1992 (Stutzman, 1995).

2.2.5.3 Marketing Tilapia

When tilapia production was still in its early phases, the primary consumers were of oriental descent (Engle, 1997). Tilapia production was met through small producers since the demand was for live fish only. As the number of Asian consumers in Canada and the U.S. increased, frozen tilapia began to be imported from Taiwan (Fitzsimmons, 2004). Today, tilapia is sold in a number of forms including live, whole, fillets, fresh, frozen or smoked. Boneless fillets, yielding between 142 - 227 g, attract the majority of buyers (Engle, 1997).

Live fish prices (US dollars) have dropped from \$6.60/kg (1989) to \$1.65/kg (1993) with the cost of production being lowered by efficient farms (Engle, 1997). Retail price for frozen whole tilapias ranged between \$2.20 - 5.00/kg (1999), while fresh

fillets peaked at \$12.00/kg in 1999 (Fitzsimmons, 2004). Presently, prices for all tilapia products are much lower than in 1993 due to higher production rates (Fitzsimmons, 2004).

It is clear that there is a market for tilapia within the U.S based on the growth of the industry, but the future of this product relies heavily on the consumer. Engle (1997) summarized key elements needed for continued growth of the tilapia market, which was collected using a telephone survey of companies who sold, distributed, or imported tilapia. The first element was quality. Off-flavor fish, poor grading, and size have led to inconsistencies in tilapia quality. Secondly, pricing needs to be competitive with other fish products and the marketing of tilapia needs to have one strong voice which can promote tilapia to consumers. Finally, supply must be consistent for retailers in order to develop product assurance.

2.3 Nutrient Requirements for Tilapia

2.3.1 Energetics

Energy partitioning (Figure 2.1) in fish is significantly different than in mammals and birds. This is due to 2 factors: 1) fish are poikilothermic and 2) energy expenditure to produce urea or uric acid is avoided because fish excrete ammonia directly through their gills (De Silva and Anderson, 1995). In terrestrial livestock, the energy losses due to the heat increment can be 20-30% of the intake energy (Farrell, 1974) while for rainbow trout this is only 5-15% of intake energy (Cho et al., 1982). Furthermore, the maintenance energy requirements of fish are only 5-10% of those for similar sized terrestrial livestock (Brett, 1973). These differences mean that fish are significantly more

efficient in converting feed into body tissues than terrestrial species if an optimal environment is provided.

The amount of energy needed for the synthesis of uric acid or urea is equal to 3.1 to 2.4 kcal g⁻¹ of nitrogen in birds and mammals respectively whereas the amount of energy fish require to release nitrogen as ammonia, through the gills, is negligible (National Research Council, 1993). With higher protein in terrestrial diets, there will be an increase in nitrogen excretion resulting in more energy needed to produce urea and uric acid, whereas fish do not have to expend more energy for nitrogen excretion when fed higher protein diets. These two factors account for the fact that feed efficiency in finfish is often less than 1 kg feed per kg body weight gain.

2.3.2 Energy Value of Feed Ingredients

2.3.2.1 Protein

Protein is relatively more important as an energy source in aquaculture diets than poultry or pig diets (National Research Council, 1993). The protein requirements of fish species are higher than for terrestrial livestock ranging from 30% for tilapia to as high as 42% for rainbow trout (National Research Council, 1993). Net protein retention for fish is between 20 and 50%, similar to values for pigs and poultry (Bowen, 1987). The reason for the higher protein requirements for fish is that their energy requirements are lower (Smith, 1989). Lower energy requirements for heat increment and maintenance mean that a higher protein: energy ratio is required in aquaculture diets (National Research Council, 1993). The ratio of digestible protein to energy required for optimal fish growth ranges from 81-117 mg kcal⁻¹ (National Research Council, 1993) which is

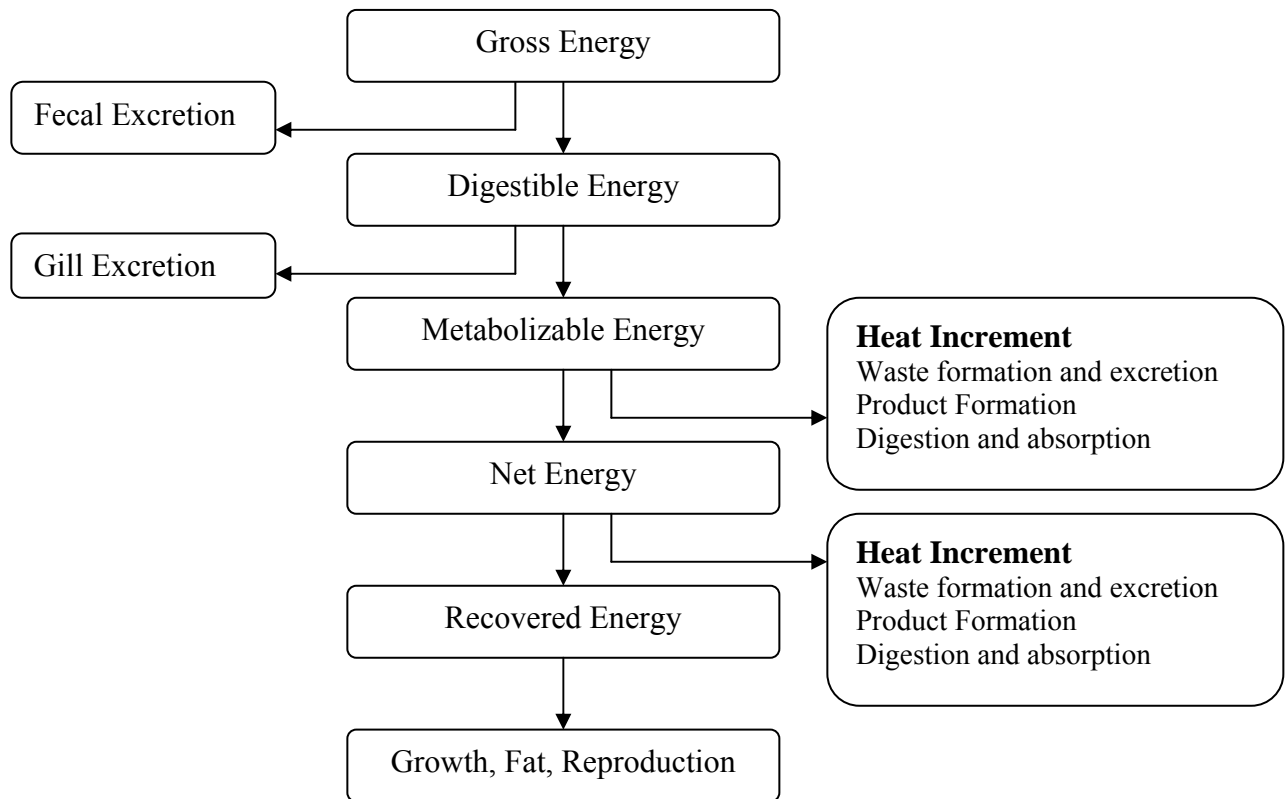


Figure 2.1. Energy flow through fish (National Research Council, 1993).

significantly higher than for pigs and broiler chickens which range from 40-60 mg kcal⁻¹ (National Research Council, 1993).

Like terrestrial animals, fish eat to meet their energy requirements (National Research Council, 1993). When energy is in excess, fish may reduce feed intake thereby limiting the intake of amino acids needed for growth, while deficient energy leads to the utilization of protein for maintenance instead of growth (National Research Council, 1993). A study by Cho and Jo (2002) examined the effect of feeding a high energy diet (4.27 kcal g⁻¹) compared with a low energy diet (3.84 kcal g⁻¹) with similar protein levels. The results of this study showed that high energy diets did not improve the performance of Nile tilapia even under limited feed allowance. Visceral fat content of fish was significantly higher for fish fed the higher energy diets (Cho and Jo, 2002).

In most fish species, protein is utilized for energy. However, once this requirement has been fulfilled, the remaining protein can be utilized for growth and protein accretion (National Research Council, 1993). In terrestrial animals, the amount of protein retained for growth is similar to that of fish and is estimated to be around 20 – 50 % for birds and mammals and 30% for fish (National Research Council, 1993). Protein requirements vary with each species as well as individual weight (National Research Council, 1993). As the weight of an individual increases with maturity, protein requirements decrease (National Research Council, 1993). The most economical dietary protein levels for different stages of *Oreochromis niloticus* are shown in Table 2.3. Hafedh (1999) showed that tilapia fry, weighing 0.5 grams, had the best growth and matured earlier when fed protein levels around 40%. As tilapia increased in age (100 – 200 grams), optimal dietary protein level was shown to decrease to 30%, resulting in

Table 2.3 Inclusion rates of dietary protein for tilapia species at varying stages of growth.

Stage	Dietary Protein Level (g kg ⁻¹)
Tilapia Fry (0.51 g)	400
Juvenile Tilapia (45 g)	400
Adult Tilapia (96-264 g)	300
Source: Hafedh, 1999	

Table 2.4 Amino acid requirements for tilapia.

Amino Acid	Protein In Diet (%)	g kg ⁻¹ of feed DM
Arginine	28	11.8
Histidine	28	4.8
Isoleucine	28	8.7
Leucine	28	9.5
Lysine	28	14.3
Methionine ^a	28	7.5
Phenylalanine ^b	28	10.5
Threonine	28	10.5
Tryptophan	28	2.8
Valine	28	7.8

^a Cystine 1.5 g kg⁻¹ of feed DM.

^b Tyrosine 5.0 g kg⁻¹ of feed DM.

Source: National Research Council, 1993

higher relative fecundity for females compared with higher dietary protein levels (Hafedh et al., 1999).

While Table 2.3 lists the most economical dietary protein levels, Twibell and Brown (1998) argue that fish weighing approximately 21 grams, which is generally the size at which fish enter the grow out phase in production operations, require a minimum crude protein allowance of 28% for maximum growth and feed efficiency when fed 100% plant based diets in tank culture systems. Similarly, Hanley et al. (1997) reported that 28% protein for adult tilapia (weighing approximately 150 grams) provided optimal growth with no extra cost compared with lower protein levels.

Although protein is an important energy source for tilapia, it also provides amino acids required for protein metabolism (National Research Council, 1993). Tilapia require the same 10 essential amino acids as terrestrial vertebrates including arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (National Research Council, 1993). The amino acid requirements for tilapia can be found in Table 2.4.

Because tilapias are cultured around the world, a number of different protein sources have been used as a partial or total replacement for fishmeal. El-Sayed (1998) compared shrimp meal (SM), blood meal (BM), meat and bone meal (MBM), blood meal and meat and bone meal mix and a poultry by-product meal (PBM) as protein sources for *O. niloticus* fingerlings. This study showed that SM, MBM, PBM can replace fishmeal without adversely affect growth but feed conversion and protein efficiency ratios were significantly lower than FM diets (El-Sayed, 1998). When looking at the cost and benefit of these ingredients compared with FM, analysis

concluded that SM, MBM and PBM were better protein sources than FM (El-Sayed, 1998).

Fagbenro (1998) studied the impact of replacing fish meal protein with legume seed meals, which have low market prices and improved accessibility, would have on the replacement of fish meal in tilapia diets. The legumes winged bean, jack bean, African locust bean, velvet bean, pigeon pea and lima bean seeds were roasted in order to eliminate heat labile antinutritional factors present. The author concluded that all the legume seed meals tested were suitable for tilapia diets, if properly processed. However, the winged bean had the highest energy and crude protein when compared with the rest of the ingredients.

Alfalfa leaf protein concentrate was able to replace up to 35% of the fishmeal without negatively affecting growth parameters (Olvera-Novoa et al., 1990). Including alfalfa leaf protein concentrate at levels higher than 35% of fishmeal reduced growth likely caused by limiting sulfur amino acids.

Fontainhas-Fernandes et al. (1999) noted that extruded pea seed meal, defatted soyabean meal, full-fat toasted soyabean, and micronized wheat (wheat subjected to infrared micronization and then flaked in a roller mill) had high apparent digestibility coefficients for dry matter, crude protein and gross energy, whereas triticale, lupin seed meal, faba bean meal and pea seed meal had low apparent digestibility coefficient compared with fishmeal. The results of this study suggest that there is a possibility of partial replacement of fishmeal with plant ingredients that have high apparent digestibility coefficients. However, 100% replacement resulted in reduced performance.

Sklan et al. (2004) compared apparent digestibility coefficients for soyabean meal, rapeseed meal, sunflower meal, corn gluten meal, wheat, corn, sorghum, barley,

and wheat bran and concluded that crude protein digestibility for these ingredients ranged between 75 to 90%. The lowest crude protein value was for corn and wheat.

2.3.2.2 Lipid

Protein and lipids are well utilized sources of energy for fish (Cruz, 1975; Smith, 1976; Popma, 1982). However, protein is relatively expensive so lipids are used to spare protein and decrease the cost of tilapia diets. Chou et al. (1996) studied the effect of varying levels of lipid on growing hybrid tilapia (*O. niloticus* x *O. aureus*). Fish fed diets containing 0% lipids had comparable growth for the first two weeks of the trial followed by a sudden reduction in growth likely caused by limiting essential fatty acids. Using polynomial regression analysis, the authors estimated the optimal level of lipids at 12%, while 5% lipid did meet the dietary requirements. In another study, the addition of 5, 9 or 12% lipid (oil: yellow grease) to tilapia diets, resulted in no differences in growth and feed conversion (Hanley, 1991). Hanley (1991) concluded that improvement to tilapia diets may be better achieved through the quality and level of protein or lipid rather than the quantity of lipid added. Furthermore, Hanley (1991) noted that the addition of lipid resulted in increased levels of visceral fat in the fish carcass. Wu et al. (1999) studied the effect that two different levels of soy oil had on the growth of Nile tilapia. The results indicated no differences in weight gain, feed conversion or protein efficiency ratios between the two levels (5.5 or 7.3 %). However, when considering weight gain, the most favorable diet contained the higher level of soy oil. A study by De Silva et al. (1991) examined three different levels of protein (15, 20 and 30%) combined with one of four levels of lipids (6, 12, 18 and 24%). For all three levels of protein, the authors concluded that the best growth was obtained with the addition of 18% lipid.

2.3.2.3 Carbohydrate

Carbohydrate utilization among fish species is extremely variable. However, tilapia and catfish can digest over 70% of uncooked dietary starch (Popma, 1982; Wilson and Poe, 1985) while rainbow trout can digest less than 50% (DeSilva et al., 1995). The potential for incorporation of carbohydrates into the diets of fish depends on the species of fish, type and amount of carbohydrate and the environmental conditions available. One study compared the effect of carbohydrate composition on absorption efficiency and reported that monosaccharides had greater absorption efficiency followed by disaccharides, cooked starch and finally raw starch (Bergot, 1979). Starch digestibility decreased with increasing levels of starch in rainbow trout diets (Bergot and Breque, 1983). As well, environmental factors such as water temperature also affected digestibility. Warm-water fish can utilize higher levels of carbohydrates than cold-water and marine species (National Research Council, 1993).

The objective of a number of studies has been to include carbohydrates in fish diets with the idea that protein will be spared as an energy source and used for growth (Anderson et al., 1984). Anderson et al. (1984) observed an increase in growth for Nile tilapia fed diets containing either starch or dextrin as compared with carbohydrate free diets. Diets containing up to 40% starch or dextrin did not decrease growth.

Testing the effect of maltose, sucrose, lactose, glucose and starch for juvenile *Oreochromis niloticus* x *O. aureus* showed that weight gain was highest for fish fed starch followed by maltose, sucrose, lactose and finally glucose (Shiau and Liang, 1995). Higher levels of body fat were found in fish fed the starch diet, intermediate body fat levels were observed in fish fed the disaccharide diets followed by the lowest levels of body fat in fish fed the glucose diets. Shiau and Peng (1993) indicated that the

storage of body fat was a direct indication that carbohydrates are sparing protein from being used as an energy source so that it may be used for growth.

Shiau and Peng (1993) studied nine diets which were formulated based on three levels of protein (24, 28 and 32%), three levels of carbohydrate (33, 37 and 41%) and three types of carbohydrates (glucose, dextrin, starch). The results of this study indicated that carbohydrates can have a protein sparing effect for tilapia if protein levels are decreased and starch or dextrin is added to the diet at levels no higher than 41%.

Tung and Shiau. (1991) reported that dextrin and starch provided better growth, feed efficiency, protein efficiency and energy retention compared with glucose. This study also indicated that carbohydrates were better utilized when fish were fed six times a day rather than two times a day.

Feeding schedule was not a factor when *Oreochromis niloticus* x *O. aureus* were fed either a 30% starch or a 30% glucose diet (Lin et al., 1997). Continuous feeding versus two meal feeding had no effect on growth parameters such as specific growth rate, feed efficiency, or protein efficiency ratio. However, the carbohydrate source did. Similar to the findings of Shiau and Peng (1993), Tung and Shiau (1991), Shiau and Liang (1995) and Anderson et al. (1984), Lin et al. (1997) results indicated that starch was utilized better than glucose.

Shiau and Lin (1993) observed an increase in glucose utilization in tilapia when chromium chloride was added to the diet. Higher weight gain was seen in tilapia fed 0.5% chromium chloride compared with 2 % in a study by Shiau and Liang (1995). It was determined in this study that glucose utilization was highest when fed chromic oxide as opposed to other forms of chromium (Shiau and Shy, 1998). From this point, the dietary requirements of chromium needed to be determined in order to allow

maximum glucose utilization, Shiau and Shy (1998) studied the difference between 0, 2, 10, 50, 100, 300, 1000 and 5000 mg kg⁻¹ of chromic oxide. The dietary requirement of chromium was decided by the broken-line model and was estimated to be 204 mg kg⁻¹ of feed for maximum growth. No significant effects on growth were seen when chromium picolinate, an organic form of chromium, was added to diets with either dextrin or glucose and fed to a hybrid tilapia (Pan et al., 2003).

Excessive carbohydrates in the diet can lead to liver cell degeneration, hyperglycaemia and poor growth (Roberts, 1978). The occurrence of hyperglycaemia is determined by the level of glucose ingested. However, it can be effectively regulated within 24 hours (Bergot, 1979). Severe hyperglycaemia and elevated liver glycogen was reduced when insulin injections were administered following an introduction of glucose into the stomach of rainbow trout (Palmer and Ryman, 1972 as cited by Steffens, 1989). Austreng et al. (1977) fed rainbow trout 17, 25 and 38% of metabolizable energy as carbohydrates for 24 weeks. Their results showed heavier livers and a higher percentage of discolored livers in fish fed the highest level of carbohydrate.

Anderson et al. (1984) studied the effect of α -cellulose in Nile tilapia diets. No reduction in growth was observed at low levels (10%). However, as α -Cellulose (produced by Sigma Chemical Co.) was increased, growth, feed conversion efficiency, and net protein retention were considerable reduced.

2.4 Peas, Canola and Flax as Ingredients in Tilapia Diets

2.4.1 Peas

Field pea is a pulse crop grown in Western Canada with over 2 million acres seeded and 1.5 million tonnes produced in Saskatchewan in 2003 (SAFRR, 2004). Peas

have low lipid content ($< 10 \text{ g kg}^{-1}$), and an average protein content of 22 g kg^{-1} (Nwokolo, 1996). Globulins, albumins and glutelins make up the protein structure of leguminous plants such as peas (Nwokolo, 1996). The majority of the protein is in the form of globulins which makes up approximately 70% of the protein (Nwokolo, 1996). Legumes tend to be low in the sulfur containing amino acids cystine and methionine (Nwokolo, 1996).

Other dietary components of legumes include carbohydrates and fiber. Carbohydrates, mainly starch, account for roughly 70% of the seed weight (Nwokolo, 1996). Smaller carbohydrates include sucrose and some oligosaccharides (Nwokolo, 1996). A noticeable amount of fiber can be found in legumes, making them an excellent choice for human consumption (Nwokolo, 1996), but this may be a cause of concern for tilapia species.

2.4.1.1 Antinutritional Factors

Peas contain some antinutritional factors that may hinder its ability to provide protein to fish. They contain small amounts of tannins, protease inhibitors, and phytic acid in the testa and kernel (Castell et al., 1996). As well, large amounts of oligosaccharides and polysaccharides can be found in the cell wall (Castell et al., 1996).

2.4.1.1.1 Protease Inhibitors

A number of digestive enzymes including trypsin, chymotrypsin, α -amylase and lipase may be inhibited by plant compounds that will ultimately reduce the digestion of nutrients, which can lead to an impairment of body metabolism, growth and overall health (Grant, 1998). Referred to as protein protease inhibitors, they are classified into

two distinct categories; Kunitz as well as Bowman and Birk (Castell et al., 1996).

Kunitz generally targets trypsin and has a molecular weight of 20 kilodaltons with two disulfide bridges and one reactive site whereas Bowman and Birk is capable of inhibiting chymotrypsin, and a few other enzymes (Liener, 1994). Five Bowman and Birk type inhibitors have been identified with similar characteristics such as molecular weight between 6 and 10 kilodaltons, a high number of disulfide bonds and seven disulfide bridges, and two reactive sites (Liener, 1994).

Protein protease inhibitors are found in peas, with 10% of the primary activity found in the hulls (Owusu-Ansah, 1991). The concentration and activity of the protease inhibitor depends on species, season, and physiological status of the plant (Grant, 1999). For example, winter varieties of white peas display 2-4 times more activity than spring varieties, and smooth peas have higher concentrations than wrinkled peas (Gatel, 1994).

Reduced food conversion efficiency and weight gain are seen when protease inhibitors are fed to young animals. However, body weight is unaffected in older animals (Grant, 1999). The reason for the reduction in food conversion and weight gain in young animals is due to a loss of amino acids in the form of pancreatic enzyme secretions by a hyperactive pancreas (Booth et al., 1960). Enzymes originating from the pancreas are especially high in the sulfur containing amino acids methionine and cysteine. Therefore, a hyperactive pancreas will divert these essential amino acids away from body tissue protein towards the synthesis of pancreatic enzymes (Liener, 1994).

2.4.1.1.2 Tannins

Tannins have been found to produce adverse nutritional effects in animals fed tannin rich diets (Mueller-Harvey, 1999). Some common nutritional effects include

reduced weight gain, poor feed conversion efficiency, reduced apparent digestibility of protein and reduced feed intake (Jansman, 1993). These effects are not seen in all animals, and the extent is variable depending on the source of the tannins, concentration, duration of exposure, diet composition, species of animal, age of the animal and production level (Jansman, 1993).

Tannins play an important role in the biological activities of the plant (Jansman, 1993). Because of their bitter and astringent taste, herbivores and birds may refrain from eating the plant (Jansman, 1993). As well, some plants may produce tannins in response to stressors such as defoliation, low soil fertility, water deficits or adverse temperatures (Mueller-Harvey, 1999).

Tannins are described as water-soluble polymeric phenolics with high molecular weight between 500 and 3000 daltons (Nackz et al., 1998). Two types of tannins exist in nature, mainly condensed and hydrolysable. Condensed tannins consists of linked flavanol units that are susceptible to a large number of permutations depending on the total number and positioning of OH groups, stereochemistry and the total number and positioning of the flavanol units (Mueller-Harvey, 1999). Hydrolysable tannins are formed from a number of gallic acids attached to a common sugar. Like condensed tannins, hydrolysable tannins are variable depending on the type of sugar, and the number and linkage of gallic acid units (Mueller-Harvey, 1999).

Tannins have the ability to bind to amide groups of enzymes and other proteins using hydrogen bonds and create large blocks of amino acids that are resistant to digestive enzymes (Sosulski, 1979 as cited by Wang et al., 1998). Because of this factor, it is important to consider the concentration of tannins in fish diets. Commonly, tannins are reported in the Leguminosae family where they can be found in the

condensed form. The testa layer generally contains the tannins and is related to the color of the pericarp (Jansman, 1993).

A study involving seventeen cultivars of field peas commonly grown in Western Canada concluded that there was a significant difference in the phenolic levels between cultivars (Wang et al., 1998). The phenolic concentrations ranged from 162 to 325 mg/kg (dry matter). However, very little condensed tannins were reported. It was apparent that genotype was the deciding factor determining phenolic concentrations whereas the environment played a relatively small role (Wang et al., 1998).

2.4.1.2 Digestibility of Peas in Aquaculture

The digestibility of pea seed meal by Nile tilapia was examined by Fontainhas-Fernandes et al. (1999) who reported that this ingredient had the lowest apparent digestibility coefficients for dry matter (46.1 %), protein (77.6 %) and energy (1.18%) observed in the study compared with lupin seed meal, triticale, faba bean meal, defatted soyabean meal, full-fat toasted soyabean and micronized wheat. However, when the pea seed meal was extruded, the apparent digestibility coefficient was similar to the highest tested vegetable proteins including: micronized wheat, full-fat soyabean and defatted soyabean meal.

Booth et al. (2001) looked at the difference in digestibility between field pea, dehulled field pea and field pea concentrate for silver perch (*Bidyanus bidyanus*). Dehulling field peas significantly improved protein digestibility. However, field pea concentrate was significantly higher than whole or dehulled field peas for protein, dry matter and energy digestibility (Booth et al., 2001).

Peas are a viable option, to replace soyabean meal, in rainbow trout diets if they are dehulled, or subjected to air classification with pea protein concentrate as the end product (Thiessen et al., 2003). The optimum level of inclusion for dehulled peas or pea protein concentrate was found to be 25% or 20% respectively without negatively affecting growth (Thiessen et al., 2003).

2.4.2 Canola

Canola is an important oil seed for Western Canadian crop production. In 2003, production rates reached well over 2.6 million tonnes (Saskatchewan Agriculture Food and Rural Revitalization, 2004). The whole seed contains approximately 21% crude protein while canola meal contains approximately 36% crude protein (Naczek et al., 1998; Uppström, 1995). Similar to peas, globulins make up 70% of the protein, with albumins and oleosins representing the remaining protein (Uppström, 1995). However, unlike peas, canola is rich in sulfur amino acids and lysine (Uppström, 1995). The fiber content of canola is primarily found in the hull and ranges from 12 – 30% in canola meal (Uppström, 1995).

2.4.2.1 Antinutritional Factors

Similar to the field pea, canola and canola meal have antinutritional elements that need to be considered before inclusion into fish diets. Canola contains tannins, glucosinolates, phytic acid, and a high level of fiber (Bell, 1993).

2.4.2.1.1 Glucosinolates

With the development of low erucic acid rapeseed, commonly referred to as canola, a reduction in glucosinolates was obtained (Bell, 1993). Canola contains almost ten times fewer glucosinolates with 10-12 μ moles of glucosinolates per gram of oil free dry matter, compared with the rapeseed varieties with 110-150 μ moles of glucosinolates per gram of oil free dry matter (Bell, 1993). However, the level of glucosinolates in canola is still a concern in animal feeding regimes.

Glucosinolates are concentrated mainly in the seed embryo at full maturity which is shown by the increase in glucosinolate levels after dehulling, but they can be found virtually anywhere in the plant (Bell, 1993). After ingestion, glucosinolates will decompose into toxic derivatives such as isothiocyanates, nitriles and thiocyanates through the help of plant enzymes and lower gastrointestinal microorganisms (Orginsky et al., 1965). In order for this to occur, the plant enzyme myrosinase must be present; otherwise glucosinolates are more or less harmless (Orginsky et al., 1965).

2.4.2.1.2 Phytic Acid

Phytic acid is a cyclic inositol ester containing six phosphate groups and occurs in cereals, grain-byproducts and oilseed meals (Castell, 1996). Phytate can be located in the seed embryo where it is the main storage site of phosphorus (Likuski and Forbes, 1965). Canola meal may contain approximately 1.22% total phosphorus with 0.53% bound to phytic acid (Bell, 1993).

Phytic acid negatively affects the utilization of minerals which can be seen by its ability to bind up to 75% of all phosphorus (National Research Council, 1998). It can chelate di and trivalent metals including calcium, magnesium, zinc and iron into

compounds that are less easily absorbed in the intestine. (Liener, 1994). Phytic acid also has the ability to nonselectively bind to protein and inhibit activities of a number of digestive enzymes such as pepsin, trypsin, and alpha-amylase (Liener, 1994).

Negative effects were seen when rainbow trout were fed purified diets containing phytic acid. Phytic acid was included in the diets at levels typical of salmon diets (0.5%), leading to a reduction in growth and feed conversion between 8-10% over 150 day period (Spinellie et al., 1983). Spinelli et al. (1983) hypothesized that a protein phytate complex was formed that was only partially digestible by pepsin.

2.4.2.1.3 Tannins

Tannins have also been detected in the hulls of a number of canola cultivars. A study by Naczek et al. (1994) observed the total content and activity of tannins in hulls of canola varieties. Tannin concentrations between 144 to 797 mg 100 g⁻¹ of canola hulls were observed, which is 8 times higher than previously cited (Mitaru et al., 1982; Leung et al., 1979). This leads to the conclusion that both genotype and environmental growing conditions have a significant impact on tannin levels in canola hulls. Biological activity is determined by the ability of tannins to bind/precipitate proteins (Naczek et al., 1994). Low-tannin cultivars (Delta) displayed greater binding to proteins with a precipitation index of 17.7 to 40.7 mg of blue BSA solution/mg of tannins, compared with the high-tannin cultivars (Westar) which did not exceed 5.0 mg of blue solution/mg of tannins (Naczek et al., 1994).

2.4.2.2 Digestibility of Canola in Aquaculture

Rapeseed meal with 7.49 mg g^{-1} of total glucosinolates was fed to tilapia for nine weeks (Jackson et al., 1982). Despite the good amino acid profile, inclusion levels of greater than 50% decreased growth rate by 22.7% in tilapia (Jackson et al., 1982). Similarly, juvenile tilapia (*Oreochromis mossambicus* Peters) fed a double low variety of rapeseed meal displayed poor growth parameters when included at levels higher than 15% replacement for soyabean meal (Davies et al., 1990). However, Soares et al. (2001) showed that the replacement of 48.17% soyabean meal with 35.40% canola meal did not affect feed: gain ratio or protein efficiency ratio of growing tilapia (*O. niloticus*).

Higgs et al. (1982) was able to replace 13 to 16% of the dietary protein in Chinook salmon diets with feedstuffs derived from canola. However, the best performance was found with Bronowski rapeseed protein concentrate, which was able to be included in the Chinook salmon diet at 25% (Higgs et al., 1982). In the same study, they observed higher inclusion levels of canola meal (30 to 32%) with the addition of 3,5,3'-triiodo-L-threonine (T_3) to reduce impairment of the thyroid gland caused by glucosinolates found in the canola (Higgs et al., 1982).

A study conducted by Higgs et al. (1983) concentrated on the inclusion levels of canola meal in the diets of Chinook salmon. Provided that the dietary glucosinolate content of the canola meal was less than $2.65 \text{ } \mu\text{moles/g}$ of dry diet, canola meal could provide 25% of the required dietary protein.

Canola meal fines, with lower levels of fiber, glucosinolates and phytate produced by sieving conventional canola meal through a mesh screen and then washing with a solvent, were found to be a good substitution for soyabean meal in juvenile rainbow trout diets (Thiessen et al., 2003). Results indicate that canola meal fines can

be included in rainbow trout diets up to 20% without negatively affecting growth and feed intake (Thiessen et al. 2003).

When thin distiller's solubles, a byproduct of ethanol production that is high in glutamic acid and proline, was added to rainbow trout diets containing 15% canola meal at a level of 3.3 or 3.9%, feed intake was increased in a short term study (Thiessen et al., 2003).

2.4.3 Flax

Flaxseed is a good source of omega 3 and omega 6 fatty acids with concentrations of linolenic acid (18 : 3n-3) greater than 50 g 100g⁻¹ of the total fatty acid concentration (Cunnane et al., 1993). Omega 6 fatty acids can be found in the linseed at concentrations of 12.7% which would be adequate to fulfill tilapia fatty acid requirements. As well, linseed contains approximately 23% crude protein with a good amino acid balance (Lee et al., 1991).

2.4.3.1 Antinutritional Factors

While whole flaxseed has desirable nutrient characteristics for aquaculture diets, it contains antinutritional factors, the main ones being pyridoxine antagonist (linatine), cyanogenic glycosides and mucilage (Bhatty, 1993). Extruding linseed or any other processing which involves heat will destroy linatine and cyanogenic glycosides. Flax contains 5-8% mucilage (DeMiller, 1986 as cited by Fedeniuk, 1994) and is a heterogenic polysaccharide that can be divided into acidic and neutral components (Cunnane et al., 1993; Fedeniuk et al., 1994). Mucilage also has a large capacity to bind

to water and increases intestinal viscosity thus, reducing nutrient digestibility (Fedeniuk et al., 1994). However, mucilage is heat stable and must be removed from the seed by other processing to eliminate its negative nutritional effects.

Mucilage has been used for thickening and stabilizing a number of commercial products (Wanasundra and Shahidi, 1997). However, it has caused problems with nutrient digestibility (Wanasundara and Shahidi, 1997). Increasing the level, up to 240 g kg⁻¹ of ground flaxseed caused a severe reduction in metabolizable energy and fat digestion when fed to growing broiler chickens (Ortiz et al., 2001). In a study conducted by Alzueta et al. (2002), demucilaged flaxseed was exchanged for flaxseed and an improvement in metabolizable energy, digestibility of fat and major fatty acids were seen. Furthermore, protein yield was increased when flaxseed was dehulled by enzymatic treatments (Wanasundara and Shahidi, 1997).

2.5 Processing Aquatic Feeds

Feed formulation must take into account the differences between terrestrial animals and aquatic species in order to maximize growth and feed efficiency. Not only do fish have different sensory systems, they also have different feeding behaviours, which require different feed densities (floating versus sinking) and pellet sizes. Water stability of pelleted feeds should also be considered when manufacturing diets for aquatic species.

Extrusion has proven to be a valuable technology in the aquaculture industry because it can increase nutrient availability of plant ingredients, and destroy micro-organisms and antinutritional compounds found in plant sources (Woodroffe, 2003).

Buoyancy, size, pellet quality, and ingredient variability can also be manipulated during the extrusion process.

2.5.1 Chemoreception

Fish sensory systems are different than terrestrial animals. Fish have four main types of sensory systems including visual, chemical, mechanical and electrical (Hara, 1992). All of these senses play a role in feeding. However chemoreception is likely the most important because it is the sensory system related to olfaction and gustation (Hara, 1992). The differences between terrestrial animals and fish in terms of gustation and olfaction lies with the media of transport namely air versus water and distant contact versus close contact (Kanwal and Finger, 1992).

2.5.1.1 Olfaction

With olfaction, fish can detect odours through the nares, located in front of the eyes, which are connected to sensory cells found on the bottom of a pit (Reebs, 2001). As water is moved over the pit by water currents, swimming or respiratory movements, it passes over the sensory cells which can then notify the brain through olfactory nerves of a chemical stimulant, such as food (Reebs, 2001).

2.5.1.2 Gustation

Gustation in fish is mediated by taste buds located in or around the mouth of fish. However, they can be located all over the body including fins, gills and barbells (Finger, 1992). Taste buds in fish have two distinct roles. One is a long range detection of food,

while the other is short range which is used for acceptance or rejection of food (Kanwal and Finger, 1992).

A number of compounds such as aliphatic acids, bile salts, nucleotides, and amino acids are known to be excitatory stimuli in fish (Lamb, 2001). Fish tend to be the most sensitive to amino acids, and more specifically L-amino acids (Marui and Caprio 1992). The taste receptors for amino acids can also have one of two distinct roles namely a wide range, which responds to several amino acids, or a small range, which responds to only specific amino acids (Hara, 1992).

2.5.2 Fish Feeding Characteristics

The species of fish being cultured will determine what type of pellet is needed. Each fish species has a unique feeding method such as biters (Cichlidae), suction feeders (Cyprinidae), scoopers (Cichlidae), filterers (Mugilidae), luring (Centrophrynidae), stalkers (Lepisosteidae), chasers (Scombridae) and ambushing (Esocidae) (Gerking, 1994). Tilapia generally feed on slow sinking pellets, but they will also consume feed that has fallen to the bottom of the culture tank (Gerking, 1994). Rainbow trout, on the other hand, like to chase their food and will not eat any pellets that have settled on the bottom of the culture tank (Gerking, 1994).

2.5.3 Pellet Characteristics

2.5.3.1 Dry vs. Moist Feed

Aquaculture diets generally fall into one of two categories, dry and moist feeds. Dry feeds contain 6-10% moisture and can be further divided into meals or pellets.

Moist feeds contain anywhere between 45 - 70% moisture and can also be further divided into moist or wet feeds (De Silva and Anderson, 1995).

The moisture difference between moist and wet feeds can be explained by the amount of wet ingredients present in the diet. Wet feeds contain fish byproducts, slaughterhouse waste and undried forages whereas moist feeds contain these ingredients mixed with a blend of dry ingredients (DeSilva and Anderson, 1995). In some coastal regions, fish byproducts are readily available and economical and as a result they have high inclusion rates in diets. They also contribute to increased diet palatability (Bureau and Cho 2003). Moist and wet diets can present a problem with pathogens however, because they are usually only subjected to mild heat treatment (Bureau and Cho 2003).

Dry feeds and more specifically pellets, are the most commonly used feed types in aquaculture diets because they can be manipulated to produce target specification such as particle size, density, surface area and size distribution (Jobling, 2001). These factors will ultimately produce a pellet that can either float or sink.

2.5.3.2 Physical Characteristics of Dry Feed

Pellet characteristics can influence whether or not fish will be interested in eating a particular diet (Jobling, 2001). Physical characteristics such as size, shape, colour and texture are important factors to consider when developing fish feeds (Jobling, 2001). Desirable pellet size tends to range for each species of fish depending on the mouth width and gape. Linner & Brannas (1994) reported that 20-25% of the mouth width is the optimal pellet size.

The effect of particle size was tested on Atlantic salmon by feeding either coarse (3-5 mm), standard (>1mm) or micronized (0.3-0.1 mm) feeds (Sveier et al., 1999). Feed intake was highest with the standard ground feed followed by micronized and coarse ground respectively. As well, growth rate was higher with the standard diet compared with the coarse diet. By studying the dry matter and chromic oxide contents of the gastrointestinal tract, coarse ground fish meal proved to have a higher evacuation time compared with the other two diets. Sveier et al. (1999) concluded that fish feed particle size has an important influence on growth and feed utilization.

2.5.4 Extrusion

Extrusion utilizes several operations including heat applied externally or by friction, mixing and shearing that are all executed simultaneously (Camire, 1998). Camire (1998) has summarized the major changes that occur in feed ingredients as it passes through the extruder as: 1) Chemical: thermal degradation, depolymerization and recombination of fragments and 2) Physico-chemical: binding, volatilization and change in native structure.

2.5.4.1 Types of Extruders

There are a number of different types of extruders available for use including single or twin screws. The major difference between the two is the level of sophistication and the energy requirements (Strong, 2001). Single screw extruders are limited by the rate at which the feed is entering the extruder, the speed of the screw, melting characteristics of the ingredients and viscosity (Harper, 1989). Twin screw

extruders are more flexible in processing conditions such as increased capacity, superior energy efficiency and a larger range of operating conditions than single screw extruders, which leads to a higher quality end product (Strong, 2001).

2.5.4.2 Flavors

Along with chemical changes is the production of various flavors which will give the product its finishing touch (Riha and Ho, 1989). Flavor compounds are formed through a number of reactions including lipid oxidation, Maillard reactions, and the breakdown of carotenoids, glutamine, and asparagines all derived from the starting material (Riha and Ho, 1989). However, some of the volatile flavor components may be lost during extrusion. Volatile flavor compounds can be degraded by the high temperatures associated with extrusion, become bound to proteins or starch or, they may be stripped away by steam (Riha and Ho, 1989).

2.5.4.3 Nutrients

2.5.4.3.1 Proteins

When proteins are extruded, the primary effect is from heat. A more texturized product is the result of increasing temperature during extrusion causing structural changes such as hydrolysis of peptide bonds, the formation of new covalent isopeptide cross-links, amino acid chain modification, enzymes that have become inactivated and denatured proteins (Stanley, 1989). Overall functioning of the protein is altered because protein solubility is reduced (Camire, 1998).

Extrusion can also create the formation of new isopeptide cross-links between amino acids such as lysine, methionine, asparagine, aspartic acid, cysteine, glutamic acid

and histidine (Stanley, 1989). If these amino acids enter into this reaction, the result may be decreased digestibility. For example, the level of methionine in soyabean meal was reduced from 1.54% to 1.45% after extrusion processing (Jeunink and Cheftel, 1979)

Three types of canola meal, commercial canola meal, low temperature extruded canola meal and high temperature extruded canola meal were used to replace herring meal in juvenile chinook salmon diets (Sato et al., 1998). Low and high temperature canola meal had an improvement in nutritive value when compared with the commercial canola meal. Burel et al. (2000) reported that rainbow trout were able to digest pea protein, lupin and heat-treated rapeseed meal after extrusion more efficiently than a solvent-extracted meal. However, Cheng and Hardy (2003) found that the apparent digestibility coefficient for crude protein was reduced when barley, corn gluten meal and whole wheat were extruded when compared with the nonextruded ingredients fed to rainbow trout but no explanation was given as to why this occurred.

2.5.4.3.2 Starch

Starch (found in feed ingredients such as peas, canola or flax) is transformed into smaller molecules upon extrusion (Colonna et al., 1989), because amylose and the branched structure of amylopectin are susceptible to shearing (Camire, 1998). During extrusion, shearing activity is increased and starch molecules are degraded to produce dextrins and free glucose (Camire, 1998). The degradation of starch is directly related to the final texture or expansion of the product. Optimal expansion for starch that contains 50% amylose is obtained when the barrel temperature is 150 ° C with low moisture (Camire, 1998).

Beside the shearing activity in the extruder, starch is also subjected to heat and water. Starch absorbs water, swells from the heat and pressure and eventually breaks (Woodrooffe, 2003). This process is known as gelatinization and leads to smaller molecules such as glucose to form a gel (Woodrooffe, 2003). Once the starch has been gelatinized, the surface area is increased allowing for greater enzymatic activity to occur and as a result, increased starch digestibility occurs (Allan and Booth., 2004).

Conflicting results have been reported regarding the digestibility of starch after extrusion. Burel et al. (2000) found the digestibility of starch from extruded peas was lower than gelatinized wheat starch which was not extruded. While Cruz-Suarez et al. (2001) reported positive improvements on digestibility of gelatinized starch from extruded whole and dehulled peas compared with non-extruded whole and dehulled peas fed to blue shrimp.

When the digestibility of extruded and nonextruded peas and lupins were compared, the results showed that extrusion had a positive effect on the digestibility of starch for peas in silver perch diets (Allan and Booth, 2004). There was a marked difference between the peas and lupins with the primary difference between the two being that lupins do not contain starch or heat-labile anti-nutrients.

Gouveia and Davies (2000) saw an increase in carbohydrate digestibility for pea seed meal when fed to European sea bass, which was thought to be a direct result of the extrusion process. As well, Carter and Hauler (2000) also reported high energy digestibility from peas likely caused by gelatinization of starch upon extrusion, allowing for increased surface area allowing for enzymatic reactions to occur.

The digestibility coefficient of gelatinized starch from SP35, soyabean meal with 35% protein (Ridley Aquafeeds, Narangba, Queensland, Australia) that was subjected to

extrusion had a significant improvement for dry matter and energy when fed to silver perch as compared to soyabean meal diets that were steam conditioned, ground or variations of the two (Booth et al., 2000).

The extent of digestibility of starch was found to be important in the excretion of ammonia. Robaina et al. (1999) measured the levels of ammonia excreted every 2 hours by European sea bass fed four diets composed of extruded or pelleted basal fish meal diet with and without wheat gluten. Results indicated that diets without wheat gluten had lower ammonia excretion. However, processing methods showed a significant difference between diets, with extruded diets producing much lower levels of ammonia. The authors suggested that the extrusion improved the availability of starch by increasing the gelatinization ratio of the starch which ultimately improved nitrogen utilization (Robaina et al., 1999).

2.5.4.4 Antinutritional Factors

A number of antinutritional factors can be reduced through extrusion. Trypsin inhibitors found in soyabean can be inactivated through heat processing in the same way myrosinase, an enzyme that activates glucosinolates, is deactivated through heat treatment (National Research Council, 1993). Studies looking at processing methods such as boiling, steaming or autoclaving, methods that are similar to extrusion, have shown that 80% of the trypsin inhibitor found in full fat soyabeans was inactivated (Wee and Shu, 1989).

Satoh et al (1998) looked at the effect that extrusion had on canola meal and the level of phytic acid in the canola products. By subjecting commercial canola meal to two different levels of heat treatment through extrusion (90°C or 150°C), protein and

lipid concentrations were increased with rising temperatures whereas the level of phytic acid was reduced by 30% with increasing temperatures leaving an estimated 7 g kg⁻¹ of phytic acid which is enough to reduce growth (Satoh et al., 1998).

2.5.4.5 Buoyancy

Extruded products that need to float or sink have specific conditions that must be met in the extruder in order for accurate buoyancy to be achieved (Woodroffe, 2003). Buoyancy is determined by the final bulk density of a pellet. The bulk density can be controlled by changing the level of expansion of the pellet which is directly related to the level of starch, lipids, and moisture content (Woodroffe, 2003). A pellet will not expand if starch is not present. Fat can help regulate whether or not a pellet will float due to lubricating characteristics and starch interactions (Woodroffe, 2003).

Expanded pellets, with moisture levels at approximately 22%, are extruded at 125-150 ° C for 20 seconds in a pressurized chamber (De Silva and Anderson, 1995). After emerging from the die, pressure is released and the water in the feed flash evaporates due to the decreased pressure and the gelatinized starch expands, forming air pockets (De Silva and Anderson, 1995).

Floating pellets generally require 20% starch, a bulk density of approximately 550 gm L⁻¹ for a 4-6 mm pellet and less than 6% lipids (Woodroffe, 2003). In some cases, expansion can be increased with different sources of starch. For example, potato and rice starch will expand easier than corn or wheat starch (Woodroffe, 2003).

The bulk density for a sinking pellet is above 650 gm L⁻¹ (Woodroffe, 2003). A number of other factors can be adjusted to create a sinking pellet such as higher lipid levels and moisture content (too high will create tough skinned pellets) and thick dies

(Woodroofe, 2003). A thick die will reduce the degree of expansion by allowing the material to expand longitudinally (Woodroofe, 2003).

2.5.4.6 Stability of Feed in Water

A very important aspect to consider when developing aquaculture feeds is there effects on water quality. Extruded feeds need to be fairly stable in water so as to minimize leaching of nutrients due to the disintegration of the pellet and ultimately the loss of nutrients (De Silva and Anderson, 1995). This loss of nutrients pollutes the water and causes stress to fish due to high nitrogen and organic matter as well as low oxygen levels (Bureau and Cho, 2003). High temperatures during extrusion coupled with low moisture, will result in a final product that will readily dissolve in water due to high levels of dextrinization (Woodroofe, 2003).

A study by Rout and Bandyopadhyay (1999) analyzed pellets for water stability by placing a number of pellets on a wire mesh basket that was then immersed in saline water with mild agitation for periods between 30 and 240 minutes. The pellets were then dried and expansion ratios (dry diameter: die hole diameter) were determined. Settling velocity was also determined by measuring terminal velocity as pellets were dropped into a water column. Results showed that density and settling rate were the highest for the extruded diets compared with the commercial diet (pellet mill) and a meat mincer. Pellet stability was also measured and it was determined that the commercial diet had the highest water stability. However, the authors speculated that this was due to the inclusion of a special quality binder. In spite of this, extruded diets had a lower loss of nutrients compared with the commercial diet (Rout and Bandyopadhyay, 1999).

Hilton et al. (1981) also found extruded diets to be of higher quality. They studied the difference between extrusion and steam pelleting for diets fed to rainbow trout. Extruded diets were reported as being more durable, higher water stability and absorbed more water than steamed pellets.

The following considerations should be kept in mind when producing aquaculture feeds. Grinding can increase hardness and overall pellet quality by increasing surface area that allows more steam conditioning (Hasting and Higgs, 1990). Diet composition can be manipulated to increase water stability by including ingredients that are easy to grind and have good binding properties such as starch (De Silva and Anderson, 1995; Woodroffe, 2003). Because salt, sugar and molasses tend to absorb moisture, they can prevent the pellet from drying, making it more susceptible to crumbling (De Silva and Anderson, 1995). Binders, ingredients that can reduce the void space in the pellet and eventually improve pellet quality, should be added into the mix (De Silva and Anderson, 1995). Because of its protein functionality, wheat gluten has been proven to be an excellent binder (Woodroffe, 2003).

3.0 Effect of replacing fish meal with simple or complex mixtures of vegetable ingredients in diets fed to Nile tilapia (*Oreochromis niloticus*)

3.1 Introduction

Nile tilapia are excellent candidates for intensive aquaculture production because of their rapid growth, tolerance to high stocking densities and poor water quality, high

reproductive rates, and low susceptibility to diseases (Chamberlain, 1993; Stickney, 1986). Furthermore, tilapia feed low on the trophic level in nature, and are therefore accustomed to gaining much of their nutritional needs from plant sources (Fitzsimmons, 1997).

Despite the ability of wild tilapia to utilize plant proteins efficiently, commercial tilapia diets have primarily focused on using fish meal as the primary source of protein. Fishmeal is a desirable ingredient in aquaculture diets due to its essential amino acid profile, and its high content of essential fatty acids, digestible energy, vitamins and minerals (Tacon, 1993). However, fish meal usage in aquaculture, world wide, was over two million tonnes in 1999 and is estimated that it will reach well over four million tonnes by 2015 (New and Wijkström, 2002). Given that total production of fish meal is approximately 6 million tonnes per year and that this level of production is expected to remain constant or decrease slightly in the future, there are concerns regarding the long term sustainability of this resource (Tacon, 1993).

A number of studies have examined the effects of replacing fish meal with plant proteins in diets fed to tilapia including soyabean meal (Wee and Shu, 1989, Shiau et al., 1989, Webster et al., 1992), maize gluten meal (Wu et al., 1995), lupins, (Fontainhas-Fernandes et al., 1999) rapeseed (Davies et al., 1990), cottonseed meal (Rinchard et al., 2002) and distillers dried grains with solubles (Coyle et al., 2004). However, complete replacement of fish meal with individual plant proteins has generally resulted in a decrease in fish growth performance (Sklan et al., 2004; Mbahinzirek et al., 2001). This decrease has been attributed to the presence of antinutritional factors in plant protein, particularly soyabean meal (Bureau et al., 1998).

Strategies to overcome this limitation include the fractionation of feed ingredients to reduce the level of antinutritional factors and replacing fish meal with a complex mixture of plant protein sources rather than one or two ingredients to reduce the exposure to individual antinutritional factors. Studies with fish have demonstrated that fractionation of soyabean, pea or canola to produce protein concentrates improves the protein and energy digestibility of these ingredients in salmonid fish compared with unprocessed soyabean, pea or canola meal (Rumsey et al., 1994; Thiessen et al., 2003; Mwachireya et al., 1999). Increasing diet complexity also appears to be a feasible method for replacing fish meal in aquaculture diets. El-Sayed et al., (2003) reported that when fish meal was completely replaced with a mixture containing 25% soyabean meal, 25% cottonseed meal, 25% sunflower meal and 25% linseed meal in diets fed to Nile tilapia, performance was not significantly impaired.

The following studies were done to assess the effect of fractionation on the digestibility of experimental diets with single ingredients produced in Western Canada including flax, peas and canola in Nile tilapia and to determine the effect of replacing fish meal with simple (soyabean and maize gluten meal) or complex (soyabean meal, maize gluten meal, dehulled flax, pea protein concentrate and canola protein concentrate) mixtures of plant proteins on the performance of Nile tilapia.

3.2 Materials and Methods

3.2.1 Digestibility Trial

The digestibility of the feed ingredients was assessed using the method of Bureau and Cho (1994). This method uses a reference diet (Table 3.1) that is mixed with the test

Table 3.1 Composition of reference diet used in digestibility trial.

Ingredient	Inclusion (g kg ⁻¹)
Fish meal ^a	300
Soyabean meal	170
Maize gluten meal	130
Wheat flour	280
Vitamin mineral premix ^b	10
Fish oil ^d	100
Celite ^c	10
(Total)	1000

^aSouth American aquagrade; EWOS Canada Ltd.

^b Vitamin Mineral premix (mg kg⁻¹ dry diet unless otherwise stated): vitamin A (as acetate), 7500 IU kg⁻¹ dry diet; vitamin D3 (as cholecalciferol), 6000 IU kg⁻¹ dry diet; vitamin E (as dl- α -tocopheryl-acetate), 150 IU kg⁻¹ dry diet; Vitamin K (as menadione Na-bisulfate) 3; vitamin B12 (as cyanocobalamin), 0.06; Ascorbic acid (as ascorbyl polyphosphate), 150; d-biotin, 42; choline (as chloride), 3000; folic acid, 3; niacin (as nicotinic acid), 30; pantothenic acid, 60; pyridoxine, 15; riboflavin, 18; thiamin, 3; NaCl, 6.15; ferrous sulfate, 0.13; copper sulfate, 0.06; manganese sulfate, 0.18; potassium iodide, 0.02; zinc sulfate, 0.3; carrier (wheat middling or starch)

^cCelite 545, <125 μ m; Celite Corporation, World Minerals Co., Lompoc, CA, USA

^dMixed variety fish oil; EWOS Canada Ltd.

ingredients in a 70% reference: 30% test ingredient ratio. Celite (Celite Corporation, Lompoc, CA) was added to the basal diet at 10 g kg^{-1} as a nonabsorbable marker. All of the diets were produced by cold extrusion using the Hobart mixer at the University of Saskatchewan with a 4.0 mm die. The diets were dried in a forced air oven (55°C) for a minimum of 2 hours, and chopped to an acceptable size (3mm). Digestibility of ten feed ingredients grown in Western Canada including canola meal, canola protein concentrate (MCN Bioproducts Ltd., Saskatoon SK), whole flax seed and dehulled flax seed (Prairie Agriculture Machinery Institute, Humboldt SK) whole pea, pea protein concentrate (Parrheim Foods, Portage La Prairie MB), coextruded flax: pea and coextruded canola: pea (Oleet Processing, Regina SK), fishmeal and soyabean meal were evaluated.

3.2.2 Environmental Conditions and Fish Management

The fish used in all experiments were maintained in accordance with the guidelines of the Canadian Council on Animal Care (Canadian Council on Animal Care, 1993). Male and female Nile tilapia were acquired from Greenview Aquafarms Ltd. (Calgary AB). The fish were housed in 350 L tanks in a recirculating system using biological filtration. Water temperature was maintained at $28 \pm 1^{\circ} \text{C}$ and water quality (oxygen, nitrate, nitrite ammonia and pH) were monitored daily. The photoperiod used was a 14 h light/10 h dark cycle.

The digestibility of the ingredients was assessed in two separate trials with five tanks per ingredient (complete randomized design), consisting of 40 fish per tank weighing 12 ± 1.2 grams per fish for the first trial and 57 ± 2.4 grams per fish for the second trial. The fish were fed to apparent satiation with two daily feedings (800 and

1600 hours) throughout the length of the trials and were acclimated to the diets for seven days before fecal collections commenced. Feces were collected over a seven day period using a settling column and were centrifuged (5000 x g; 15 min), frozen (0°C) and freeze dried prior to analysis.

3.2.3 Laboratory Analyses

Feces, ingredients (Table 3.2) and experimental diets (Table 3.3) were ground using a Retsch mill (Model SR 200; Retsch Inc, Newtown PA; 1.0 mm screen). Analysis of these samples included moisture (AOAC 1990, method no. 934.01), ash (AOAC 1990, method no. 924.05), acid ether hydrolysis (AOAC 1995, method no. 954.02). Gross energy was obtained by oxygen bomb calorimetry (Parr Adiabatic Calorimeter, Model 1281; Parr Instrument Company, Moline IL). The nitrogen content of samples was obtained using a combustion nitrogen analyzer (Leco FP-528, Leco Corporation, St. Joseph MI; AOAC 1995, method no. 990.03). Crude protein was estimated by multiplying nitrogen content by 6.25. Acid insoluble ash was analyzed using the method of Vogtmann et al. (1975).

3.2.4 Statistical Analysis

Statistical analysis was preformed using the General Linear Models procedure of SPSS (v.12.0, SPSS Inc, Chicago IL, USA) using the following least squares model.

$$Y_i = \mu + F_i + e_i$$

Where Y_i = the dependent variable (Apparent Digestibility Coefficient of Crude Protein, Gross Energy, Dry Matter)

μ = the overall mean

F_i = the effect of the i^{th} feed ingredient

Table 3.2 Nutrient analysis of test ingredients incorporated into the experimental diets
used in digestibility trial (n=2).

Ingredient	Dry Matter (g kg ⁻¹)	Crude Protein (g kg ⁻¹)	Gross Energy (MJ kg ⁻¹)	Acid Ether Extract (g kg ⁻¹)	Ash (g kg ⁻¹)
Fish meal	929.3	719.6	20.70	132.6	180.7
Soyabean meal	886.3	521.3	19.50	41.9	72.4
Whole pea	857.6	230.5	18.61	36.3	29.3
Pea protein concentrate	954.7	825.3	23.32	103.9	63.0
Canola meal	885.8	439.4	19.93	53.7	77.5
Canola protein concentrate	975.0	700.4	20.74	46.1	108.9
Whole flax	946.8	221.8	27.84	406.4	33.9
Dehulled flax	957.5	235.3	29.99	435.3	33.7
Canola: pea coextrudate	913.8	251.7	22.78	251.7	40.2
Flax: pea coextrudate	905.8	240.8	23.26	240.8	41.0

Table 3.3 Nutrient analysis of reference diet and experimental diets with 30% of the test ingredient used for the digestibility trial
(n=2).

Experimental Diets	Crude Protein (g kg ⁻¹ DM)	Ether Extract (g kg ⁻¹ DM)	Gross Energy (kcal kg ⁻¹ DM)	Ash (g kg ⁻¹ DM)	Acid Insoluble Ash (g kg ⁻¹ DM)
30% Fish meal	518	153	5205	113	10
30% Soyabean meal	460	123	5109	80	11
30% Whole peas	372	886	4948	68	10
30% Pea protein concentrate	551	145	5363	75	9
30% Canola meal	444	146	5237	82	11
30% Canola protein concentrate	519	130	5194	92	10
30% Whole flax	367	155	5639	69	13
30% Dehulled flax	368	273	5806	67	10
30% Canola: pea coextrudate	380	170	5331	70	11
30% Flax: pea coextrudate	378	192	5340	69	15
Reference diet	433	178	5278	82	13

e_i = the residual error

Treatment means were separated using the Student-Newman-Keuls test and differences were considered significant when $P < 0.05$.

3.2.5 Growth Trial

The eight diet formulations used in the growth trial (Table 3.4) were arranged in a 2 x 4 factorial design with two types of plant protein mixtures used to replace fish meal (simple: soyabean meal and maize gluten meal or complex: soyabean meal, maize gluten meal, dehulled flax, pea protein concentrate and canola protein concentrate) and four substitution levels of protein originating from fish meal (100%, 66.6%, 33.3% and 0%). Diets were formulated to contain equal amounts of digestible protein (380 g kg⁻¹) and digestible energy (17.63 MJ kg⁻¹). Furthermore, the diets were formulated based on the digestible protein and energy of canola protein concentrate, pea protein concentrate, dehulled peas, fish meal and soyabean meal reported in the digestibility trial. Digestibility coefficients for wheat, corn gluten and fish oil in tilapia were taken from Pezzato et al., (2002).

The diets were first mixed at the University of Saskatchewan using a Hobart mixer and then processed using a co-rotating twin screw extruder (Werner & Pfeiderer, Model ZSK 57-M 50/2, Stuttgart, Germany) with a 6-hole strand die. The screw speed was held between 200 and 225 rpm and diets were subjected to temperatures between 70°C to 116°C (die plate), depending on which part of the barrel the feed is passing through, with barrel pressure averaging 2400 kPa and cooled using a continuous fluid bed dryer (Niro Atomizer, Model VB-0,3, Soeborg, Denmark) for approximately 15 minutes at 40° C resulting in a moisture content of 8%. Fish oil was added to the diets,

following the drying process, using a vacuum tumbler (Daniels Food Equipment, Model R2-50, Parkers Prairie MN) with a mixing time of 15 minutes at 11 rpm.

3.2.6 Environmental Conditions and Fish Management

Tilapia (same strain and source as digestibility trial) were housed in twenty-eight 360 L and twenty-eight 150 L tanks in the same recirculation system and experimental conditions described above. The fish were sexed and only male fish were used in this experiment. Each tank was stocked with 10 fish, resulting in four 360 L tanks and four 150 L tanks per experimental diet. Fish were fed to apparent satiation twice daily for a total of 56 days. The total tank weight of the fish was recorded on days 0, 14, 28, 42 and 56 and feed intake was recorded daily. Growth was assessed by calculating average daily gain, specific growth rate ($[\ln \text{ final weight} - \ln \text{ initial weight}] / \text{days} \times 100$), average daily feed intake, feed conversion ratio (average daily feed intake/average daily gain) and protein efficiency ratio (average daily gain/average daily protein intake) on a per fish basis. Because some of the tanks lost weight throughout the trial, feed conversion ratio resulted in better performance than was actually seen. Therefore, overall feed conversion ratio for the level of protein originating from fish meal and the complexity of the diet was calculated by using only the positive feed: gain values.

On day 56 of the experiment, one fish per tank was euthanized and a one cm segment of small intestine obtained from the mid point of the intestinal tract was submerged in 10% neutral buffered formalin for 24 h. Thereafter, the tissue was immersed in 70% ethanol until embedded in paraffin, sectioned and stained with hematoxylin and eosin. Images of the intestinal cross-sections were captured with a DVC digital camera (Digital Video Camera Company, Austin, TX) mounted on a BH-2

Table 3.4. Growth trial experimental diet formulation for simple and complex diets with varying levels of protein originating from fishmeal (g kg⁻¹).

	100% FM	Simple			Complex		
		66.6% FM	33.3% FM	0% FM	66.6% FM	33.3% FM	0% FM
Fish meal	584.4	389.6	194.8	0	389.6	194.8	0
Wheat	277.6	248.8	220.4	191.6	237.2	216.8	186.0
Maize gluten meal	0	113.6	226.8	340.4	66.8	100.0	120.0
Soyabean meal	0	120.0	240.0	360.0	40.0	100.0	172.4
Canola protein concentrate	0	0	0	0	40.0	120.0	247.2
Dehulled flax	0	0	0	0	60.0	60.0	60.0
Pea protein concentrate	0	0	0	0	60.0	112.4	120.0
Choline	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin / mineral ^a	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Fish oil	123.0	113.0	103.0	93.0	91.4	80.6	79.4

^aSame formulation as shown in Table 3.1.

Olympus light microscope (Olympus America Inc., Melville, NY) and analyzed using Northern Eclipse Software (Empix Imaging, Inc., Mississauga, ON). Ten well- oriented villus height measurements per intestinal cross-section were measured and the mean of these measurements were calculated. A single person, blinded to treatment assignment, conducted all measurements.

3.2.7 Experimental Diet Analysis

Experimental diets were analyzed (Table 3.5) for moisture, crude protein, gross energy, acid ether extract, and ash as described previously on page 67. Amino acid analysis (AOAC Official Method 982.30; AOAC 1995), was conducted by the Animal Nutrition Analytical Lab of Degussa Corporation (Allendale NJ).

3.2.8 Statistical Analysis

The experiment was initially analyzed using the General Linear Models procedure of SPSS (v.10.0.5, SPSS Inc, Chicago IL, USA). An initial analysis determined that the effect of tank size was not significant, thus, the experiment was analyzed using the following least squares model.

$$Y_{ij} = \mu + F_i + C_j + F_iC_j + e_{ij}$$

Where Y_{ij} = the dependent variable (Average Daily Gain, Average Daily Feed Intake, Specific Growth Rate, Feed:Gain, Protein Efficiency)

μ = the overall mean

F_i = the effect of the i^{th} level of fish meal protein inclusion

C_j = the effect of the j^{th} level of diet complexity

F_iC_j = the interaction between the i^{th} level of fish meal inclusion and the j^{th} level of diet complexity

Table 3.5 Nutrient analysis of experimental diets used for the growth trial (n=2).

Experimental Diets	Crude Protein (g kg ⁻¹ DM)	Ether Extract (g kg ⁻¹ DM)	Gross Energy (kcal kg ⁻¹ DM)	Ash (g kg ⁻¹ DM)
0% Simple	469	140	5370	45
33.3% Simple	476	171	5416	74
66.6% Simple	487	185	5391	87
0% Complex	474	152	5409	60
33.3% Complex	468	172	5442	64
66.6% Complex	485	197	5483	88
100% Complex	496	137	5364	109

Differences between least square means were separated using Student-Newman-Keuls

test and means were considered significantly different when $P < 0.05$.

e_{ij} = the residual error term

3.3 Results

3.3.1 Digestibility Trial

Fractionation of flax, pea and canola resulted in increases in crude protein and gross energy values (Table 3.2). Pea protein concentrate (825 g kg⁻¹ crude protein) and canola protein concentrate (705 g kg⁻¹ crude protein) obtained through aqueous extraction, had higher crude protein levels than the starting ingredients. Removal of the flax seed hull resulted in a very small increase in crude protein (222 g kg⁻¹ to 235 g kg⁻¹).

Apparent digestibility coefficients for crude protein, energy and dry matter of both control diets were not significantly different between the two trials so results from the two digestibility trials were analyzed as one experiment (Table 3.6). The apparent digestibility coefficients of crude protein for whole flax, dehulled flax and coextruded flax: pea were significantly lower than for the other feed ingredients tested and whole flax had a significantly lower ($P < 0.05$) apparent digestibility coefficient for crude protein (-0.38) compared with dehulled flax (0.46). The coextruded flax: pea product had an apparent digestibility coefficient for crude protein of 0.61 which was significantly higher than that of dehulled flax. All other products tested had apparent digestibility coefficients for crude protein that were not significantly different from each other ranging from 0.76 for coextruded canola: pea to 0.95 for pea protein concentrate.

Processing improved digestibility for energy and dry matter for pea, canola and flax products. Pea protein concentrate had an apparent digestibility coefficient of 0.95 for energy and 0.93 for dry matter which was significantly higher than the unprocessed whole peas with 0.58 and 0.59 for energy and dry matter respectively. Similarly, canola

Table 3.6 Apparent digestibility coefficients of experimental diets with test ingredients
(%) determined in digestibility trial.

Ingredient	Crude Protein	Gross Energy	Dry Matter
Fish meal	.93 ^a	.92 ^a	.85 ^{ab}
Soyabean meal	.91 ^a	.82 ^{ab}	.74 ^{abcd}
Whole pea	.86 ^a	.58 ^{cd}	.59 ^{cde}
Pea protein concentrate	.95 ^a	.95 ^a	.93 ^a
Canola meal	.82 ^a	.68 ^{bc}	.54 ^{de}
Canola protein concentrate	.86 ^a	.84 ^{ab}	.78 ^{abc}
Whole flax	-.38 ^d	-.27 ^e	-.45 ^f
Dehulled flax	.46 ^c	.48 ^d	.41 ^e
Canola: pea coextrudate	.84 ^a	.76 ^{de}	.69 ^{de}
Flax: pea coextrudate	.61 ^b	.53 ^{cd}	.41 ^e
Reference diet (trial 1)	.92 ^a	.90 ^a	.87 ^{ab}
Reference diet (trial 2)	.89 ^a	.89 ^a	.85 ^{ab}
SEM	.05	.05	.05

^{a-f} Means within columns with different superscripts are significantly different ($P < 0.05$).

protein concentrate had significantly higher apparent digestibility coefficients for energy and dry matter than canola meal. Whole flax had negative apparent digestibility coefficients for gross energy and dry matter (-0.27 and -0.45 respectively) and these values were significantly lower than those for dehulled flax with apparent digestibility coefficients for energy and dry matter of 0.48 and 0.41.

3.3.2 Growth Trial

The amino acid contents of the diets are shown in Table 3.7. The level of lysine and threonine were lowest in the 0% fish meal level simple diet but still exceeded the requirements for these amino acids (National Research Council, 1993).

The effect of fish meal level on average daily gain, specific growth rate, average daily feed intake, feed: gain ratio and protein efficiency ratio was significant ($P < 0.05$) (Table 3.8). The average daily gains, specific growth rates and feed efficiencies of fish fed diets with 0% fish meal were significantly lower than fish fed diets with the 33.3, 66.7 or 100% fish meal levels. Average daily feed intakes were significantly decreased in fish fed the 0% fish meal diets compared with the 66.7 or 100% fish meal diets. The fish fed the 33.3% fish meal diets had feed intakes that were not significantly different than fish fed 0 or 66.6 and 100% fishmeal. Protein efficiency ratios of the fish fed at the 0 and 33.3% fish meal levels were significantly lower than fish fed the 66.6 or 100% fish meal levels.

The effect of diet complexity was significant for average daily gain, specific growth rate, feed: gain ratio and protein efficiency ratio but not for average daily feed intake. The fish fed the complex diets had significantly higher average daily gains, specific growth rates and protein efficiency ratios as well as decreased feed: gain ratios compared with those fed the simple diets.

Table 3.7 Amino acid content of the diets fed in the growth trial (g kg⁻¹ as-fed basis) (n=1)^a.

	100%	Simple			Complex			Requirements ^b
		66.6 %	33.3%	0 %	66.6%	33.3%	0%	
Protein								
Amino Acid	448	444	441	404	457	445	425	
<i>Essential</i>								
Arginine	17.0	16.5	15.0	14.7	17.4	16.5	16.1	11.8
Histidine	10.7	10.8	9.7	9.5	11.0	11.1	10.5	4.8
Isoleucine	16.6	17.4	17.1	17.5	17.0	17.9	18.5	8.7
Leucine	28.8	35.7	40.9	46.5	34.0	36.9	37.8	9.5
Lysine	27.3	23.8	18.6	15.7	25.0	23.2	20.1	14.3
Methionine	10.9	9.9	8.3	7.4	9.9	8.7	7.4	
Cystine	4.3	5.2	5.7	6.2	5.4	6.3	7.3	
Methionine + Cystine	15.2	15.1	14.0	13.6	15.3	15.0	14.7	9.0
Phenylalanine	16.3	18.7	19.9	21.6	18.6	19.6	20.6	15.5 ^c
Threonine	27.1	25.7	22.2	21.0	27.9	28.7	27.3	10.5
Valine	20.3	20.7	19.7	19.8	20.4	21.3	21.8	7.8
<i>Non Essential</i>								
Alanine	27.3	27.5	26.1	26.7	26.8	24.6	22.0	
Aspartic Acid	36.1	36.4	34.0	34.3	37.6	37.5	36.7	
Glutamic Acid	63.5	71.6	76.1	82.6	72.7	78.4	82.2	
Glycine	37.3	30.1	21.3	16.1	31.3	25.5	19.2	
Serine	18.6	19.5	19.2	20.4	20.5	20.0	19.8	

^a Amino acid analysis by AOAC Official Method 982.30 (AOAC, 1995)

^b Requirement for tilapia from National Research Council (1993).

^c Requirement for phenylalanine + tyrosine.

Table 3.8 Performance of tilapia based on simple and complex diets with varying levels of fishmeal for days 0-56 of the growth trial.

	Average Daily Gain (g d ⁻¹)	Specific Growth Rate (%)	Average Daily Feed Intake (g d ⁻¹)	Feed Conversion (g d ⁻¹)	Protein Efficiency Ratio (gain protein intake ⁻¹)	Villus Length (mm)
Fishmeal Level						
0	0.97 ^a	1.79 ^a	2.35 ^a	3.28 ^b	0.96 ^a	302.1
33	2.00 ^b	3.06 ^b	2.96 ^{ab}	1.79 ^a	1.30 ^a	341.9
67	2.50 ^b	3.66 ^b	3.09 ^b	1.30 ^a	1.69 ^b	352.7
100	2.68 ^b	3.66 ^b	3.16 ^b	1.23 ^a	1.82 ^b	354.1
SEM	0.82	1.15	0.28	1.98	0.24	24.7
Diet Complexity						
Simple	1.79	2.68	2.72	2.50	1.27	320.9
Complex	2.29	3.40	3.00	1.46	1.59	354.5
SEM	0.35	0.72	0.09	1.50	0.14	11.35
<i>P</i> -value						
Fishmeal	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.19
Complexity	0.04	0.02	0.20	< 0.01	0.01	0.08
Interaction	0.23	0.03	0.07	< 0.01	0.14	0.31

^{ab}Means within columns with different superscripts are significantly different.

There were significant interactions between fish meal level and diet complexity for specific growth rate and feed: gain ratios so the individual treatment means and differences between the individual means are shown for these parameters in Figure 3.1. The fish fed the 0% fish meal complex diet had significantly higher specific growth rates and lower feed: gain compared with the fish fed the 0% fish meal simple diet.

Decreasing villus length was observed as the levels of protein originating from fish meal was decreased while villus length increased with increasing diet complexity (Table 3.8). However, the main effects of fish meal level ($P = 0.19$) and diet complexity ($P = 0.08$) were not significant.

3.4 Discussion

Energy and dry matter digestibility was significantly increased when pea, canola and flax was processed. However, there was less effect on the protein digestibility of these ingredients when fed to Nile tilapia. Processing to remove antinutritional factors such as fiber and improve starch digestibility found in peas has been shown to increase the apparent digestibility coefficients for crude protein, gross energy and dry matter for pea products. Fontainhas-Fernandes et al., (1999) observed an increase in the apparent digestibility coefficients for gross energy and dry matter for extruded pea seed meal compared with pea seed meal when fed to Nile tilapia. However, no increase was seen in the digestibility of crude protein. The authors hypothesized that the increase in digestibility for energy and dry matter is a result of the reduction in antinutritional factors from processing. Theissen et al., (2003) observed higher digestibility coefficients

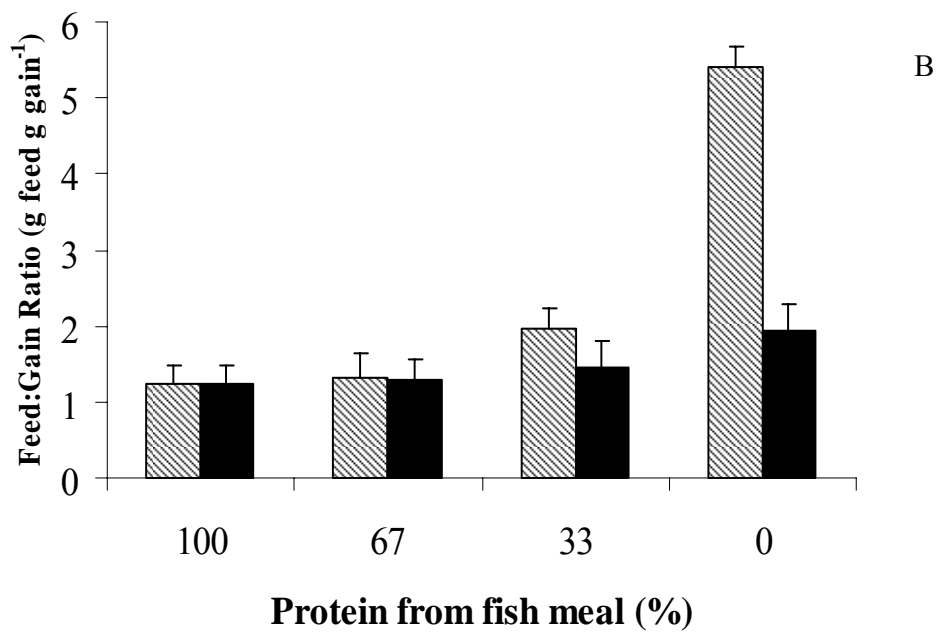
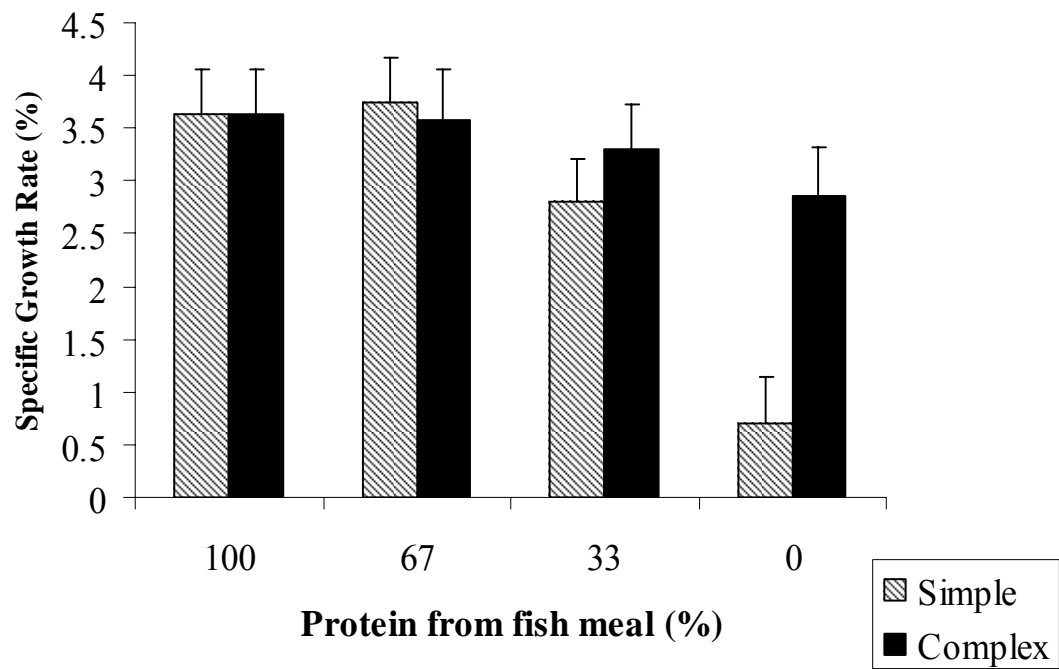


Figure 3.1 Interaction between diet complexity and the level of protein originating from fish meal for specific growth rate (A) and feed: gain ratio (B) of tilapia for days

0-56 in the growth trial. Means with different superscripts are significantly different ($P < 0.05$).

for gross energy and dry matter when rainbow trout were fed pea protein concentrate prepared by air classification compared with extruded/dehulled peas, raw/dehulled peas or raw/whole peas. In contrast to the present study, they also noted an increase in protein digestibility of the pea protein concentrate compared with the other three pea products. Booth et al. (2001) also indicated that pea protein concentrate had a greater potential to be incorporated into the diets of silver perch, compared with whole peas, due to the removal of indigestible carbohydrates found in the hull.

Canola protein concentrate has reduced levels indigestible fiber and lower phytic acid which is an antinutritional factor. In a previous study, canola protein concentrate was shown to have an apparent digestibility coefficient for crude protein of 0.896 and 0.861 for gross energy when fed to rainbow trout (Thiessen et al., 2004). These results are similar to those found in the present study which showed canola protein concentrate to have an apparent digestibility coefficient of 0.86 for crude protein and 0.84 for gross energy. Mwachireya et al., (1999) also reported that canola protein isolate had significantly higher apparent digestibility coefficients for crude protein, gross energy and dry matter compared with canola meal and various canola meal fractions. The authors indicated that the removal of phytic acid, and glucosinolates had less effect on improving the digestibility of canola meal than the removal of indigestible fibre fractions.

Whole flax had negative apparent digestibility coefficients for crude protein, gross energy and dry matter which may have been caused by high levels of mucilage

resulting in increased intestinal content. Low consumption of this diet coupled with high gut viscosity may have increased endogenous nutrient losses in the gut of the fish resulting in the negative apparent digestibility coefficients observed. Sklan et al., (2004) reported lower digestibility with plant ingredients high in fiber such as rapeseed meal and barley. They speculated that high fiber content in feedstuffs may reduce enzymatic activity in the gut resulting in poor digestion.

The canola: pea and flax: pea co-extrudates had low protein levels and high levels of oil producing a high energy ingredient. The digestibility was lower for canola: pea co-extrudate than the individual ingredients. However the canola: pea co-extrudate is comprised of whole canola not canola meal. The flax: pea co-extrudate had higher digestibility than the individual ingredients. In contrast, Gomes et al., (1995) studied a co-extruded rapeseed and pea product and found that the digestibility of this ingredient was improved compared with the individual ingredients. This suggests that the coextruded canola: pea product may have been subjected to excessive heat during extrusion processing, thus reducing nutrient digestibility. The coextruded ingredients were not used in the growth trial because their digestibility's and nutrient densities were not as high as for the fractionated ingredients.

Recent efforts to incorporate plant ingredients into tilapia diets have concentrated on replacing fishmeal with single ingredients. The general trend of these trials has shown replacement of fishmeal with a single plant source higher than 25-35% of diet dry matter resulted in poor growth which was mainly attributed to antinutritional factors (Davies et al., 1990; Olvera-Novoa et al., 1988; 1990; Jackson et al., 1982). Replacement of fish meal with a complex mixture of plant proteins might reduce the

exposure of fish to individual antinutritional factors and improve performance.

Furthermore, utilization of processed ingredients like pea protein concentrate, canola protein concentrate and dehulled flax further reduces the level of individual antinutritional factors.

In the present study, feeding complex diets significantly improved all growth parameters with the exception of average daily feed intake indicating that tilapia will eat both simple and complex diets. However, suboptimal growth was achieved with simple diets especially at the 0% fish meal level. Fontainhas-Fernades et al. (1999), incorporated a number of plant ingredients including extruded pea meal and defatted soybean meal into tilapia diets replacing 0%, 33%, 67% and 100% of fish meal. They reported significant increases in all growth parameters with increasing levels of fishmeal. Gomes et al. (1995) also studied the effect of replacing fish meal with a mixture of plant proteins including coextruded rapeseed: peas, full-fat toasted soyabeans and maize gluten in rainbow trout. They reported a significant decrease in weight gain and specific growth rate in diets containing 0% fish meal compared with those containing 100, 66 or 33% fish meal. These ingredients were not as highly processed as the ingredients used in the present trial and contained more antinutritional factors, particularly fibre.

Although intestinal villus length was not significantly affected by diet in experiment two, there was a trend to shorter villi in the simple diets compared with the complex diets. Soyabean meal has been reported to result in intestinal hypersensitivity reactions in salmon (Rumsey et al., 1994; Bureau et al., 1998; Burrells et al., 1999). Burrells et al., (1999) observed that feeding diets containing 600-700 g kg⁻¹ of soybean

meal to rainbow trout resulted in the loss of integrity of the villus tips and increased inflammatory cell infiltration into the lamina propria. In the present study, the simple diets contained more than twice as much soyabean meal as the complex diets and this may have contributed to the shorter villi seen in fish fed the simple diets.

The experimental diets used in this study were formulated to have identical levels of digestible energy and protein. However, amino acid analysis showed that lysine and threonine levels in the simple diets were lower than in the complex diets. However, it should be noted that the levels of lysine and threonine as well as all the other essential amino acids were above National Research Council requirements for tilapia (National Research Council, 1993) for every diet. Since amino acids in excess of requirement are deaminated and used for energy, the amino acid levels in the diets should not have contributed to the treatment differences seen in this trial.

The present study used an all male population to reduce variability. Male tilapia grow faster than females (Toguyeni et al., 2002; Beardmore et al., 2001) but a study conducted by Fauconneau et al. (1997) showed that a mixed group (male and female) of Nile tilapia had a higher feed intake and lower growth compared to an all male or all female population. However, the all male population had a higher protein efficiency ratio and lower feed conversion ratio compared with the all female population (Fauconneau et al. 1997). Even though most small scale production systems do not sort their fish, it was beneficial to this experiment in order to reduce variability (Beardmore et al., 2001).

The results of this study indicate that replacement of fish meal with a complex mixture of processed plant ingredients results in superior performance compared with

replacement with soyabean meal and corn gluten meal. This strategy may reduce the requirement of the aquaculture feed industry for fish meal and enhance the sustainability of the industry. It will also provide another market for Saskatchewan grown products such as canola, peas and flax as well as increasing the need for further processing of these ingredients.

4.0 Conclusions

In order for aquaculture to continue growing, it needs to reduce its reliance on fish meal. The rapid decline of the world fish stocks which continues to decline through overfishing and poor management is resulting in a reduced amount of fish for human consumption, and fish meal and fish oil for aquaculture feed production. The use of plant protein sources is the obvious solution to this problem to reducing the amount of fishmeal used in fish diets but optimal fish growth and efficiency must be maintained at the same time.

One way in which this can be accomplished is through selection of fish species to be reared. Tilapia are excellent choice for production in intensive aquaculture systems because they feed low on the trophic level, have high stocking densities and are susceptible to a limited number of diseases. A second way to accomplish this goal is the development of improved plant-based feed ingredients for aquaculture through fractionation and processing. Such a strategy can improve the nutritive value of plant protein sources. The ingredients chosen for our digestibility which were then incorporated into our growth trial all had received one form of processing prior to the trials. Abrasion was applied to flax to remove fiber found in the hull, pea and canola protein concentrate was subjected to aqueous extraction in order to deactivate

antinutritional compounds and the canola: pea and flax: pea coextrudates were subjected to extrusion in hopes of improving starch digestibility through heat treatment. Lastly, formulating aquaculture diets to contain a large number of plant protein sources lowers the inclusion rate of each single ingredient and reduces the exposure of fish to individual antinutritional factors present in each ingredient.

The present studies demonstrate:

- 1) Fractionation of flax, pea and canola significantly improved the apparent digestibility coefficient of energy and dry matter in tilapia
- 2) Performance was significantly improved by feeding fish complex mixtures of fractionated ingredients compared with diets containing only soybean and corn gluten meals
- 3) It is feasible to totally replace fish meal in tilapia diets using complex mixtures of fractionated plant ingredients.

Direct implications of this research may not be seen immediately by tilapia producers. However, this research should serve as a good starting point to develop future studies in this area. Future studies should concentrate on improving methods for fractionating and processing Saskatchewan feed ingredients and in turn developing more products that are suitable for tilapia diets. Future research might take into account new plant protein sources produced in Saskatchewan such as faba beans as well as alternative processing methods of these new products and of existing products such as flax. Dehulling flax resulted in a product with a large portion of the hull still present. Aqueous extraction followed by air classification of flax may be a better choice to produce flax protein concentrate. Another area of research may be determining the

optimal level of fish meal replacement to maximize the growth and health of Nile tilapia to produce a high quality product with nutritional benefits for consumers. The current research shows that lower fish meal levels are possible, but an optimum level has yet to be identified. This level may lie between 0% and 30% of protein originating from fish meal.

Suggestions for future research of tilapia in a recirculating facility would be to ensure that all tanks are secured with black netting in order to avoid any escapes or mortality. As well, behavioural observations should be accounted for, due to the aggressive behavior of dominant fish. In order to try and eliminate aggressive behaviour, using smaller fish with a higher stocking density may be warranted. And finally in order to eliminate large differences in growth, with no statistical significance detected, an increase in the number of tanks used or increasing the stocking density may be necessary.

5.0 References

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6.0 APPENDICES

Appendix A: Extrusion process used to manufacture the feed used in the tilapia growth trial.



Figure A1. Mixed feed is placed into a hopper bin where it drops onto a conveyor belt at a pre-determined rate.

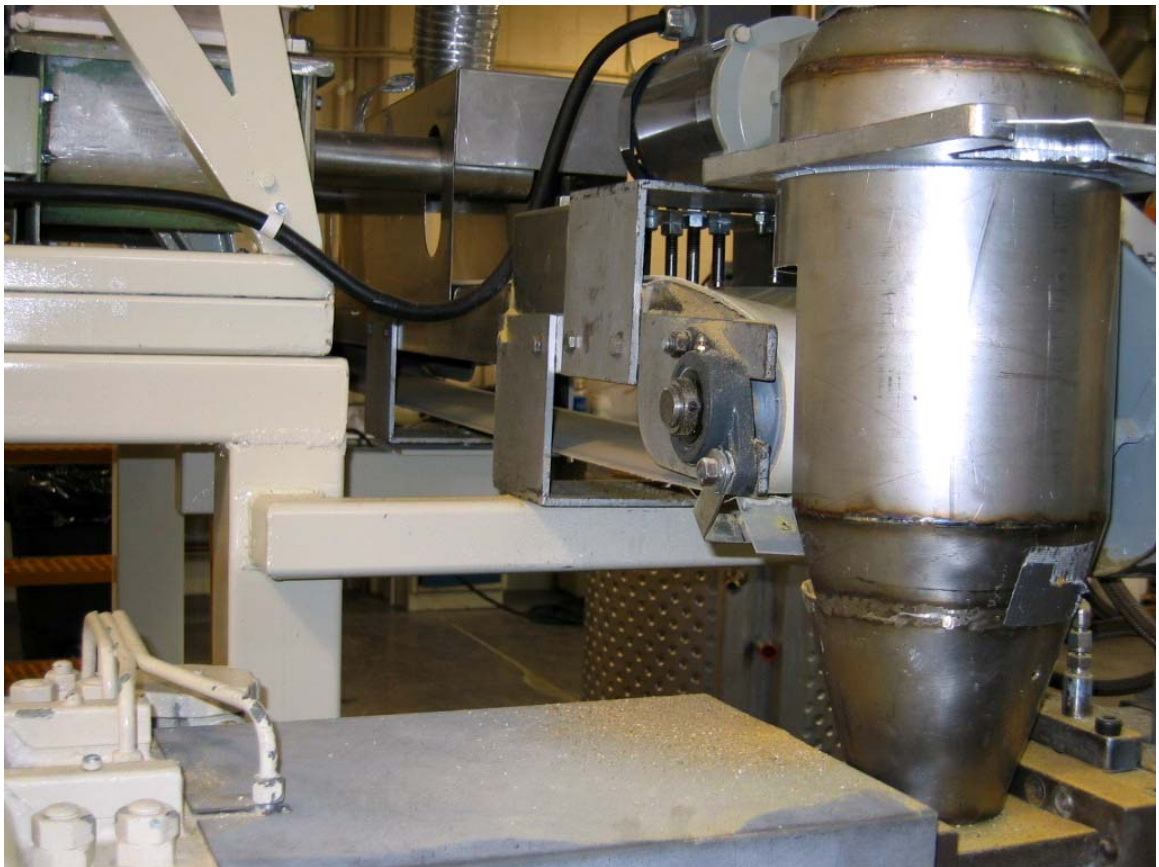


Figure A2. The mixed diet travels along the conveyor belt to the pre-conditioner and then into the barrel of the extruder.

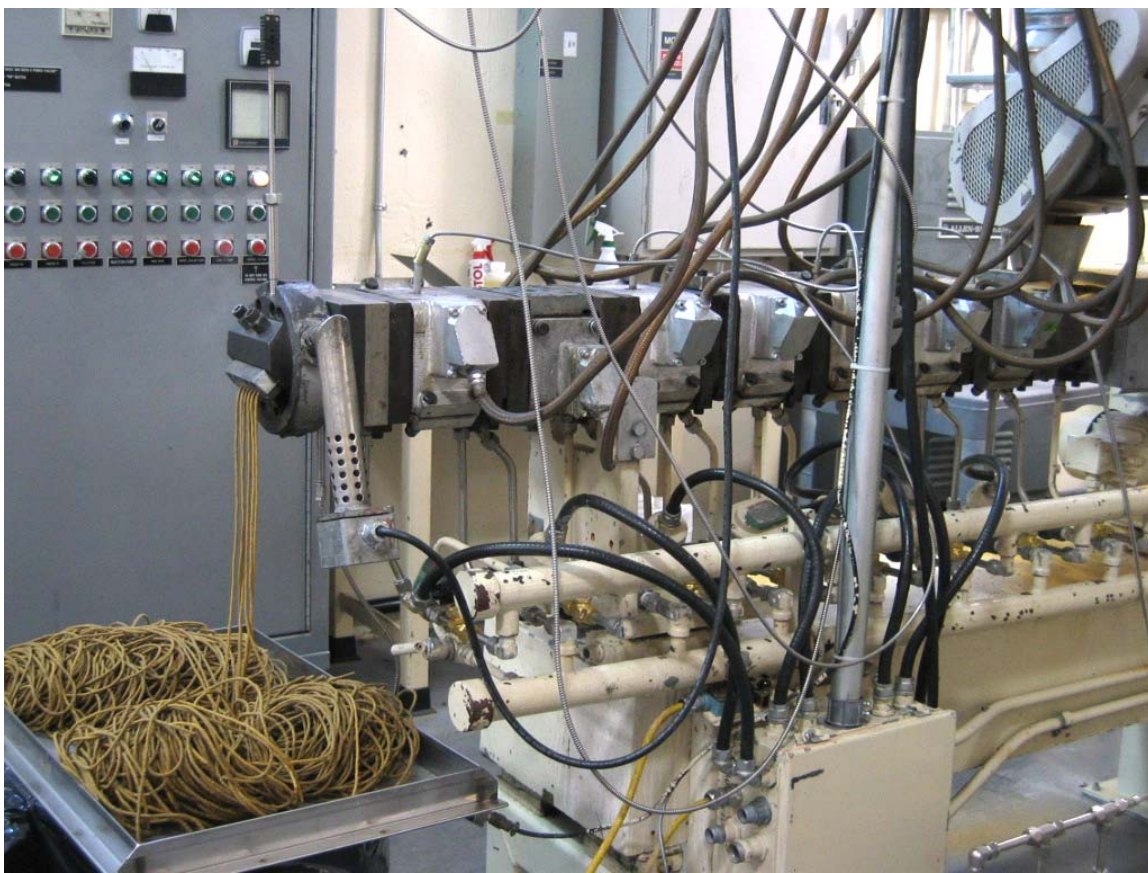


Figure A3. The barrel consists of 8 different sections with 6 different temperature probes, water injection sites, a twin co-rotating screw and a 6-hole strand die plate. The screw speed was held constant between 200 and 225 rpm and the temperature ranged between 70 - 116° C between the 8 sections of the barrel.



Figure A4. A single screw consists of 38 elements making a total length of 1.5 meters.



Figure A5. The elements of the screw consist of left hand and right hand kneading blocks, concave and non-concave elements.



Figure A6. Feed is moved through the barrel being exposed to shearing, high temperatures and pressure averaging 2400 kPa and is then released through the 6 hole strand die.



Figure A7. Drying the extruded product in a continuous fluid bed dryer for approximately 15 minutes at a temperature of 40°C.



Figure A8. Feed dropping out of the continuous fluid bed dryer with a moisture content of approximately 8%.



Figure A9. Adding fish oil following the drying procedure. The diet is placed into a rotating vacuum tumbler along with the fish oil and is mixed for 15 minutes at 11 rpm. Because the feed expanded as it exited the die, it becomes porous allowing for the fish oil to be absorbed during the vacuum process.



Figure A10. Pelleting the diets using a quadro mill.

APPENDIX B: Methods used to process ingredients used in this study.

Table B1. Processing methods used to prepare the pea, canola and flax products used in this study.

Ingredients	Products	Processing
Peas	Whole Peas	no processing
	Pea Protein Concentrate	aqueous extraction
Canola	Canola Meal	
	Canola Protein Concentrate	aqueous extraction
Flax	Whole Flax	no processing
	Dehulled Flax	abrasion
Miscellaneous	Canola: Pea Coextrudate	extrusion
	Flax: Pea Coextrudate	extrusion

APPENDIX C: Fish Management for digestibility and growth trial.

Digestibility Trial

Fish Handling

- Fish were placed into a holding tank for approximately 1 week upon arrival at the Prairie Aquaculture Research Center
- The fish weighed around 10 grams
- Fish were collected from the holding tank and placed into Rubbermaid tubs with aeration
- Hand sorted to collect the largest fish
- 40 fish were weighed in a bucket and then placed into a tank

Fish Tanks

- All tanks had a mesh coving held down with clips in order to prevent fish from jumping out
- An additional mesh covering was placed over the tanks to prevent escapes
- Some fish still managed to jump out, however they generally survived the night and were then placed back into the tank that they were found closest to and had a missing fish
- A quick count of fish in each of the tanks every morning ensured that there was the right number of fish per tank

Table C1. Fish mortality in the tilapia digestibility trials.

Digestibility Trial 1

Diet	Nov 24 -Dec 8/03 Pre-Trial Period	Dec 8 - Jan 1 /04 Trial Period
Whole peas	4	
Pea protein concentrate	2	4
Canola: pea coextrudate	2	
Flax: pea coextrudate	2	1
Control	2	
Fish meal	3	
Total	15	5

Digestibility Trial 2

Diet	Jan 1 - Jan 12 /04 Pre-Trial Period	Jan 12 - Jan 27 /04 Trial Period
Canola meal		
Canola protein concentrate		1
Whole flax		
Dehulled flax		
Soya bean meal		
Control		
Total	0	1

Fish Feeding

- Fish were fed twice a day once at 800 hours and again at 1600 hours
 - A small amount of feed is thrown into the tank. Once this feed is gone, another small amount of feed is thrown in. This continues until the fish slow down and do not show interest in eating.
- Feed intake was measured by weighing the container for each tank at the end of each day

Fecal Collections

- Each night during the collection period, all tanks on trial were cleaned carefully to remove any food that may have not been eaten and any fecal material present
- Collection tubes were placed under the tanks and the valves opened
- The next morning the valves were closed and the collection tubes taken off
- The fecal material settles on the bottom of the collections tubes and the excess water is poured out
- Fecal material is then poured into vials and frozen in a freezer
- Once these vials are full they are defrosted and centrifuged and placed into containers

Growth Trial

Fish Handling

- Following the digestibility trial, fish were placed into the holding tank for a couple of days
- Fish were then collected and placed into Rubbermaid tubs, with aeration, and were anesthetized using ??
- Fish were then hand sorted in order to obtain an all male population
 - Male - two opening in front of the anal fin
 - Female - three openings in front of the anal fin
- Applying ink or a dark dye to this area was suppose to increase the accuracy but we felt that it made a big mess and was not helping us to determine the sex

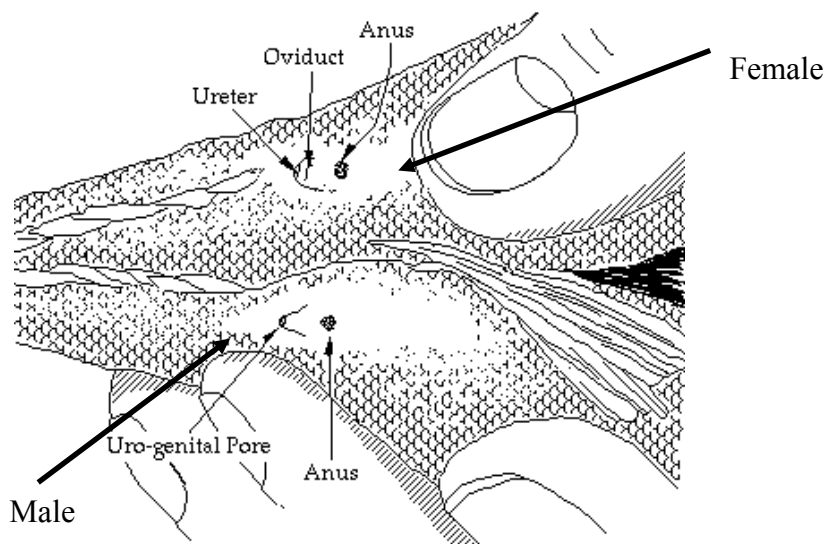


Figure C1. Anatomical differences between male and female tilapia (Auburn University, 2005).

Fish Feeding

- The same procedure for feeding was used as in the digestibility trial

Weighing Tanks

- Every two weeks each tank was weighed
- Fish are collected from the tanks and placed into Rubbermaid tubs with aeration
- They are anesthetized in these tubs order to take an accurate reading (they jump around a lot if they are not anesthetized making it hard to get an accurate weight)
- Once the fish are asleep, they are netted and placed into a bucket that has been tarred off already
- Fish are weighed on the scale and then put back into their original tank
- Within a few minutes of being back into their tanks

Table C2. Fish mortality in the tilapia growth trial.

Diet	March 9 - March 23 /04	March 23 - April 6 /04	April 6 - April 20 /04	April 20 - May 4
100%	4			1
66.6% Simple				2
33.3% Simple	7	1	1	1
0% Simple			1	2
66.6% Complex	3	1		1
33.3% Complex	3			
0% Complex			1	
Total	17	2	3	7

Stocking Density

- Stocking density is determined by a number of factors
 - feeding rate
 - size of tank
 - size of fish
- The stocking density for the digestibility trial was not an issue according to the following table (Rakocy, 1989).

Table C3. Stocking density of tilapia with different weights and feeding rates (Rakocy, 1989).

Stocking Rate (number of fish 1000 L ⁻¹)	Weight Fish-1 (grams)	Feeding Rate (%)
8,000	0.02	20 -15
3,200	0.5 - 1.0	15 -10
1,600	5	10 - 7
1,000	20	7 - 4
500	50	4 - 3.5
200	100	3.5 - 1.5
100	250	1.5 - 1.0

- In the digestibility trials, we placed 40 fish weighing between 10 - 50 grams in 350 L tanks
- In the growth trial we placed 10 fish weighing between 50 - 150 grams with final weights around 200 - 300 grams
- Feeding rate was determined to be 2 - 3% at the beginning of the digestibility trial

APPENDIX D: Growth trial data arranged in 2 week periods.

Table D1. Performance of tilapia based on simple and complex diets with varying levels of fishmeal for days 0-14 of the growth trial.

	Average Daily Gain (g d ⁻¹)	Specific Growth Rate (%)	Average Daily Feed Intake (g d ⁻¹)	Feed Conversion ¹ (g d ⁻¹)	Protein Efficiency Ratio (gain protein intake ⁻¹)
Fishmeal Level					
0	0.80 ^a	1.92	1.89 ^a	-0.21	0.77
33	1.77 ^{ab}	3.23	2.49 ^{ab}	1.33	1.17
67	2.10 ^{ab}	3.73	2.36 ^{ab}	0.46	1.99
100	3.03 ^b	5.10	2.59 ^b	0.88	2.92
SEM	0.42	0.98	0.19	0.72	0.78
Diet Complexity					
Simple	1.78	3.27	1.92	0.28	1.52
Complex	2.08	3.70	2.04	0.95	2.01
SEM	0.33	0.63	0.13	0.57	0.59
<i>P</i> -value					
Fishmeal	0.04	0.13	0.02	0.50	0.19
Complexity	0.72	0.74	0.50	0.41	0.52
Interaction	0.07	0.04	< 0.01	0.32	0.14

¹The feed conversion ratios were calculated using all the observations including tanks where there were negative average daily gains.

Table D2. Performance of tilapia based on simple and complex diets with varying levels of fishmeal for days 14-28 of the growth trial.

	Average Daily Gain (g d ⁻¹)	Specific Growth Rate (%)	Average Daily Feed Intake (g d ⁻¹)	Feed Conversion ¹ (g d ⁻¹)	Protein Efficiency Ratio (gain protein intake ⁻¹)
Fishmeal Level					
0	1.12 ^a	2.05 ^a	2.19 ^a	-0.37	1.31
33	2.43 ^b	3.57 ^{ab}	3.15 ^b	1.92	1.38
67	3.44 ^b	5.44 ^b	3.37 ^b	0.06	2.06
100	2.77 ^b	3.81 ^{ab}	3.20 ^b	1.78	1.64
SEM	0.34	0.62	0.15	0.91	0.33
Diet Complexity					
Simple	2.09	3.16	2.81	1.09	1.42
Complex	2.67	4.29	3.23	0.60	1.77
SEM	0.27	0.41	0.15	0.72	0.25
P-value					
Fishmeal	< 0.01	< 0.01	< 0.01	0.24	0.051
Complexity	0.24	0.11	0.04	0.63	0.28
Interaction	0.07	0.02	< 0.01	0.30	0.57

¹The feed conversion ratios were calculated using all the observations including tanks where there were negative average daily gains.

Table D3. Performance of tilapia based on simple and complex diets with varying levels of fishmeal for days 28-42 of the growth trial.

	Average Daily Gain (g d ⁻¹)	Specific Growth Rate (%)	Average Daily Feed Intake (g d ⁻¹)	Feed Conversion ¹ (g d ⁻¹)	Protein Efficiency Ratio (gain protein intake ⁻¹)
Fishmeal Level					
0	0.90 ^a	1.48 ^a	2.67	2.87	0.72 ^a
33	1.89 ^b	2.46 ^b	3.09	0.03	1.25 ^{ab}
67	2.23 ^b	2.82 ^b	3.14	-0.19	1.31 ^b
100	2.52 ^b	2.99 ^b	3.20	1.98	1.31 ^b
SEM	0.28	0.31	0.27	1.47	0.18
Diet Complexity					
Simple	1.56	2.04	2.90	2.50	0.84
Complex	2.16	2.83	3.32	-0.15	1.25
SEM	0.22	0.25	0.21	1.16	0.14
P-value					
Fishmeal	< 0.01	< 0.01	0.14	0.41	< 0.01
Complexity	0.15	0.06	0.13	0.11	0.28
Interaction	0.37	0.18	0.04	0.84	0.29

¹The feed conversion ratios were calculated using all the observations including tanks where there were negative average daily gains.

Table D4. Performance of tilapia based on simple and complex diets with varying levels of fishmeal for days 42-56 of the growth trial.

	Average Daily Gain (g d ⁻¹)	Specific Growth Rate (%)	Average Daily Feed Intake (g d ⁻¹)	Feed Conversion ¹ (g d ⁻¹)	Protein Efficiency Ratio (gain protein intake ⁻¹)
Fishmeal Level					
0	1.05 ^a	1.71 ^a	2.65 ^a	3.22 ^b	1.04
33	2.21 ^b	2.97 ^b	3.11 ^{ab}	1.36 ^{ab}	1.40
67	2.23 ^b	2.65 ^b	3.49 ^{ab}	1.80 ^{ab}	1.40
100	2.41 ^b	2.74 ^b	3.66 ^b	0.13 ^a	1.42
SEM	0.18	0.18	0.31	0.92	0.18
Diet Complexity					
Simple	1.72	2.25	3.25	2.39	1.30
Complex	2.24	2.77	3.41	0.87	1.34
SEM	0.15	0.14	0.24	0.47	0.16
P-value					
Fishmeal	< 0.01	< 0.01	0.03	0.02	0.06
Complexity	0.054	0.05	0.58	0.03	0.76
Interaction	0.34	0.42	0.09	0.43	0.51

¹The feed conversion ratios were calculated using all the observations including tanks where there were negative average daily gains.

APPENDIX E: Tilapia villi micrographs (50X).



Figure E1. Tilapia intestinal section from fish fed the 0% Simple diet.



Figure E2. Tilapia intestinal section from fish fed the 0% Complex diet.

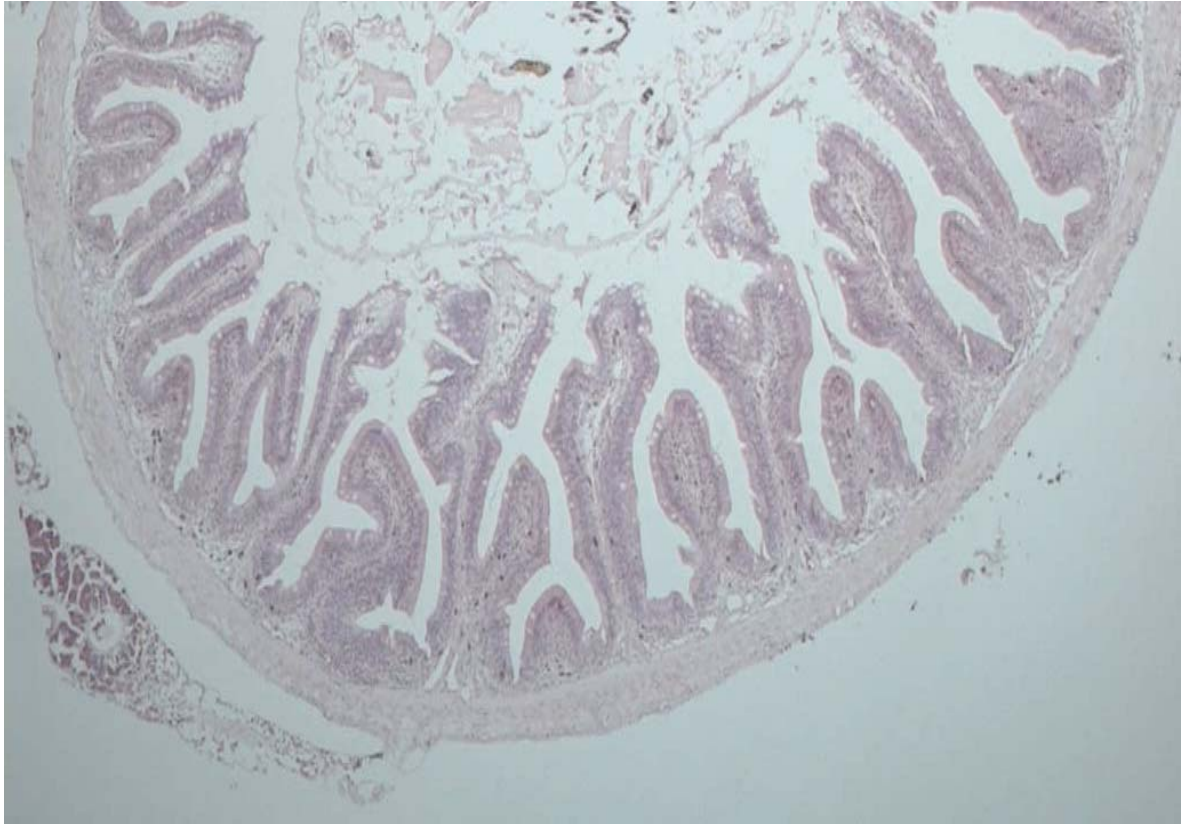


Figure E3. Tilapia intestinal section from fish fed the 100% Fishmeal diet.