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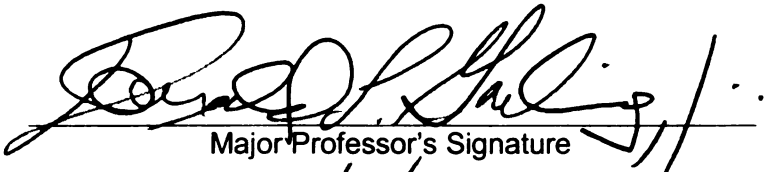

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REPLACEMENT OF FISH MEAL WITH SOYBEAN MEAL IN DIETS FOR ATLANTIC SALMON, *SALMO SALAR*, EFFECTS ON GROWTH, PROTEASE ACTIVITY, DIGESTIBILITY AND INTESTINAL HISTOLOGY

By

Christopher Todd Weeks

A DISSERTATION

Submitted to
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In partial fulfillment of the requirements
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ABSTRACT

REPLACEMENT OF FISH MEAL WITH SOYBEAN MEAL IN DIETS FOR ATLANTIC SALMON, *SALMO SALAR*, EFFECTS ON GROWTH, PROTEASE ACTIVITY, DIGESTIBILITY AND INTESTINAL HISTOLOGY

By

Christopher Todd Weeks

Wild harvested seafood resources are at or near maximum sustained yields worldwide. This has, in part, caused a significant increase in aquaculture production over the last 50 years. Further expansion of farmed fish production is likely in order to meet nutritional needs of a growing global human population. Since aquaculture depends heavily on high quality fish meal (FM) as a feed ingredient, demand for fish meal is expected to exceed global supplies in the near future. This could have major economic impact on commercial aquaculture facilities raising carnivorous fish such as Atlantic salmon. There has been increasing effort aimed at utilizing alternate protein sources to help alleviate the growing demand for FM. Soybean meal (SBM), in particular, is considered by many as a leading alternative to FM in formulated feeds for aquaculture.

Extensive research on SBM diets for carnivorous fish has identified various anti-nutritional factors inherent to soya and other plant-based products. Among SBM anti-nutritional factors, trypsin inhibitors can be a serious problem for salmonids because they bind with protein digestive enzymes and interrupt nutrient absorption.

A series of experiments were conducted to examine effects of soybean trypsin inhibitors (SBTI) and high nutrient dense (HND) practical SBM diets on Atlantic salmon fingerlings and smolts. In the trypsin inhibitor studies, stock SBTI were added to

standardized semi-purified diets containing 50% crude protein and 19% crude fat at graded levels from 0 - 60% SBM equivalencies. In one trial, small Atlantic salmon (17.5 ± 1.4 grams) were fed in triplicate either a commercial control or test diets containing 0, 15, 30, 45, and 60% SBM equivalency TI for a period of 8 weeks. No significant differences were observed between growth rates of Atlantic salmon fed SBTI diets, although the commercial control diet resulted in significantly lower growth rates than those fed the test diets. There were no differences in feed conversion rate (FCR, 0.78–0.83), protein efficiency ratio (PER, 0.24–0.27), or apparent protein retention (APR, 32.9–35.7%) between diets. Slight differences were observed in proximate body composition data, but they did not appear to be related to SBTI levels. In another trial, similar experimental diets containing an inert marker were fed to Atlantic salmon smolts (89.2 ± 4.1grams) for a period of 21 days. Slight differences were observed between dietary SBTI levels in digesta dry matter and intestinal trypsin activity, and small intestine trypsin activities varied over time. Neither apparent protein digestibility nor body lipid compositions showed any affects from SBTI over the 21-day trial.

For the final experiment HND diets containing 30% FM and 0, 20, 25, 30% SBM, or 24% FM with 20 and 30% SBM were fed to Atlantic salmon for 12 weeks. No differences were observed in growth (SGR, 1.88–1.94), FCR (0.78-0.82), PER (2.20–2.32), trypsin activity, or intestinal histology. A negative linear response was observed between SBM content, body lipid composition, and fecal dry matter. Fish whole body lipid composition decreased from 22.0% to 12.9% for 0% SBM and 30% SBM diet groups respectively. Study findings indicate use of HND SBM diets may contribute to protein sharing functions of Atlantic salmon to SBM carbohydrates.

This work is dedicated to all individuals contributing to the development for sustainable aquaculture - past, present and future, especially my Mom - simple things like the unrelenting support for a son, and aquaculture news article clippings from places out west...

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Lastly I give a very special thanks to my mentor and friend Harry Westers. I would not be where I am today if it were not for him.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

$\#Fish_f$ - number of fish alive at end of study

#TU - number of thermal units in ^0C above 0.0^0C

$\%\ P_{Feed}$ - percent protein in diet as fed basis

%BW - percent body weight

^0C - temperature in Celsius

ALA - alanine

ANF - anti-nutritional factor

ANOVA - analysis of variance

APD - apparent protein digestibility coefficient

APR - apparent protein retention

Arg - arginine

ASN - asparagine

BAPA - Benzoyl-DL-arginine-p-nitranilide

Ca - calcium

CF - crude fat

CFbr - crude fiber

CHO - carbohydrate

CP - crude protein

Cr_D - percent marker (Cr_2O_3) in diet

Cr_F - percent marker (Cr_2O_3) in faeces

Cu - copper

Cum Feed - cumulative weight of feed

Cys - cystine

d - time

DE - digestible energy

DM_{Feed} - percent dry matter of feed

DNR - Department of Natural Resources

DP - digestible protein

E - energy

EVol - extract volume

F - fecal

FAO - Food and Agriculture Organization of the United Nations

FCE - feed conversion equivalencies

FCR - feed conversion rate

FD_f - final percent freeze-dried matter of fish samples

FD_i - initial percent freeze-dried matter of fish samples

FE - feed efficiency

Fe - iron

FM - fish meal

FPG - fish protein gain

$FRC_{1.0}$ - theoretical feed conversion rate of 1.0

FWS - Fish and Wildlife Service

GLU - glutamic acid

GLY - glycine

His - HIS - histidine

HND - high nutrient dense

HSI - hepatic somatic index

HYP - hydroxyproline

IAA - indispensible amino acid

Ile - ILE - isoleucine

k - condition factor

k_i - condition factor at the start of the trial

Leu - LEU - leucine

L_F - final length

L_i - initial length

LI - large intestine

Lpm - liters per minute

LW - liver weight

Lys - LYS - lysine

Magn - Maganese

Met - MET - methionine

Mg - magnesium

mmt - million metric tonne

MS222 - tricaine methanesulfonate

MSE - mean square error

MSU - Michigan State University

N - nitrogen

Na - sodium

N_{Morts} - number of mortalities removed

NSP - non-starch polysaccharide

OMNR - Ontario Ministry of Natural Resources

ORN - ornithine

Panta-Acid - pantothenic acid

P_D - percent protein of diet

PER - protein efficiency ratio

P_f - final percent protein in body compositions of freeze-dried sample

P_F - percent protein of feces

Phe - PHE - phenylalanine

Phos - phosphorous

P_i - initial percent protein in body compositions of freeze-dried sample

PI_{Feed} - protein intake from feed

Pot - potassium

P(reg) - P-value for regression analysis

PRO - proline

Pyrdox - pryidoxine

Ribo - riboflavin

SBM - soybean meal

SBTI - soybean trypsin inhibitor

Se - selenium

SER - serine

SGR - specific growth rate

SI - small intestine

TA - trypsin activity

TAPD - total apparent protein digestion

TAU - Taurine

Thia - thiamine

Thr - THR - threonine

TI - trypsin inhibitor

TP_{Feed} - total protein in feed

TRIS - Tris (hydroxymethyl) aminomethane

Trp - TRP - tryptophan

Try - trypsin value

TUG - temperature unit growth rate

Tyr - TYR - tyrosine

USB - United Soybean Board

Val -VAL - valine

W - wet weight of fish at time of sampling

W_f - average final wet weight

WGM - wheat gluten meal

W_i - initial wet weight

W_{Morts} - wet weight of mortalities removed

Zn - zinc

Chapter 1 – Introduction

Soybean meal (SBM) has been widely considered as a viable alternative protein source for fish meal in formulated fish feeds for aquaculture (Hardy 1996, 2003, Storebakken et al. 1998). Studies have shown, however, that SBM diets can lead to significantly reduced growth rates and cause severe health problems for certain, mainly carnivorous, fish species such as salmonids (Arnesen et al. 1989, Refstie et al. 2000, Krogdahl et al. 2003).

Atlantic salmon are an important commercial aquaculture species in the European Union, and North and South America with total production in 2005 estimated at over 1.2 million metric tonnes (mmt) and an value of $4.7 billion US (FAO 2002–2007). Due to increasing demands in salmon production and fish meal, a relatively large number of studies have been conducted in effort to determine the maximum amount of dietary fishmeal that can be replaced by SBM in Atlantic salmon and rainbow trout feeds (Hardy 2003). After over a decade of research the maximum safe level of dietary SBM in formulated diets for these salmonid species remains uncertain.

In 2002, a collaborative project was funded by the United Soybean Board (USB), Soy-in-Aquaculture Program, to examine the extent of SBM anti-nutritional properties in formulated feeds for salmonids. The project was designed to develop a commercially acceptable SBM based formulated feed for Atlantic salmon and other species. Michigan State University (MSU), Department of Fisheries and Wildlife, was awarded a 2-year project funded through the USB Soy-in-Aquaculture Program to examine effects of

trypsin inhibitors and SBM on Atlantic salmon. This dissertation describes the experimental design, methods and results of studies conducted at MSU.

The research conducted at MSU was originally intended as a 2-phase experimental design to examine affects of trypsin inhibitors in SBM-based diets on Atlantic salmon. Phase I of the design focused on the effect of growth, feed consumption, digestibility, and pancreatic proteolytic enzyme activity of juvenile and smolting Atlantic salmon (*Salmo salar*) fed purified diets with graded levels of trypsin inhibitors. The second phase of this research project was initially intended to examine effects of trypsin inhibitors in SBM-based diets containing practical feed ingredients under different processing conditions.

Based on results obtained by other researchers in the Soy-in-Aquaculture Program in 2003 and an extensive literary review, MSU expanded the focus of the research scheduled in phase II to include a detailed assessment of commercial SBM diets for Atlantic salmon. The overall goal of this study was to develop a potentially commercially viable, practical diet for Atlantic salmon containing the highest level of SBM possible based on best available knowledge.

Study Hypothesis

Hypothesis:

Based on literature review and recent unpublished study data the maximum level of SBM incorporation into formulated diets for Atlantic salmon is expected to be in the range of 20-30% wet ingredient weight without adverse affects on growth, feed efficiency, and/or other observable fish health characteristics.

Study Description

This dissertation is presented in 7 chapters. This first chapter provides a brief introduction of the research undertaken at MSU and the hypothesis developed by the researcher in regards to SBM diets for Atlantic salmon. Chapter 2 is a literature review providing relevant background information of the status of SBM as an alternative protein source in formulated diets for Atlantic salmon.

Chapters 3 and 4 describe two phase I trypsin inhibitor feed trials completed in 2003. Chapter 5 - *Fixed Formulation Model Development of Practical High Energy SBM Diets for Atlantic Salmon (Salmo salar)*, draws upon widely dispersed information available specifically on Atlantic salmon diet formulations to develop a practical feed formulation model. Diet formulation model parameters are provided in the Appendix. Chapter 5 was added for potential use as a "blueprint" for anyone initiating a similar exercise. Chapter 6 - *Replacement of FM with SBM in High Energy Practical Diets of Smolting Atlantic Salmon (Salmo salar)*, provides details and results of the 2004 practical SBM diet study on Atlantic salmon. This study was designed to determine maximum levels of SBM that can be safely added to commercially viable diets for Atlantic salmon based on best available knowledge.

Chapter 7 summarizes the research results and hypothesis testing. The chapter also provides conclusions and recommendations for further research.

Chapter 2 - Literature Review: Effects of Soybean Meal as an Alternative Protein Source in Formulated diets for Atlantic Salmon (*Salmo salar*)

Introduction

Global production from capture fisheries and aquaculture supplied about 140 million metric tonnes (mmt) of food fish in 2004 (FAO 2006). According to Wijkström (2003), human consumption of seafood products is expected to increase to approximately 121 mmt by the year 2010, and 271 mmt by 2050. Most experts agree that wild capture fisheries are at maximum yield at about 100 mmt per year. Global aquaculture production must increase then in order to meet projected demands in seafood consumption.

According to the Food and Agriculture Organization of United Nations (FAO), current expansion rates observed in world aquaculture production combined with anticipated needs for human consumption of seafood products are expected to cause a global shortage of fish oil by the year 2010 and fish meal by 2015 (New and Wijkström, 2002). In a recent survey of 600 fish species, 77% of the world's marine fish stocks are estimated to be either fully exploited, over exploited or depleted (FAO 2004). Adding to this dilemma are reports and highly publicized news events linking elevated contaminant levels found in farmed Atlantic salmon destined for human consumption to fish meals and oils used to manufacture aquaculture feeds (Hites et al. 2004, Foran et al. 2005). Based on current trends, future demands for high quality fish meals and oils are likely to have severe economic impacts on global aquaculture production. Clearly, incentives exist for the development of alternate protein and lipid sources for use in formulated feeds for aquaculture.

Atlantic Salmon

Indigenous to the North Atlantic, the native range of Atlantic salmon (*Salmo salar*) extends from the Arctic Ocean and Baltic Sea to Portugal in the east, and from Iceland, to Southern Greenland, Canada, and Northern US in the western Atlantic (Netboy 1974 in Danie et al. 1984). Original landlocked populations existed in Maine, New Brunswick and Nova Scotia, and transplanting has occurred to literally hundreds of inland lakes (Danie et al. 1984). Distinct segments of Atlantic salmon from Maine have been listed as either endangered or threatened under the Endangered Species Act of 1973.

Atlantic salmon are cold water carnivorous species with a preferred water temperature range of 8-18^{0}C (Sedgewick 1988, Jensen et al 1991). Adults can survive in both fresh and salt water. They are an anadromous - wild adults return from the ocean or landlocked lakes to spawn in gravel areas of freshwater streams, and iteroparous - spawn more than once. Various populations migrate into rivers anytime from the spring to late fall, and peak spawning season is typically from mid-October to November (Bigelow and Schroeder 1953). Their egg incubation period is temperature dependent (Ojanguren et al. (1999), and may range from 2-3 months in a hatchery to several months in the wild under normal winter conditions. Wild newly hatched fry (alevins), remain in the gravel approximately 6 weeks until their yolk sacks are depleted of nutrients (Bigelow and Schroeder 1953). At this time they must emerge and begin foraging for food.

At approximately 4.0 cm in length, normally achieved in the first summer, young Atlantic salmon are classified as parr or fingerlings (Danie et al. 1984). Most parr remain in the stream for 2-3 years (125-150 mm length), although Schaffer and Elson (1975) have reported populations in Ungava Bay region of Canada may remain in fresh water for

4-8 years (180 mm long). Prior to seaward migration Atlantic salmon undergo a physiological transformation called smoltification. These changes allow for a variety of salmonids to survive in a salinity environment (Sedgewick 1988), and occurs whether smolt migration is from river to either sea or fresh water.

Wild fish typically grow to maturity in 2-3 years in the sea or landlocked lakes before returning to their home stream to spawn. Returning Atlantic salmon usually range between 3-9 kg in weight, although much larger fish have been observed and recorded (Scott and Crossman 1973).

Atlantic salmon culture began in the 19th century in the United Kingdom in freshwater as a means of stocking waters with parr in order to enhance wild returns for anglers (FAO 2000–2007). North Atlantic commercial salmon catch data indicated a high abundance cycle from the mid-1960s to the 1970s with a maximum harvest of 12,000 tonnes in 1967 (Mills 1989). Numbers of returning fish declined greatly over this time frame, and presently nearly all commercial fisheries for wild Atlantic salmon are closed (Parrish et al. 1998). According to these authors, over fishing, dam construction, pollution, and dewatering of streams caused the decline and extirpations of Atlantic salmon.

Atlantic salmon are a widely popular commercially farmed aquaculture species in Europe, North and South America and Australia. Commercial aquaculture of Atlantic salmon first began in the early 1960s, and production has increased dramatically over the past few decades (Figure 2.1). In 2005, total annual production from commercial Atlantic salmon farms exceeded an estimated 1.2 mmt worth for $4.7 billion US (FAO 2002–2007).

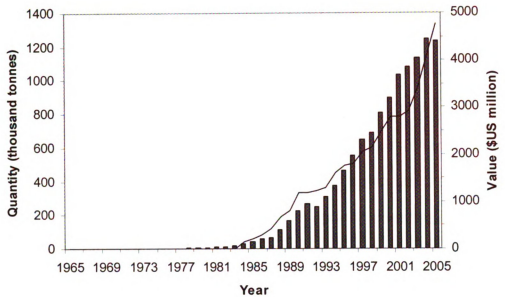

Figure 2.1 Global commercial Atlantic salmon production (bars) and value (line) from aquaculture 1964-2004. Source: FAO FIGIS data base (FAO 2002–2007).

Current intensive rearing programs utilize culture techniques that provide a great deal of control over production cycles (AAFC 2004). Temperature manipulation, for example, can significantly speed up the time required to grow from egg to fingerling stages (Ojanguren et al. 1999). Salmon eggs are collected from either captive or wild female broodstock during fall spawning cycles. Eggs are mixed with milt from males and incubated at around 6-10^{0}C optimal water temperatures (Massachusetts Office of Coastal Zone Management 1995, FAO 2000–2007). Newly hatched fry (sac fry) typically emerge in 2-4 months depending on incubation water temperatures (AAFC 2004). Sac fry use up oil sack energy reserves in approximately 3-6 weeks, at which

point they become swim-up fry and readily begin to feed on formulated starter diets. Cultured Atlantic salmon are kept on formulated diets throughout the entire production cycle. Fish are usually graded several times to maintain fish of the same size together in individual rearing units (AAFC 2004). This practice reduces cannibalism and facilitates product uniformity. Photoperiod manipulation can shorten the fresh water production phase to about 6 months (IFREMER 2005). Smolts are transferred to ocean grow out facilities (cages or net enclosures) in the spring, where they are raised to a market size of between 8 and 10 pounds. The overall production cycle of farmed Atlantic salmon takes approximately 20 months from hatch to harvest (AAFC 2004).

The Fish Meal Dilemma

Significant expansion of the commercial aquaculture industry faces a major dilemma. Most processed feeds for aquaculture contain varying amounts of fish meal and oils. Carnivorous species, such as trout and salmon require up to 45–75% fish meal of very high quality. High quality fish meals used to make commercial feeds for aquaculture are obtained from wild harvested small pelagic fish species including Peruvian anchoveta, Icelandic herring, menhaden (Gulf of Mexico), and Norwegian capelin. These species are currently being harvested at or close to maximum yields.

New and Wijkström (2002) examined global projections of aquaculture production rates and projected 15 and 30 year fish meal usage requirements for aquaculture (Table 2.1). Their conclusions indicate that a supply and demand crisis for fish meal is highly likely over the projected timeline. This crisis is further compounded by the point that aquaculture uses a disproportionate amount of fish meal as compared to

Table 2.1 Estimated fish meal and oil supply and usage in 1999 compared to 15 and 30 year projections. Source: New and Wijkström (2002).

Year	Fish Meal		Fish Oil	
	Global Supply (thous. mmt)	Usage by Aquaculture (%)	Global Supply (thous. mmt)	Usage by Aquaculture (%)
1999	6548	32	1360	49
2015	6526	70	1283	145
2030	6526	159	1283	460

other forms of agriculture. While aquaculture makes up only approximately 3% of global animal feed production, 45% of fish meal usage goes into aquaculture feeds (Gill 2005, Pike 2005). In order for aquaculture to successfully meet projected needs for food fish for humans, ways must be found to reduce the amount of wild fish required to feed fish, livestock and poultry. This realization has stimulated a rather intensive international effort aimed at reducing global reliance on fish meal and oils in animal feeds. Most of the focus of research to date has been placed on improving feed utilization of cultured species, and replacement of fish meal by alternative protein sources.

Feed Utilization in Intensive Fish Culture

Feed utilization in intensive aquaculture is most often measured by feed conversion ratio (FCR) or by feed efficiency (FE). FCR is equal to feed fed divided by weight gain. FE is equal to the reciprocal of FCR expressed in percent. For example, a FCR of 1.4 provides a reciprocal of 0.71 and FE equal to 71%. The lower the FCR, the higher the FE and hence feed utilization efficiency. One important aspect of FCR is that it does not account for differences between feed and fish dry matter content. For

example, an FCR of 1.0 (FE = 100%) is considered very good by industry standards (Harry Westers, Aquaculture Bioengineering Corporation, personal communication). For dry matter contents of 95% and 28% for feed and fish respectively, the true feed conversion would equate to 3.4, or 30% efficiency.

Fish feed utilization rates in the aquaculture industry have substantially improved over the past few decades due to advances made in feed formulation methods, feed manufacturing technology, and feed management practices. According to Tacon (2005), the average FCR for commercial Atlantic salmon farming operations has been reduced from >2.0 before 1985, to 1.3 in 2003. This author also points out that FCRs for farmed salmon and large rainbow trout are the lowest of all the major aquaculture species.

Another way to assess feed utilization is in terms of fish conversion equivalencies. Approximately 4 to 5 tons of whole fish are required to produce 1 ton of dry fish meal (Miles and Chapman, 2006). Feed conversion equivalencies (FCE) then is the apparent conversion efficiency of pelagic fishes (wet weight basis; calculated by summing total fishmeal and fish oil consumption figures and then multiplying by 4 or 5) to farmed fish (Tacon 2004, 2005). According to Tacon, FCE's should continue to decrease over the next several years (Table 2.2)

Protein Sparing Effects of Aquaculture Feeds

Carnivorous fish are notably very efficient at using protein as an energy source. Gaitlin III (1995), attributes this due to the ability of fish to efficiently deaminate ammonia from protein and excrete it through the gills with limited energy expenditure.

Table 2.2 Estimated 2003 and projected 2010 feed conversion efficiencies (FCE) for various farmed fish species.

FCE (Fish input:output)	2003[1]	2010[2]
Marine eels	3.1 – 3.9	1.8-2.3
Salmon	3.1 - 3.9	1.2-1.5
Marine fish	2.5 - 3.2	1.5-1.9
Trout	2.5 – 3.1	0.8-1.0
Marine shrimp	1.6 - 2.0	1.0-1.2
Freshwater crustaceans	0.9 - 1.1	0.5-0.6
Milkfish	0.30 - 0.37	0.11-0.14
Tilapia	0.23 - 0.28	0.11-0.14
Catfish	0.22 - 0.28	0.16-0.20
Feeding carp	0.19 - 0.24	0.02

[1] FAO Fisheries Department, Fishery Information, Data and Statistics Unit. Fishstat Plus (2005) in Tacon (2005)
[2] Tacon (2004)

The concept of protein sparing typically refers to the protein utilization efficiencies of monogastric animal to feeds containing carbohydrates. With a greater fraction of energy supplied by carbohydrates, more protein is utilized for protein anabolism. Studies have shown that inclusion of carbohydrates improves protein sparing effects for nearly all species (Hemre et al. 1993, Sanchez-Muros et al. 1996). The later authors suggested protein sparing and growth promotion is aided by depression of gluconeogenic activity by dietary carbohydrates, which divert amino acids away from oxidative pathways. Carnivorous fish species, however, such as salmonids are limited to approximately 10% starch before growth is depressed (Hemre et al. 1993, 1995). Increased levels of indigestible carbohydrates have shown to increase hepatic lipogenesis (Brauge et al.

1994, 1995). Signs of negative impacts on protein sparing include decreased protein deposition, increased liver size and increased liver lipid content.

Alternative Protein Sources

In terms of commercial aquaculture production, high valued carnivorous species (salmon, trout, eels) require much greater amounts of high quality protein from fish meal (FM) than omnivorous species (catfish, tilapia). Salmonids, for instance, require 40-50% protein (Hardy 1996) and approximately 35% FM (Tacon 2005), compared to channel catfish requirements of 32-36% protein (Garling and Wilson 1976) and only 3% FM (Hardy 2000). Further reduction of fish meal in carnivorous fish diets requires development of nutritionally and environmentally acceptable, low cost alternative protein sources.

Major feed ingredients for salmonids must be high in protein, have high digestibility value, provide necessary essential amino acids, provide essential fatty acids, and contain relatively low levels ash, carbohydrates and fiber. From an environmental standpoint, fecal output, and ammonia and phosphorus content in effluent water must satisfy facility discharge requirements. Obviously, the ingredient or ingredients must also be available at a lower cost than fish meal. According to Hardy (1996), the selective properties required of protein sources for salmonid diets limit potential choices to a small list of ingredients.

Alternative practical ingredient protein sources best suited for salmonid diets are listed in Table 2.3. Note that each of the ingredients listed has one or more negative attributes when compared to a standard anchovy meal. Plant-derived meals are usually

Table 2.3 Comparison of ingredient properties between anchovy meal and potential alternative protein sources for use in commercial salmonid diets. Proximate compositions include crude protein (CP), crude fat (CF), crude fiber (CFbr) and ash.

Ingredient	Proximate composition (%)				Negative qualities[1]
	CP	CF	CFbr	Ash	
Anchovy meal	70	5.3	1.0	16.9	-- -- -- -- -- --
Soybean meal	50	0.9	3.4	5.8	antinutritional factors
Canola meal	38	3.8	11.1	6.8	high fiber, phytic acid
Corn gluten meal	60	1.8	1.5	2.1	fiber, colors fish flesh yellow
Wheat gluten meal	80	1.5	0.5	0.7	cost
Soy protein concentrate	70	0.75	4.2	7	low protein solubility, cost
Rapeseed protein concentrate	77	0.8	--	14.2	cost, availability, phytic acid
Pea protein concentrate	76	3	1	4	cost, antinutritional factors?
Feather meal	83	5.4	1.2	2.9	variable digestibility, [2]
Poultry by-product meal	60	13.6	2.1	14.5	low essential amino acids, [2]
Meat meal	55	8.7	2.3	27	high ash, phosphorus, [3]
Blood meal	89	0.7	1.0	2.3	cost, [3]

[1] Modified from Hardy (1996)
[2] Avian flu concerns
[3] Mad cow and TB concerns

the least expensive, but most are lower in protein than fish meal. In addition, all of the plant meals listed in Table 2.3 have been shown to contain various anti-nutritional properties in association with use in salmonid diets (Carter and Hauler 2000, Buttle et al. 2001, Francis et al. 2001).

Of all the alternative ingredients examined for use in aquaculture feeds, SBM has probably received the most attention. Soybeans constitute about 50% of the total oilseed crops worldwide and have become the most important source of plant proteins in the diets of monogastric animals (Alexis and Nengas 2001). SBM is a cost effective alternative to

fish meal and is an established agriculture product in the US. SBM was first tested in diets for trout in the early 1940s (Hardy 2003), and today is a common ingredient in many formulated feeds for aquatic and terrestrial animal species. Presently SBM may comprise 50% or greater of all dietary ingredients for omnivorous aquaculture species such as catfish, tilapia and carp (ElSaidy and Gaber 2002, Peres et al. 2002, Jahan et al. 2003). Its use in diets of carnivorous species like Atlantic salmon, however, has been severely limited due to a number of associated anti-nutritional properties. These will be discussed in more detail in following sections of this review.

Animal by-product meals have been commonly used in conjunction with fish meal in salmon diets. However, these ingredients are also expensive and can vary substantially in digestibility properties from batch to batch. Fairly recently, global threats of highly contagious emerging diseases (e.g. mad cow, avian flu), have resulted in international quarantines of beef and poultry products (EC Regulation 999/2001, CDC 2007).

SBM Manufacturing Process

"Dehulled solvent extracted" SBM is the most common form used in aquaculture feeds. The processing method for this product is described in an American Soybean Association Technical Bulletin by Behnke entitled *U.S. Soybean Meal Extraction, Processing and Specifications* (K. Behnke, Kansas State University, personal communication) and is described as follows:

Soybeans are cracked and dehulled on special rollers, then heat conditioned at a temperature of 60^0C for approximately 10 minutes. The conditioned beans are ground into a "flake" through another series of rollers, cooled, and solvent extracted. In this

process, the solvent, normally hexane, passes through the flake in a counter current exchange, removing most of the soybean oil and other soluble materials. Extracted flakes are dried under heat to volatilize and remove the solvent, and dried flakes are cooled and ground into meal. The oil rich material, miscella, is most often heated (distilled) to recover the solvent. The remaining miscella can be refined into oils for cooking and other purposes.

SBM Nutritional Characteristics

Dehulled solvent extracted SBM typically contains 47-50% protein and 3-4% crude fiber. In comparison, high quality fish meal contains 64-72% protein, 0.5-1.0% fiber (NRC 1993, Hertrampf and Piedad-pascual 2000). Differences in fat content between SBM and fish meal has, until recently, been of low concern in diet formulations because of the high energy supplied by fish oil. It is fairly clear by the projections of New and Wijkström (2002, Table 2.1), that future demands for fish oil will exceed that of fish meal. Researchers have begun to look for alternative energy sources for aquaculture diets. For example, Torstensen et al. (2005), reported that 100% of fish oil can be replaced with a vegetable oil blend without compromising growth or flesh quality of Atlantic salmon. The current challenge in this area is finding an energy source rich in polyunsaturated omega-3 fatty acids, EPA (eicosapentaenoic acid, C20:5n-3), and DHA (docosahexaenoic acid, C22:6n-3). Presently, high levels of EPA and DHA can only be obtained commercially through marine fish oils.

SBM has one of the best amino acid profiles of all protein-rich plant-based ingredients for meeting essential amino acid requirements of fish (Mohsen 1989 in NRC

1993). According to Wilson (2002), there are 10 indispensable amino acids (IAA) for all fish species known to date. Anchovy meal and SBM compositions of these amino acids are compared in Table 2.4. SBM contains much lower levels of Met, which is often considered the first limiting IAA in fish diets. SBM diets formulated for salmonids may be deficient in Met and thus require supplementation. While SBM appears to have a fairly good IAA composition, questions remain as to whether the essential amino acids are sufficiently balanced to replace large amounts of fish meal. Nutritional studies have shown that an imbalance of amino acids may affect feed utilization and growth of Atlantic salmon. Berge et al. (1999) examined effects of Lys:Arg ratios and found that lysine had both a stimulatory and inhibitory effect on the uptake of arginine on Atlantic salmon, depending on the relative concentration of the two amino acids. In another study on rainbow trout, Davies et al. (1997) concluded that the optimal Lys:Arg ratio is 1:1 for SBM diets.

SBM Anti-nutritional Factors

Researchers have identified several anti-nutritional factors (ANFs) in SBM and other plant meals which have shown to severely impair the health of carnivorous fish. These researchers have observed negative impacts including poor growth, inflammation of the cellular lining of the distal intestine (enteritis), mortality and disease (Dong et al. 2000, Storebakken et al. 2000, Krogdahl et al. 2003). ANFs of concern for Atlantic salmon include protease inhibitors, non-starch polysaccharides, oligosaccharides, saponins, lectins, antigenic proteins, isoflavones and phytic acid.

Table 2.4 Essential amino acid content of Peruvian anchovy meal and soybean meal.

Amino Acid	Peruvian anchovy meal (%)	Soybean meal (%)
Arg	3.85	3.67
His	1.61	1.22
Ile	3.17	2.14
Leu	5.05	3.63
Lys	5.04	3.08
Met	1.99	0.68
Cys[1]	0.60	0.75
Phe	2.78	2.44
Tyr[1]	2.24	1.76
Thr	2.82	1.89
Trp	0.75	0.69
Val	3.50	2.55

[1.] Considered as semi-essential amino acids

Protease inhibitors

Protease inhibitors are proteins that inhibit proteolytic enzymes, or protease activity, in the digestive track of animals (Krogdahl and Holm in Krogdahl et al. 1994). Inhibition occurs when inhibitors bind with the enzymes forming compounds unavailable for hydrolysis. The primary protease inhibitor of concern in SBM diets are trypsin inhibitors (TIs). Trypsin inhibitors have been isolated in two forms: Kunitz inhibitors, which inhibit mainly trypsin and is heat labile, and Bowman-Birk inhibitors, which

inhibit both trypsin and chymotrypsin (Liener 1980). In raw soybeans, TIs account for about 6% of the protein content (Alexis and Nengas 2001), and 2-6 mg TI/g in commercial soybean products (Snyder and Kwon 1987). Heat treatments in SBM processing results in values of about 3.0-3.5 mg TI/g meal (Tacon et al. 1983 in Hardy 2003). Further TI deactivation occurs in cooking-extrusion processes that occur during diet manufacturing. Atlantic salmon have been shown to tolerate up to 5 mg TI/g meal (Krogdahl et al. 1994).

Non-starch polysaccharides

Non-starch polysaccharides (NSPs), also referred to as dietary fiber, form about 14-18% of the total carbohydrate content of defatted SBM (Alexis and Nengas 2001), and up to 200 g/kg meal (Snyder and Kwon 1987). Insoluble NSPs such as cellulose and hemicelluloses are structural polysaccharides that do not dissolve in water. Soluble NSPs do not dissolve in water completely, but swell to form a gel in the presence of water (NRC 2003). While fiber is important for nutrient passage of various omnivorous fish species (e.g. catfish), finfish have no capacity to digest most fibrous material (De Silva and Anderson 1995, Roberts 2002).

Studies conducted on Atlantic salmon have identified NSPs as probable causes for reduced lipid absorption and increased fecal water content (Reftsie et al. 2000, 2001). According to Storebakken et al. (2000) NSPs likely impair diffusion, convective transport and/or micelle formation within the gastrointestinal tract of Atlantic salmon. These structural carbohydrates are not readily destroyed by normal heat treatments or solvent extraction. Fairly recently, scientists have been exploring the development of low NSP

soybeans for use in aquatic animal feeds through rapid detection methods such as infrared spectroscopy and proteomics (Hollung et al. 2005), and through genetic modification (Sanden et al. 2006).

Oligosaccharides

SBM contains approximately 10-15% oligosaccharides (sucrose, raffinose and stachyose, Dersjant-Li 2002, Alexis and Nengas 2001). Like NSPs, oligosaccharides are a carbohydrate fraction of SBM not digested by endogenous fish enzymes and thus expected to be an energy loss to the fish (Alexis and Nengas 2001). Oligosaccharides impair nutrient absorption, increase water content in feces, and may cause enteritis in Atlantic salmon (Reftsie et al. 2000, Storebakken et al. 2000). Since oligosaccharides are alcohol soluble, they can be removed from SBM via alcohol solvent extraction. This does not appear, however, to be a common industry practice at this time, perhaps because of the added cost.

Saponins

Saponins are steroid or triterpene glycosides found in many plant-derived feed ingredients and present in commercial SBM from 0.43-0.67% (Ireland et al. 1986). They are bitter in taste and are highly toxic to fish in sufficient doses. Bureau et al. (1998) identified soya saponins as a causative agent for reduced feed intake and intestinal damage to Chinook salmon. Krogdahl et al. (1995 in Francis et al. 2001), however, did not find any negative effects of dietary saponins on Atlantic salmon up to 30-40% SBM

equivalencies. Saponins are highly water soluble and can be removed rather easily by aqueous extraction.

Lectins

Plant lectins are glycoproteins found in many legume seeds (Chrispeels and Raikhel 1991). These compounds interfere with absorption and transport of nutrients by binding with membrane receptors of carbohydrates (Tacon 1995 in Alexis and Nengas 2001). Lectins, also known as phytohaemagglutinins, or agglutinins, have been shown to bind in vivo to the intestinal epithelium of Atlantic salmon and rainbow trout, contributing to the pathological events in the distal intestine associated with fish feeds containing high levels of SBM (Buttle et al. 2001). Since lectins, like trypsin inhibitors are proteinic, they can be destabilized by heat treatment and are destroyed to large extent in typical heating processes used to manufacture practical SBM-based aquaculture diets (Alexis and Nengas 2001).

Antigenic proteins

Antigenic proteins in SBM include active immunoglobular compounds, glycinin and β-conglycinin (Alexis and Nengas 2001). Inclusion of soya products containing high levels of these compounds reduced growth and caused enteritis in the distal intestine of rainbow trout (Rumsey et al. 1994). These same compounds also stimulate non-specific defense mechanisms in trout, but it is unknown if antigens in SBM result in higher disease resistance (Rumsey et. al. 1995). Soybean meal antigens are heat stable and alcohol soluble, and thus could be removed via alcohol solvent extraction.

Isoflavones

Soybean isoflavones are estrogenic compounds (phytoestrogens) both with and without a sugar molecule attached (Eldridge and Kwolek 1983). According to You et al. (2002), isoflavone concentration in raw soybeans ranges from 0.3 to 6.0 mg/g. The two primary isoflavones in soybeans are daidzein and genistein and their respective glucosides, genistin and daidzin. Little is known about the effect of isoflavones on salmonids, although Mambrini et al. (1999), postulates they may be responsible for reduced growth of rainbow trout fed a soy protein concentrate diet. Soy isoflavones are heat stable and alcohol soluble, and thus could be removed via alcohol solvent extraction.

Phytic acid

Phytic acid, or phytate, is a hexophosphate of myo-inositol and is common in soy beans and other legumes (Francis et al. 2001). Phytic acid is problematic for most fish species because they do not produce phytase, the enzyme required to hydrolyze phytate (Alexis and Nengas 2001). Approximately 75% of the phosphorus in SBM is in the form of phytic acid (Hardy 2003) and thus unavailable for digestive processes. Phytate can chelate with mineral ions (Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{3+} and Fe^{3+}) making these ions also unavailable for use by consumers (NRC 1993). Sajjadi and Carter (2004) showed significantly reduced protein digestibility for Atlantic salmon fed fish meal diets with addition of synthetic phytic acid. These same authors, and others, have postulated that this effect was negated with addition of dietary phytase.

Dephytinized plant protein concentrates have resulted in good growth for Atlantic salmon and rainbow trout in feed trials (Thiessen et al. 2004, Carter and Hauler 2000, Brown et al. 1997), but currently protein concentrates are too costly for large-scale commercial use. Heat treatment and fermentation can also reduce phytate content in meals and grains (Francis et al. 2001). Presently the level of SBM inclusion in diets for carnivorous fish does not necessitate supplemental phytase, and phytic acid does not appear to be a major factor limiting SBM usage.

Soybean meal-induced enteritis

Subacute enteritis of the distal intestine has been reported by several researchers as a common side effect on Atlantic salmon and rainbow trout fed SBM diets (Bakke-McKellep et al. 2000, Refstie et al. 2000, Krogdahl 2003). Observed pathological changes include hypertrophic and hyperaemic mucosa, shortened secondary mucosal folds, and loss of supranuclear vacuolization in epithelial cells (Beaverfjord and Krogdahl 1996). Those researchers also describe a widening of the lamina propria with infiltration of a mixed layer of leucocyte populations. While the causative agent in SBM is unknown, one or more of the alcohol soluble components is suspected since alcohol-extracted soy protein concentrate did not induce enteritis in salmonids at concentrations equal to SBM diets (Olli and Krogdahl 1994). Similar symptoms have also been observed with enteric disease of poultry and piglets (Morin et al 1983, Dekich 1998), which has been attributed to coccidiosis, feedborne toxins, bacteria and viruses. Since problems observed with salmonids are believed to be of non-infectious origin (Beaverfjord and Krogdahl 1996), feedborne toxins may be a common factor across

species. Examples of feedborne toxins include mycotoxins, which are molds and fungi found in cereal grains and SBM, biogenic amines, and ingredient impurities (Dekich 1998).

Continued Development of SBM Diets for Atlantic salmon

Soybean meal is widely accepted as a standard ingredient in most commercial aquaculture feeds. However, its use in commercial Atlantic salmon feeds likely remains below 8-10% (Hardy 2003). Diets containing as little as 10% SBM resulted in moderate intestinal histopathological changes in Atlantic salmon (Krogdahl et al. 2003). These authors express that caution should be taken with use of even low levels of extracted SBM in salmon feeds.

Extensive research has been designed to identify the causative agents in SBM that negatively affect salmonids. Feed trials on salmon and trout have identified various ANFs in SBM related to specific nutritional deficiencies. We now understand that heat labile factors, such as trypsin inhibitors, lectins, and perhaps phytic acid, may be of less concern because they can be destroyed or partially inactivated through meal and diet extrusion fabrication processes (Alexis and Nengas 2001, Hardy 2003). According to several authors, additional problems appear to be linked to dietary carbohydrate factions (NSP and oligosaccharides, Refstie et al. 2000, 2001), and alcohol soluble components. The later is supported by findings that alcohol extracted soya concentrate is of high nutrition value to salmonids and can replace up to 50% of dietary fish meal (Olli and Krogdahl 1994 in Krogdahl et al. 2003).

Improvements in feed processing technology have likely contributed to increased usage of SBM in aquaculture feeds. Diet formulations and pellet extrusion methods have improved substantially over the past 10-20 years resulting in very water stable, low polluting commercial fish feeds (Todd Prowless, Ziegler Feed, personal communication). Heat treatments and solvent extraction methods are physical processes which, through advancements in research and technology, may be affordable means to overcome SBM anti-nutritional properties in carnivorous fish. In the late 1990s, high energy diets were developed (Einen and Roem 1997), and made commercially available for salmon and trout. These diets improved feed efficiencies of intensively grown Atlantic salmon (Einen and Roem 1997, Azevedo et al. 2002), and are becoming widely incorporated in production cycles across the industry. Further research with high energy diets may provide valuable information between SBM carbohydrate factions in fish diets and protein sparing effects.

Chapter 3 – Trypsin Inhibitor Effects on Atlantic Salmon (*Salmo salar*) Fingerlings

Introduction

Protease inhibitors are a class of proteins that react with specific proteolytic enzymes in the digestive process of animals (Krogdahl and Holm in Krogdahl et al. 1994). Under controlled settings, feeds containing anti-nutritional factors such as proteinase inhibitors have shown to effect growth rate and feed utilization of animals and livestock. TIs are a class of proteinase inhibitors found in SBM, which is currently a leading alternate protein source candidate for fish meal in formulated feeds used in aquaculture (Hardy 2003, Alexis and Nengas 2001). Omnivorous fish species such as carp, tilapia and catfish have shown to accept rather high amounts of SBM in formulated diets (ElSaidy and Gaber 2002, Jahan et al. 2003). This has led to the development of commercial SBM based diets for a number of cultured fish species and contributed to reducing the global demand for expensive high quality fish meal. Ongoing research in the area of fish nutrition continues to focus on SBM anti-nutritional properties for other aquaculture species, particularly carnivorous species such as salmonids (Ollie et al. 1994, Sveier et al. 2001, Krogdahl et al. 2003).

This study was part of an integrated research project funded by the United Soybean Board, Soy-in-Aquaculture Program. The project was designed to develop a commercially acceptable SBM based formulated feed for Atlantic salmon and other species. The main objective of this study was to examine effects of soybean trypsin inhibitors (SBTIs) on fingerlings (young-of-year) Atlantic Salmon.

Materials and Methods

Experimental Diets

Five experimental diets containing graded levels of TIs and one standard commercial salmonid diet control were used to study effects of TIs on Atlantic salmon fingerlings. The commercial control (Zeigler Brothers Finfish Starter, slow sinking) contained a minimum of 50% crude protein (CP) and 19% crude fat (CF). Test diets were formulated and manufactured by research collaborators at Purdue University under the supervision of Dr. Steve Hart. Diets were formulated to contain the same CP and CF levels as the control feed (Table 3.1). Crude protein content of commercial and experimental diets were confirmed using a Leco nitrogen/protein analyzer. Trypsin inhibitor (Soybean Trypsin Inhibitor CAS #9035-81-8, USB Corporation) inclusion rates were 0, 0.975, 1.950, 2.925, and 3.900 gTI/kg feed representing estimated SBM equivalencies of 0, 15, 30, 45, and 60% respectively. TI inclusion rate SBM equivalencies were based on the average value of 6.5 mgTI/g SBM from the range of 5.0 – 8.0 mgTI/g SBM (Russet 1998).

Experimental System and Animals

Young-of-the-year Atlantic salmon were obtained from the Michigan Department of Natural Resources Lake Superior State University rearing facility in Sault Saint Marie, Michigan in August, 2003. The fish were transported to Michigan State University's Aquaculture Research Laboratory and acclimated to water conditions in a 710-L flow-through tank culture system over a 30-day period. Fish were fed the commercial control diet over the acclimation period.

Table 3.1 Formulations of semi-purified test diets containing graded levels of stock soybean trypsin inhibitors in g/kg. Diets are identified based on percent soybean meal equivalency.

Diet	TI0	TI15	TI30	TI45	TI60
SBM Equivalency	0%	15%	30%	45%	60%
Ingredient					
Casein	469	469	469	469	469
Gelatin	110	110	110	110	110
L-Methionine	5	5	5	5	5
Dextrin	130	130	130	130	130
α-Cellulose	10.5	9.525	8.55	7.575	6.6
Carboxymethylcellulose	20	20	20	20	20
Salmon Mineral Premix	33	33	33	33	33
Salmon Vitamin Premix	20	20	20	20	20
Ascorbic Acid (Stay C 35%)	3.5	3.5	3.5	3.5	3.5
Choline Chloride (74%)	4	4	4	4	4
Trypsin Inhibitor (TI)	0	0.975	1.95	2.925	3.9
Lecithin	5	5	5	5	5
Menhaden Oil (500 ppm ethoxy)	190	190	190	190	190
	1000.0	1000.0	1000.0	1000.0	1000.0

Note: Commercial control was Zeigler Brothers Finfish Starter, slow sinking.

A total of 450 fish were randomly distributed in eighteen, 110-L tanks, at 25 fish per tank. Fish were acclimated to feed trial rearing units and fed the commercial control diet for an additional 30-day period. Flow rates were maintained between 2.8–3.6 Lpm fresh well water based on target exchange rates of 1.5–2.0 water exchanges per hour. Water temperature for all tanks remained between 11.7–12.2 ^{0}C for the duration of the study with one potential exception on day-2 when the well went down for approximately 2 hours due to power failure. Dissolved oxygen varied from 8.9 to 9.1 mg/l; total ammonia nitrogen concentrations remained below 1.0 mg/L (0.006 mg/L unionized ammonia). All other water quality parameters fell within acceptable limits for salmonids (Piper et al. 1982).

During the acclimation period several fish showed signs of bacterial infection. Fish samples were sent to MSU's Aquatic Animal Health Laboratory for diagnosis. Isolates indicated presence of *Fexibacter columnaris* suggesting moderate chronic bacterial infection. All fish received a 10ppt salt bath for approximately 30 minutes to treat the infection. This treatment was continued on a prophylactic basis once per week through the end of the feed trial.

At the end of the rearing unit acclimation period, 3–5 fish were randomly selected and removed from each tank. Of those removed, 15 random fish were pooled, euthanized in tricaine methanesulfonate (MS-222) at a concentration of 500 mg/L (AVMA 2000), frozen, and held at -20^{0}C for subsequent whole body composition analysis prior to starting the feed trial. Weight and length data were recorded for the sample of 15. The feed trial was initiated with 20 fish in each of the 18 tanks, 360 total. Total weights of all fish from each tank were recorded at the start of the trial. The average weight of fish per tank was 17.5 ± 1.4 grams.

Triplicate tanks of fish were fed, either the commercial control diet, or one of five treatment diets, three times daily (8:00–9:00 am, 12:00–1:00 pm, 4:30–5:30 pm) for eight weeks. Total weights of fish in each tank were recorded every 2 weeks. Feed levels were maintained at a constant percent body weight (%BW), and were adjusted (by weight) based on recorded weight samples. For the trial, %BW was calculated as 90% of the theoretical optimal feed level for salmonids (Westers 1987). Feed levels fell both above and below satiation levels of the fish across feeding times based on observations of excess feed in tank bottoms at various times through the feed trial.

Mortalities were removed on a daily basis and weights of dead fish were recorded. Eighteen mortalities (5%) were observed over the course of the feed trial. At the end of the trial, total weight samples were recorded, and 10 fish per tank were selected at random for length and weight measurements. Five fish pooled from each tank were randomly selected, euthanized in MS-222 at a concentration of 500 mg/L (AVMA 2000), and frozen as a group sample at -20^{0}C for future analysis.

Sample Analysis and Calculations

Feeding levels

Trial feed levels were calculated as 90% of the theoretical optimal percent body weight (%BW) for salmonids as developed by Westers (1987):

$$\%BW = (300 \times TUG_S \times FCR_{1.0}) \times \#TU / (W/k_i)^{1/3} \times 0.90$$

$$TUG = (L_f - L_i)/(^0C \times d)$$

where:

TUG = Temperature Unit Growth Rate,

TUG_S = Theoretical TUG for Salmonids = 0.006 (cm/^{0}C/d),

$FRC_{1.0}$ = Theoretical feed conversion rate of 1.0,

#TU = Number of thermal units in ^{0}C above 0.0^{0}C (^{0}C),

W = Wet weight of fish at time of sampling (g),

k_i = condition factor at the start of the trial,

L_F = average final length (cm),

L_i = average initial length,

^{0}C = temperature in Celsius,

d = time (days).

Growth

Weight and length data were used to determine condition factor (k) and specific growth rate (SGR) over the course of the study:

$$k = W/L^3$$

$$SGR = (\ln W_f - \ln W_i)/d \times 100$$

where:

W = wet weight (g) of fish at time of sampling,

L = length (cm),

$W_{f \text{ or } i}$ = average final or initial wet weight (g), and d = time (days).

Feed conversion

Feed conversion rates were calculated as the standard apparent FCR with adjustment for mortalities:

$$FCR = \text{Cum Feed}/(\text{Net W Gained} + (W_{Morts} - (N_{Morts} \times W_i)))$$

where:

Cum Feed = cumulative weight of feed (g),

W_{Morts} = Wet weight of mortalities removed (g),

N_{Morts} = Number of mortalities removed.

Whole body composition

Whole body composition analysis included lipid, protein and ash. Pooled, frozen whole body samples were thawed quickly under cool water and homogenized in a commercial-grade food processor. The samples were then weighed and stored at -20^{0}C until lyophilization. All freeze-dried samples were finely ground and homogenized again and stored at -20^{0}C. Whole body lipid content was determined on duplicate 1.0–3.0 g samples by lipid extraction with diethyl ether (Soxtec System HT/1043 Extraction Unit, Tecator, Sweden). Nitrogen was determined on 0.5 g samples according to combustion method AOAC (2000) using a Leco nitrogen/protein analyzer (model FP-2000, Leco Corp., St. Joseph, MI). Crude protein was calculated as N x 6.25. Dry matter was obtained after oven drying 2.0 g samples at 105^{0}C for 18-24 hours. Ash content was determined after placing dry matter samples in muffle furnace at 500^{0}C for 18 hours. All body composition samples were performed in duplicate.

Protein efficiency and retention

Protein efficiency ratios were based on the formula provided by De Silva and Anderson (1995) slightly modified to account for mortality:

$$PER = (\text{Net W Gained} + (W_{Morts} - (N_{Morts} \times W_i))/ TP_{Feed}$$

$$TP_{Feed} = \text{Cum Feed} \times \% P_{Feed} /100$$

$$APR(\%) = FPG/ PI_{Feed} \times 100$$

$$FPG = (W_f \times P_f/100 \times FD_f/100) - (W_i \times P_i/100 \times FD_i/100)$$

$$PI_{Feed} = (\text{Cum Feed} \times \% P_{Feed} /100) / \#Fish_f$$

where:

PER = protein efficiency ratio,

TP_{Feed} = total protein in feed (%),

APR(%) = apparent protein retention (%),

FPG = fish protein gain (gP),

PI_{Feed} = Protein intake from feed (gP/fish),

% P_{Feed} = % protein in diet as fed basis (%P/gfeed),

$P_{f\,or\,i}$ = Final or initial % protein in body compositions of freeze-dried sample (%P/gFD),

$FD_{f\,or\,i}$ = Final or initial % freeze-dried matter of fish samples (%),

$\#Fish_f$ = Number of fish alive at end of study.

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) using SPSS (SPSS© Release 12.0, 2003) and SAS (SAS© Release 9.1, 2003) statistical software. Homogeneity of variance was confirmed by Levene's statistic analysis. Significant differences between means were compared by Duncan's multiple range test. Trend analyses were conducted using regression analysis and orthogonal contrasts. Treatment effects were considered significant at $P \leq 0.05$ unless otherwise noted.

Results

Growth Characteristics

Slight differences were observed in growth characteristics among dietary treatment groups (Table 3.2). Mean final weight for fish fed the control diet was lower than all treatment groups except TI0 ($P \leq 0.05$). The SGR value of 1.44 for the commercial control group was lower than those obtained from fish fed experimental diets which ranged between 1.58 (TI0) and 1.69 (TI45). There were no significant differences found in growth rates among treatment diets (Figure 3.1) Only the TI15 group had a higher condition factor, and k for all groups ranged between 0.0097 and 0.0105.

Feed Conversion and Protein Retention

No differences were detected in mean FCRs, PERs or APRs (Table 3.2). FCR values ranged between 0.74 and 0.83 (TI45 and TI60 respectively), PER from 2.40 (Control) to 2.67 (TI45), and APR from 32.94 (TI30) to 35.98 (TI15).

Proximate Body Composition

Slight differences were observed in proximate body compositions across diets. Overall, fish fed the commercial diet had a greater percentage of protein and less fat ($P \leq 0.05$) than fish fed semi-purified diets (Table 3.3). Whole body crude protein content was greatest for Atlantic salmon fed the control (52.94%) and least for fish fed TI0 (48.29%). Crude protein compositions were statistically insignificant across all TI levels tested.

Table 3.2 Mean final weight, specific growth rate (SGR), condition factor (k), feed conversion rate (FCR), protein efficiency ratio (PER), and apparent protein retention (APR) in fingerling Atlantic salmon fed diets containing graded levels of trypsin inhibitors (TI) at 0, 15, 30, 45, and 60% soybean meal equivalencies. Mean standard errors are in parentheses. Values in each row awarded common superscripts are not significantly different (P > 0.05).

	Control	TI0	TI15	TI30	TI45	TI60
Final weight (g)	39.55 [a]	41.28 [ab]	44.49 [b]	44.16 [b]	43.59 [b]	44.35 [b]
	(1.25)	(1.64)	(0.41)	(1.17)	(0.81)	(1.21)
SGR (%/d)	1.44 [a]	1.58 [b]	1.64 [b]	1.59 [b]	1.69 [b]	1.63 [b]
	(0.04)	(0.02)	(0.01)	(0.06)	(0.03)	(0.01)
k	0.0101 [ab]	0.0100 [ab]	0.0105 [a]	0.0098 [b]	0.0099 [b]	0.0097 [b]
	(0.0001)	(0.0002)	(0.0001)	(0.0002)	(0.0001)	(0.0002)
FCR	0.82	0.78	0.75	0.78	0.74	0.83
	(0.01)	(0.01)	(0.01)	(0.05)	(0.04)	(0.09)
PER	2.40	2.58	2.64	2.57	2.67	2.49
	(0.04)	(0.04)	(0.04)	(0.16)	(0.15)	(0.25)
APR (%)	35.71	34.71	35.98	32.94	34.62	33.36
	(0.44)	(0.93)	(0.59)	(2.09)	(1.91)	(2.86)

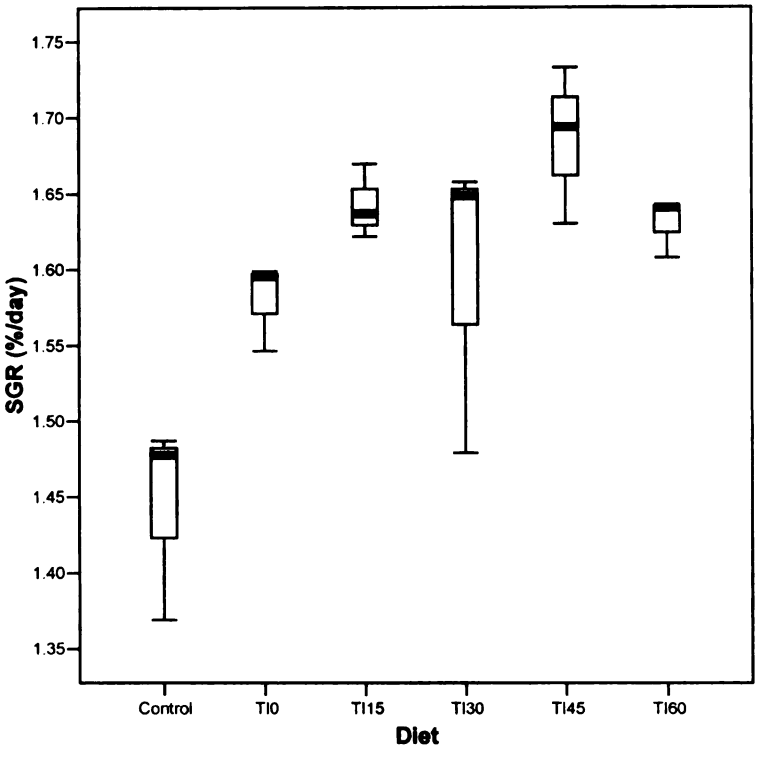

Figure 3.1 Box plot of mean specific growth rate of fingerling Atlantic salmon fed a commercial control and experimental diets containing graded levels of stock trypsin inhibitors (TI) at 0, 15, 30, 45, and 60% soybean meal equivalencies.

Table 3.3 Percent body compositions of fingerling Atlantic salmon fed a commercial control and experimental diets containing graded levels of trypsin inhibitors (TI) at 0, 15, 30, 45, and 60% soybean meal equivalencies. Mean standard errors are in parentheses. Values in each row awarded common superscripts are not significantly different (P > 0.05).

	Control	TI0	TI15	TI30	TI45	TI60
Dry matter	28.57 ^{ab}	29.67 ^{a}	29.58 ^{a}	27.54 ^{b}	28.11 ^{b}	28.74 ^{ab}
	(0.18)	(0.49)	(0.29)	(0.4)	(0.47)	(0.42)
Protein	52.94 ^{a}	48.29 ^{b}	48.20 ^{b}	50.37 ^{b}	49.20 ^{b}	48.58 ^{b}
	(0.44)	(1.07)	(0.48)	(1.21)	(0.64)	(0.6)
Fat	15.12 ^{a}	19.30 ^{b}	27.78 ^{c}	23.74 ^{d}	26.10 ^{cd}	18.62 ^{ab}
	(0.75)	(0.75)	(1.49)	(1.53)	(1.35)	(0.88)
Ash	8.28 ^{a}	8.58 ^{a}	8.62 ^{a}	9.61 ^{b}	9.35 ^{b}	9.54 ^{b}
	(0.15)	(0.13)	(0.05)	(0.3)	(0.2)	(0.3)

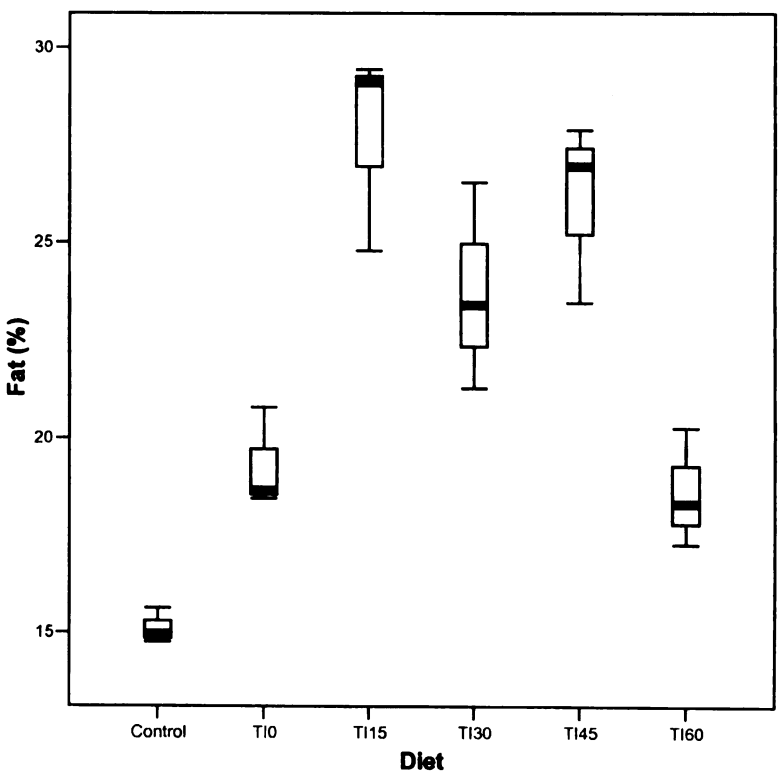

Figure 3.2 Box plot of mean body fat composition of fingerling Atlantic salmon fed a commercial control and experimental diets containing graded levels of stock trypsin inhibitors (TI) at 0, 15, 30, 45, and 60% soybean meal equivalencies.

Differences in body fat content between dietary treatments were more pronounced (Table 3.3 and Figure 3.2). Whole body crude fat content for the TI15 group (27.78%) was 12.66% higher than the commercial control group (15.12%). Atlantic salmon fed TI15, TI30, TI45 diets had 5 to 8% higher fat content than those fed TI0 or TI 60. Orthogonal contrasts showed a statistically significant ($P = 0.0003$) quadratic trend for SBTIs on body CF compositions.

Fish fed 30% SBM equivalency SBTI diets or greater had higher body ash compositions ($P \leq 0.05$) than those fed diets containing 0 or 15% SBM equivalency. Slight differences were observed in percent whole body dry matter, which ranged from 27.54 (TI30) to 29.67 (TI0). Differences detected in dry matter composition do not appear to be attributed to treatment effects.

Mortalities

Mortalities occurring over the course of the experiment were highest for fish fed TI60 (Figure 3.3). Mean mortality rates, however, were not significantly different across treatment levels, but variation within treatments was relatively high ranging from zero to five fish per tank. Five of the six mortalities occurring in the TI60 group came from a single tank. Each treatment had at least one tank that experienced no deaths. Mortalities increased as the trial progressed across all treatments: two mortalities were observed in weeks 1-3 of the trial, four observed in weeks 4-6, and thirteen in weeks 7-9.

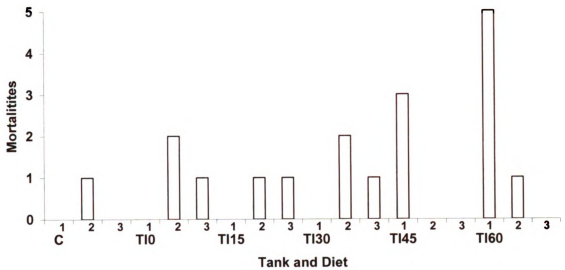

Figure 3.3 Number of mortalities by treatment (diet) and replicate (tank) of fingerling Atlantic salmon over the 8-week feed trial. Diet C is a commercial control. TI# represents percent soybean meal equivalency (0, 15, 30, 45 and 60%) of dietary trypsin inhibitor (TI).

Discussion

Experimental Diets

The experimental diets manufactured by Purdue University were similar in formulations to those used in a number of fish nutritional studies conducted at Purdue University (Dr. Steve Hart, Purdue University, personal communication). All ingredients, with exception of menhaden oil, were purchased in purified forms, and all ingredients were obtained from reputable manufacturers. The main protein source of experimental diets was ultra pure, vitamin free casein purchased from the USB chemical. Study vitamin and mineral premixes are typically > 99% pure. Based on study results, no evidence indicated that any of the ingredients, other than purified TIs, contained ANFs or

other impurities that potentially affected the outcome of the feed trial. This is in part confirmed by the inclusion of the commercial control and the results described within the following section.

Growth Characteristics

Atlantic salmon fed the semi-purified test diets containing SBTIs grew faster ($P \leq 0.05$) than fish fed the commercial control feed. The commercial control diet was obtained from a well known commercial feed supplier and considered by many to be of good to high quality. Reduced growth for fish fed the commercial control diet is likely due to differences in ingredient selection and processing methods.

Based on SGR alone, purified TIs in diets of young-of-year Atlantic salmon had no affect on growth to 60% SBM equivalency (3.9 gTI/kg feed). This finding differs slightly with results by Olli et al. (1994) who reported that Atlantic salmon were able to compensate for TI levels up to 30% SBM equivalency (2.08 gTI/kg feed), but had reduced growth at 48% SBM equivalent (3.15 gTI/kg feed). According to those researchers TI compensation by Atlantic salmon appeared to be accomplished through increased trypsin secretion, which eventually is exhausted at elevated SBTI activities. Examination of Figure 3.1 may suggest an asymptotic treatment effect of TI on Atlantic salmon growth; however, no statistical differences were detected in SGR across test diets. Increased variability in the TI30 group growth data could not be explained by study results, but could be due to treatment effects, random effects and/or sample error.

No effects of SBTIs were observed on fish condition factor. All k values fell within 0.005 of the value obtained from fish fed the control diet ($k = 0.01$). A k value of

0.01 is considered average for most salmonid species (Harry Westers, Aquaculture Bioengineering Corporation, personal communication).

Feed Conversion and Protein Retention

No differences were detected in FCR across diets. In addition there were no observable effects of TIs on feed intake, protein efficiency or protein retention. Based on these results, it appears that protein utilization by Atlantic salmon was unaffected by TIs up to 60% SBM equivalency.

Proximate Body Composition

Whole body compositions showed differences across all categories (Table 3.3). Most notably, Atlantic salmon fed the commercial control diet had more crude protein and less crude fat compared to fish fed semi-purified test diets. As previously stated, this difference is most likely due to differences in diet processing methods and ingredient digestibility.

Fish protein levels remained relatively unchanged across dietary treatments. This is in slight contrast to two related studies on salmonids. Krogdahl et al. (1994) observed minor effects of purified SBTIs on protein utilization by rainbow trout fed feed containing 57% SBM equivalence (3.7 gTI/kg feed). Olli et al. (1994) reported observing similar effects at 48% SBM equivalence (3.15 gTI/kg feed) with Atlantic salmon.

Crude fat content of TI15 and TI45 groups were approximately 8% and 12% higher than the TI60 and control group respectively. While crude protein content remained relatively constant over dietary treatments, TI levels appeared to affect lipid

digestibility and/or accretion (Table 3.3; Figure 3.2). This observation agrees with findings by several researchers examining the effects of purified trypsin inhibitors (Olli et al. 1994, Krogdahl et al. 1994) and of SBM practical diets on salmonids (Reftsie et al. 2001, Storebakken et al. 1998). Results from this study indicated a statistically significant ($P = 0.0003$) quadratic trend for SBTI level on CF. Body lipid content increased from 19% CF at 0%SBTI to a peak of about 27% CF between 15 and 45% SBTI, and then decreased to 18% for the highest SBTI (TI60) group. The peak observed between 15 and 45% SBM equivalency, then, is likely a compensatory response by Atlantic salmon to SBTIs. In the presence of the mid level amounts of SBTIs, metabolic pathways appeared to favor body lipid deposition. This suggests that internal mechanisms favored storage of additional energy reserves at mid-level SBTI's. The sharp decline at 60% SBM equivalency further suggests that more energy in the form of body lipids was required at high levels of SBTI in order to maintain similar growth and protein deposition.

Whole body ash content of fish from TI30, TI45 and TI60 groups were greater than the control, TI0 and TI15 groups ($P \leq 0.05$). Thus, while growth remained constant across treatments, internal physiological characteristics including whole body ash and lipid compositions were affected by dietary levels of TIs.

Mortalities

All of the 18 mortalities occurring over the course of the feed trial showed similar external signs of bacterial infections on the body and fins. One-way ANOVA indicated

no significant differences in mortality frequency across diets, but high variation within treatments.

Typically, wild North American populations of Atlantic salmon remain in fresh water as parr until an age of approximately 2-3 years when they undergo the physiological changes associated with smolting (Danie et al. 1984). Thus, potential stressors that may have contributed to mortalities would likely be more associated with a par stage (pre-smolt) rather than stressors associated with the smolting process. Examples of possible contributing factors include genetics, confinement, handling stress and/or presence of opportunistic bacteria.

Chapter Summary

Based on results from this feed trial, Atlantic salmon appeared to undergo physiological changes in response to increasing dietary TIs. While specific deficiencies could not be attributed to statistical significance over measured parameters, slight changes were observed in response of body fat and ash compositions to SBTIs. Specific mechanisms for these changes are currently unknown. In this study Atlantic salmon were able to consume up to 60% SBM equivalency SBTI without affecting growth rates, while a comparable study by Ollie et al. (1994) showed depressed growth at a level of 48% SBM equivalency. Both studies agree that Atlantic salmon appear to have a certain capacity to compensate for SBTIs in their diets. Results from this study support the hypothesis by Ollie et al. (1994) that TI compensation by Atlantic salmon is likely due to increase trypsin production and secretion in the pancreas. Further research is required to

assess the pathways leading to these responses, and whether the changes are detrimental to the health of the fish.

Chapter 4 – Trypsin Inhibitor Effects on Atlantic Salmon (*Salmo salar*) Smolts

Introduction

This study was part of an integrated research project funded by the United Soybean Board, Soy-in-Aquaculture Program. The project was designed to develop a commercially acceptable SBM based formulated feed for Atlantic salmon and other species. Specifically, this study focused on short term physiological effects of soybean trypsin inhibitors (SBTIs) on Atlantic salmon smolts (age-1+). Purified SBTIs were added to standardized semi-purified diets at graded levels from 0–60% SBM dietary inclusion equivalencies. Atlantic salmon intestinal digesta dry matter, trypsin enzyme activity, apparent protein digestion indices and body lipids were evaluated over a 3-week feed trial.

Materials and Methods

Experimental Diets

Five experimental diets containing graded levels of TI and one standard salmonid diet control were used to study effects of trypsin inhibitors on Atlantic salmon smolts. The commercial control (Silver Cup Extruded Salmon Feed, slow sinking) was reported to contain a minimum of 45% crude protein and 19% crude fat. Protein content was confirmed and measured to be 47.3%. Test diets were formulated to contain 50% crude protein 19% crude fat (Table 4.1), and manufactured by research collaborators at Purdue University under the supervision of Dr. Steve Hart. Measured values for crude protein were 49± 1%. Trypsin inhibitor (Soybean Trypsin Inhibitor CAS #9035-81-8, USB

Table 4.1 Formulations of semi-purified test diets containing graded levels of stock soybean trypsin inhibitors (TI) in g/kg. Diets are identified based on percent soybean meal equivalency.

Diet	TI0	TI15	TI30	TI45	TI60
SBM Equivalency	0%	15%	30%	45%	60%
Ingredient					
Casein	425	425	425	425	425
Gelatin	80	80	80	80	80
L-Methionine	5	5	5	5	5
L-Arginine•HCL	5	5	5	5	5
Dextrin	130	130	130	130	130
α -Cellulose	74.5	73.525	72.55	71.575	70.6
Carboxymethylcellulose	20	20	20	20	20
Salmon Mineral Premix	33	33	33	33	33
Salmon Vitamin Premix	20	20	20	20	20
Ascorbic Acid (Stay C 35%)	3.5	3.5	3.5	3.5	3.5
Choline Chloride (74%)	4	4	4	4	4
Trypsin Inhibitor (TI)	0	0.975	1.95	2.925	3.9
Lecithin	5	5	5	5	5
Menhaden Oil (500 ppm ethoxy)	190	190	190	190	190
Chromic Oxide	5	5	5	5	5
	1000.0	1000.0	1000.0	1000.0	1000.0

Note: Commercial control was Silver Cup Extruded Salmon Feed, slow sinking.

Corporation) inclusion rates were 0, 0.975, 1.95, 2.925, and 3.9 gTI/kg feed representing estimated SBM equivalencies of 0, 15, 30, 45, and 60% SBM, respectively. TI inclusion rate SBM equivalencies were based on the average value of 6.5 mgTI/g SBM from the range of 5.0–8.0 mgTI/g SBM (Russett 1998). Test diets also contained 0.5% chromic oxide as an inert marker for apparent digestibility analysis.

Experimental System and Animals

One-year old Atlantic salmon were obtained from the Michigan Department of Natural Resources Lake Superior State University rearing facility in Sault Saint Marie,

Michigan in August, 2003. The fish were transported to Michigan State University's Aquaculture Research Laboratory and acclimated to water conditions in a 1,890-L flow-through tank culture system over a 30-day period. Fish were fed the commercial control diet over the acclimation period. A total of 576 fish were randomly distributed in eighteen, 110-L tanks, at 32 fish per tank, and acclimated to feed trial rearing units for an additional 30-day period. Fish were fed the commercial control diet over the acclimation period. Flow rates were maintained between 2.8–3.6 Lpm well water based on target exchange rates of 1.5–2.0 water exchanges per hour. Water temperature for all tanks remained between 11.8–11.9 ^{0}C for the duration of the study with one potential exception on day-3 when the supply well was out-of-service for approximately 2 hours due to a power failure. Dissolved oxygen varied from 8.6 to 9.4 mg/L; total ammonia nitrogen concentrations remained below 1.3 mg/L (0.009 mg/L unionized ammonia). All other water quality parameters were within acceptable limits for salmonids (Piper et al. 1982).

During the acclimation period, a few fish showed signs of bacterial infection. Fish samples were sent to MSU's Aquatic Animal Health Laboratory for diagnosis. Isolates indicated presence of *Fexibacter columnaris* suggesting a moderate chronic infection. All fish received a 10 ppt salt bath for approximately 30 minutes to treat the infection. This treatment was continued on a prophylactic basis once per week through the end of the feed trial. Two mortalities occurred over the course of the 21-day feed trial.

At the end of the rearing unit acclimation period, 1-2 fish were randomly removed from each tank in order to start the feed trial with 30 fish per tank, 540 fish total. Whole body samples from 15 fish (randomly selected from those removed) were euthanized in

MS-222 at a concentration of 500 mg/L (AVMA 2000), frozen and held at -20^{0}C for subsequent analysis. Initial total weights were obtained for each tank on day-one of the feed trial. Average weight per fish in each tank was 89.2 ± 4.1g. Fish were fed three times daily (8:00–9:00 am, 12:00–1:00 pm, 4:30–5:30 pm), at a feed rate of 1.1% initial body weight per day for a period of 21 days.

On days 10 and 21 of the feed trial, 10 fish were randomly selected from each tank and euthanized in MS-222 at a concentration of 500 mg/L (AVMA 2000). Digesta were collected separately from portions of the small intestine, proximal large intestine, and fecal samples were collected from the distal portion of the large intestine. Samples were pooled within tank replicates, freeze-dried (Tri-Philizer MP, FTS systems, for 48 hours minimum), and stored frozen until analyzed. At the termination of the feed trial, three fish from each tank were randomly selected and pooled into one sample for evaluation of proximate body composition. Euthanized fish were frozen and stored at -20^{0}C for further analysis.

Sample Analysis and Calculations

Digesta dry matter

Digesta and fecal lyophilized dry matter content were determined from samples obtained from the small intestine and proximal and distal (fecal) regions of the large intestine.

Trypsin activity

Intestinal and fecal trypsin activities were determined colorimetrically using BAPA (benzoyl-DL-arginine-p-nitranilide) as a substrate as described by Kakade et al. (1969) with slight modification. Lyophilized 50.0 mg digesta samples of small intestine and proximal and distal (fecal) large intestine were extracted with 5.0 ml 0.01 N NaOH for 3 hours with periodic mixing. Supernatant 1.0 ml samples were centrifuged at 4000 rpm for 10 minutes. Tris buffer solution contained 0.60 5g Tris, 0.295 g $CaCl_2$, 100 ml distilled water (DI), pH adjusted to 8.2 using 1.0N HCL. The BAPA solution was prepared by dissolving 0.04 g BAPA completely in 1.0 ml dimethyl sulfoxide, and 100 ml Tris buffer (solution stable for 4 hours). Trypsin solution was prepared by mixing 4.0 mg trypsin (Bovine 2x Lyo, TRL3702, Worthington Biochemical Corp) with 100 ml 0.001M HCL (solution stable for 30 days at 4^0C). Absorbance readings were analyzed bichromatically by pipetting 5.0 µl extract in triplicate to a microreader plate, adding 35 µl DI, 20 µl acetic acid to blank (first cell), and adding 140 µl BAPA to all cells. The trypsin standard curve was determined similarly by placing 10, 20, 30 and 40 µl trypsin in triplicate to a microreader plate, adjusting each cell to 40 µl with DI, adding 20 µl acetic acid to blank (first cell), and by adding 140 µl BAPA to all cells. The plate was inserted into the microplate spectrophotometer (Molecular Devices Spectramax 340, Sunnyvale, CA 94089), and warmed to 37^0C for 10 minutes. The reaction was terminated after 10 minutes with 20 µl acetic acid. Absorbance was read at 410 nm. Trypsin activity in mg/g fecal sample was obtained from the trypsin standard curve. Trypsin activity (TA) in mg trypsin per gram intestinal fecal material was calculated as follows:

TA (mg/g sample) = Try (μg/vol) / wt (g)

Try (μg/vol) = Try (μg/ml from standard curve) x EVol (ml)

where:

Try = trypsin value; EVol = extract volume.

Apparent protein digestibility

Apparent protein digestibility was measured indirectly using chromic oxide as an inert marker. Chromic oxide levels in feces were obtained by digestion and atomic absorption on 10 mg samples (Williams et al. 1962). Diet and fecal nitrogen was determined on 50 mg samples according to combustion method AOAC (2000) using a Leco nitrogen/protein determinator (model FP-2000, Leco Corp., St. Joseph, MI). Protein was calculated as N x 6.25. Apparent protein digestibility coefficient (APD) was calculated as follows (Cho and Slinger 1979, NRC 1993):

$$APD = 100 \times [1 - (P_F/P_D) \times (Cr_D/Cr_F)]$$

where:

P_F = % protein of feces,

P_D = % protein of diet,

Cr_D = % marker (Cr_2O_3) in diet,

Cr_F = % marker (Cr_2O_3) in feces.

Whole body composition

Due to the short duration of the feed trial, whole body composition was not expected to differ greatly between treatments. Based on subsequent related research,

whole body lipid appears to be a sensitive parameter to SBM anti-nutritional properties. For this reason lipid analysis was used as a benchmark to assess whether additional body composition data might be warranted. Pooled, frozen whole body samples were thawed quickly under cool water and homogenized in a commercial-grade food processor. Samples were weighed, frozen and freeze-dried. All freeze-dried samples were finely ground and homogenized again and stored at -20^{0}C. Whole body crude lipid content was determined on duplicate 1.0 g samples by lipid extraction with diethyl ether (Soxtec System HT/1043 Extraction Unit, Tecator, Sweden).

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) using SPSS (SPSS© Release 12.0, 2003) and SAS (SAS© Release 9.1, 2003) statistical software. Homogeneity of variance was confirmed by Levene's statistic analysis. Significant differences between means were compared by Duncan's multiple range test. Trend analyses were conducted using regression analysis and orthogonal contrasts. Lyophilized dry matter (DM) day-10 small intestinal and fecal samples, and day-21 fecal samples tested statistically significant in homogeneity of variance and were compared using a Games-Howell test. All treatment effects were considered significant at $P \leq 0.05$ unless otherwise noted.

Results

Digesta Dry Matter

Dry matter of digesta material sampled from the small intestine (SI), proximal large intestine (LI), and distal large intestine (fecal; F) regions are provided in Table 4.2.

Generally, the control diet resulted in slightly higher DM contents than the semi-purified diets. This difference was significant in three of six cases ($P \leq 0.05$). Regarding treatment diets, the TI45 group had less DM than all other test diets including TI60. The only other differences noted were from day-21 SI samples where TI0 and TI15 were higher in DM than TI30, TI45 and TI60.

Trypsin Activity

Digesta and fecal TAs varied between intestinal sections, day of sampling, and between diets to some degree (Figures 4.1, 4.2). Overall, mean TA was higher in the SI than the LI and F regions of the large intestine.

Trypsin activity in the SI was higher for the control group than semi-purified diets ($P \leq 0.05$), and higher for fish fed TI0 than those receiving dietary SBTIs (Figure 4.1 A). No differences in TA were observed from SI samples between test diet groups TI15 through TI60 groups; however, there was a negative linear response of TA to SBTI on day-10 in the SI (linear, $P = 0.002$, $R=0.75$).

Trypsin activity in the LI on day-10 showed slight fluctuations (Figure 4.1B). Trend analysis indicated a statistically significant negative linear trend of SBTI on TA (linear, $P = 0.02$), although a linear regression showed low correlation ($R = 0.56$) of this fit. No other orthogonal contrasts were significant. Fish fed the control diet, TI0 and TI15 had higher TA levels than those fed TI45. In addition, TA from fish fed TI15 was higher than those fed 30-60% SBM TI equivalency ($P \leq 0.05$). No differences were observed in day-10 fecal TA.

Table 4.2 Dietary trypsin inhibitor (TI) affects on percent dry matter (lyophilized) of intestinal digesta from sampled regions of small intestine (SI), proximal large intestine (LI) and distal large intestine (F) on day-10 and day-21. Diet C is the commercial control. TI# represents the percent soybean meal equivalency (0, 15, 30, 45 and 60%) of purified TI in test diets. Values in each row awarded common superscripts are not significantly different (P > 0.05).

Day	Sample	C	TI0	TI15	TI30	TI45	TI60
10	SI	29.3 [a]	24.4 [a]	20.6 [a]	20.5 [a]	17.6 [b]	20.0 [a]
	LI	26.0 [a]	17.0 [b]	13.7 [b]	17.9 [b]	15.5 [b]	16.3 [b]
	F	26.2	16.5	12.8	15.1	14.6	13.2
21	SI	25.5 [a]	24.3 [a]	24.1 [a]	16.3 [b]	13.4 [b]	16.9 [b]
	LI	23.7 [a]	17.7 [b]	16.5 [b]	14.0 [b]	13.5 [b]	14.6 [b]
	F	20.1 [a]	13.3 [b]	12.6 [b]	13.3 [b]	12.8 [b]	11.4 [b]

Day-21 TA values (Figure 4.2) differed slightly to those observed for day-10. SI samples on day 21 (Figure 4.2A) again had higher TA than all test diet groups, but the negative response observed in Figure 4.1A is no longer present. Also no differences were detected in day-21 LI TA samples. Fecal TA for the TI0 group was higher than that observed for TI30 ($P \leq 0.05$), but no other differences were detected.

Apparent Protein Digestibility

Apparent protein digestibility indices and cumulative percent protein digestion occurring through the SI, LI, and F regions are shown in Figures 4.3 and 4.4. No differences were detected in total apparent protein digestibility coefficients (Table 4.3) across treatments. No differences were detected in cumulative APD across diets for individual segments of the digestive tract from which samples were taken (SI, LI, F).

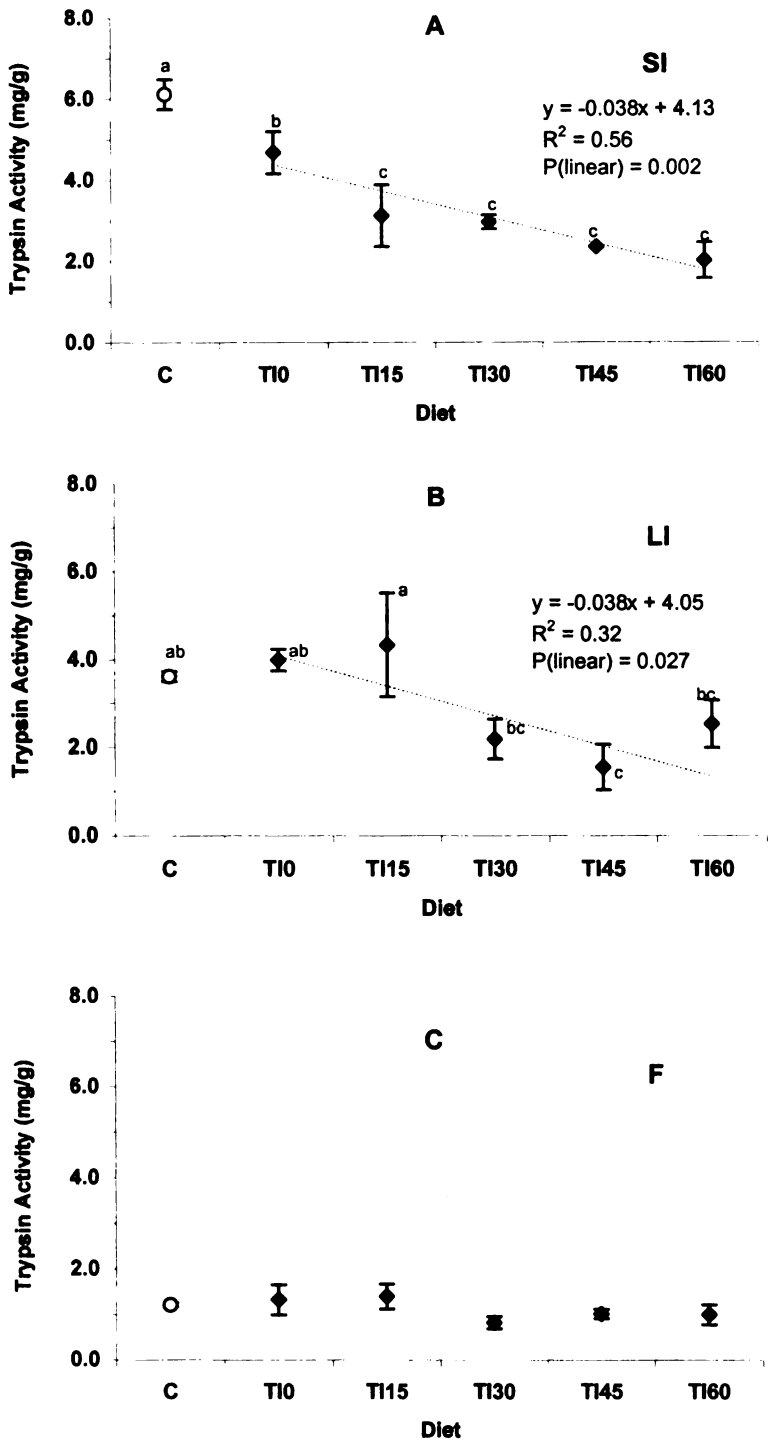

Figure 4.1 Day-10 trypsin enzyme activity by dietary treatment groups from samples of A) small intestine (SI), B) proximal large intestine (LI) and C) distal large intestine (fecal; F). Trend lines based on percent soybean meal equivalent (0, 15, 30, 45 and 60) trypsin inhibitor (TI) level in treatment diets (commercial control, C, excluded). P(linear) signifies P-value for linear regression analysis. Plotted values in graphs awarded common superscripts are not significantly different (P > 0.05).

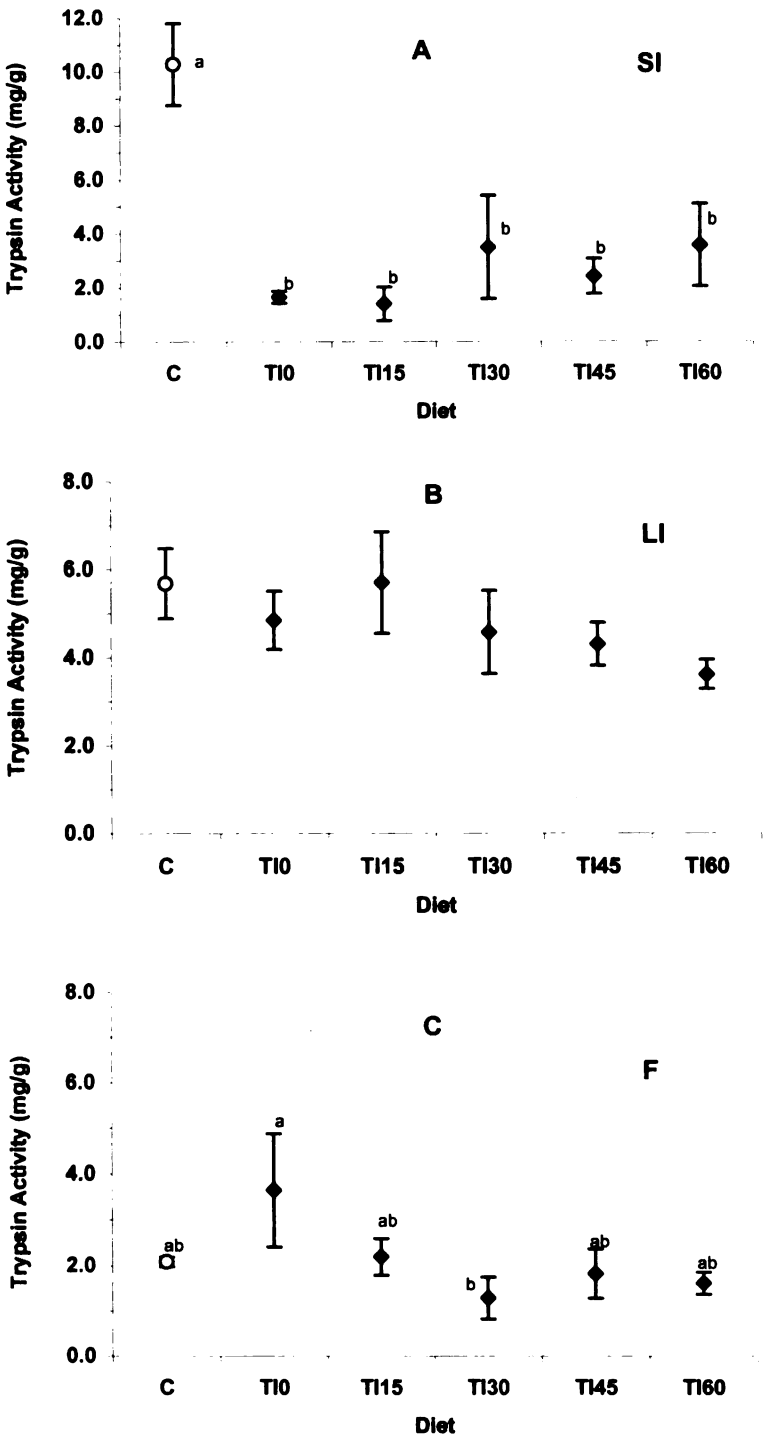

Figure 4.2 Day-21 trypsin enzyme activity by dietary treatment groups from samples of A) small intestine (SI), B) proximal large intestine (LI) and C) distal large intestine (fecal; F). Trend lines based on percent soybean meal equivalent (0, 15, 30, 45 and 60) trypsin inhibitor (TI) level in treatment diets (commercial control, C, excluded). Plotted values in graphs awarded common superscripts are not significantly different (P > 0.05).

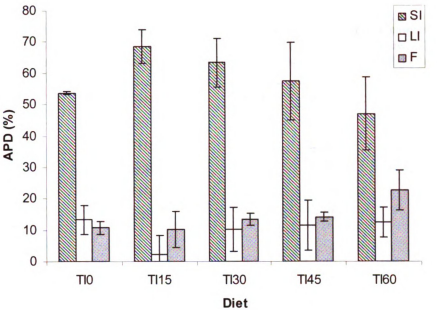

Figure 4.3 Apparent protein digestibility values (APD) of Atlantic salmon smolts based on dietary trypsin inhibitor (TI) treatments from samples taken in regions of small intestine (SI), proximal large intestine (LI), and distal large intestine (fecal; F). TI# represents the percent soybean meal equivalency (0, 15, 30, 45 and 60) of purified TI in test diets.

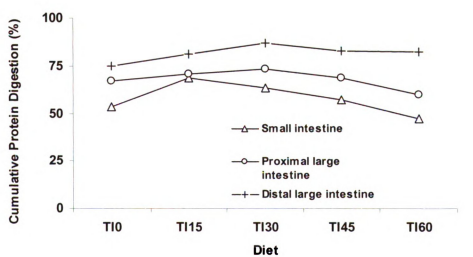

Figure 4.4 Cumulative apparent protein digestibility in intestinal segments of Atlantic salmon smolts fed experimental diets containing graded levels of trypsin inhibitor (TI) at 0, 15, 30, 45 and 60% soybean meal equivalencies.

Table 4.3 Dietary trypsin inhibitor (TI) affects on fecal sample mean total apparent protein digestibility (TAPD). MSE represents the mean square error value. TI# represents the percent soybean meal equivalency (0, 15, 30, 45 and 60) of purified TI in test diets.

Diet	TAPD	MSE
TI0	74.79	3.13
TI15	81.17	5.33
TI30	86.86	1.87
TI45	82.75	3.41
TI60	82.03	3.56

Proximate Body Composition

Body lipid compositions showed no differences between control or dietary treatment groups; however, data was highly variable within treatments (Figure 4.5). Affects of TI on fish body fat was not apparent over the 3-week feed trial. As a result ash and protein body composition assays were not undertaken under the assumption that the feed trial period was too short to produce meaningful body composition assessment results.

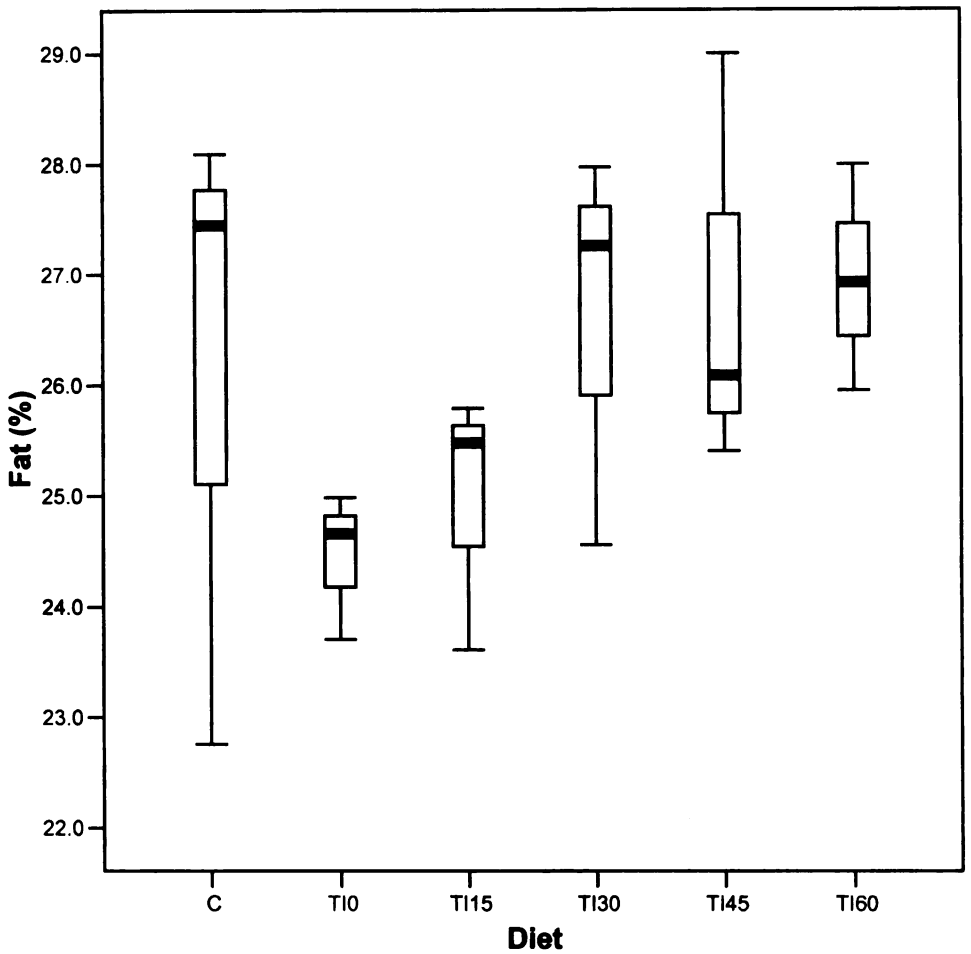

Figure 4.5 Box plot of mean body fat composition of Atlantic salmon smolts fed a commercial control (C) and experimental diets containing graded levels of trypsin inhibitor (TI) at 0, 15, 30, 45 and 60% soybean meal equivalencies.

Discussion

Experimental Animals

The one-plus year old Atlantic salmon (89.2 g) used in the feed trial were scheduled for a spring release as smolts by the Michigan DNR. The trial was conducted in early October (prior to expected spring release), which should have been relatively close to the time interval these specific fish typically undergo physiological transformations associated with smolting. Over the course of the study, parr marks were visibly receding from the sides of the fish, and fish scales were becoming more silver in color presenting a silvery sheen appearance. Based on these combined observations the fish were considered to be classified as smolts at the time of the feed trial.

Digesta Dry Matter

Differences were noted in digesta and fecal dry matter; however, it is unclear whether these differences were a result of SBTIs. At the time of sampling, digesta samples taken from the digestive tract of the commercial control group were dark brown and rather dry in appearance. In comparison, samples taken from fish fed test diets were bright green due to the marker and appeared much more liquefied. Results of weight analysis after freeze drying indicated that the commercial control diet generally resulted in higher digesta and fecal DM content than the semi-purified diets. Most likely these results are again due to differences between practical diet and semi-purified dietary ingredients and processing techniques.

Studies examining SBM replacement of fish meal in salmonid diets have reported SBM increased fecal water content and gastric emptying rates in Atlantic salmon

(Storebakken et al. 2000, Reftsie et al. 2001). In this study, SI material from fish fed TI0 and TI15 had significantly higher DM content than TI30, TI45 and TI60. This could be an expected result if SBTIs were responsible for increasing fecal wet weight in SBM diets. However, the previously mentioned studies were based on fecal material DM content, not SI digesta. In the current study neither day-10 nor day-21 fecal DM samples showed effects from SBTI. Based on data obtained from all intestinal regions, it appears that SBTIs had minimal affect on the water absorption processes in the digestive tract of Atlantic salmon.

Trypsin Activity

Trypsin activities in fish typically decrease along the digestive tract, from the pyloric ceaca and small intestine to fecal excretion (Krogdahl et al. 1994, Rust 2002). According to the last authors, this is likely due to continuous autodigestion of the enzymes taking place throughout the digestive tract. In the current study, TA from the commercial control group clearly followed this pattern. For the semi-purified diets, however, TA on day-21 was higher in the LI and possibly fecal region than those recorded for the SI. The cause for this deviation is unclear. Conceivably, nutrient characteristics of the semi-purified diets increased absorption and gastric emptying rates.

Figure 4.1A shows strong evidence of a negative dose dependent response of TI on TA in the SI of Atlantic salmon. While this was an expected result, due to the nature of trypsin enzyme/inhibitor interactions, the effect observed from this study was less severe than those previously reported (Krogdahl et al. 1994, Ollie et al. 1994, Sveier et al. 2001). Day-21 TA results (Figure 4.2) essentially show no differences in TA across

dietary SBTI. The main difference between this study and previous studies was that the primary source of protein for the earlier studies was fish meal while in this current study purified casein and gelatin were used. In this case, lack of TA differentiation across treatment diets support the previous authors' assertions that Atlantic salmon can compensate for TI activity to a certain degree.

Based on results from this study, it appears that Atlantic salmon compensated for up to 60% SBM equivalency TI (3.9 gTI/kg feed). This is somewhat higher than that reported by Ollie et al. (1994). Those authors reported a reduction in TA in digestive tract of Atlantic salmon at approximately 48% SBM equivalency and a TA of zero at 64% SBM equivalency.

Apparent Protein Digestibility

Apparent protein digestibility values showed no significant dietary effects of SBTI on protein digestion over the range 0 to 60% SBM equivalencies (Figures 4.3-4.4 and Table 4.3). In contrast, Ollie et al. (1994) reported a sharp decline in protein digestion of Atlantic salmon at a feed intake level of 48% SBM equivalency TI (3.15 gTI/kg feed). Test diets used by Ollie et al., however, consisted of 70% FM with up to 11% wheat bran, while diets from this current study were made of primarily purified protein source ingredients. Combined with the observation that test diets in the previous study out performed a commercial control in terms of growth, it would appear that the selection of protein sources of test diets has direct impact on affects of purified SBTIs in feed trials for Atlantic salmon.

Proximate Body Composition

Body lipid composition data (Figure 4.5) at the end of the 3-week trial provided no additional information regarding short term effects of SBTIs on Atlantic salmon smolts. In the 8-week fingerling feed trial (Chapter 3), the commercial diet group showed the least amount of variation in body fat. Differences observed between this and the previous study could be due to random effects, smolting and/or the short duration of this feed trial. In a short term feed trial, one would typically expect to obtain more information from measurable physiological traits such as enzymatic concentrations or protein digestion, than from growth characteristic parameters and body composition analysis.

It is important to note that commercial trout and salmon diets typically contain some level of SBM. According to Hardy (2003), this could be as high as 8% for salmonids. Commercial feed manufacturers operate under closed formula policy, and the amount of SBM in the commercial control diet was unknown. Since the control was a standard salmonid diet obtained from a highly reputable feed supplier, the likelihood that variations in body fat levels between the control and test diets observed in this study were a result of dietary effects was assumed to be low. More likely, these variations were due to the condition of the smolts when received from the DNR and/or random variations associated with tank stocking.

Fish Health and Compensatory Mechanisms

Results of this study suggest that SBTIs can cause short term affects on Atlantic salmon smolts. In the long term fingerling study (Chapter 3), SBTIs appeared to affect

body composition, to a degree, but did not show any effects on growth, feed conversion, or health conditions. Neither the short term nor long term study provided specific evidence of immediate detrimental health effects on Atlantic salmon. These results appear to support a hypothesis that Atlantic salmon are able to compensate, through physiological compensatory pathways, for up to 60% SBM equivalency dietary TIs and maintain good health and growth. Ollie et al. (1994), reported a physiological compensatory factor to SBTI in trypsin enzyme activity occurring in pyloric ceaca of Atlantic salmon. Krogdahl et al. (1994) reported a similar response in the same species occurring in the intestine. Other researchers have reported compensatory responses to SBM based diets in coho salmon (Haard et al. 1996).

Present observations may support a hormesis affect as proposed by Calabrese and Baldwin (2003), where a toxic substance acts like a stimulant in small doses, but as an inhibitor in larger doses. This point was also made by Krogdahl et al. (2003). Conversely, if the observed physiological compensations are indicators of a pending impairment, then, based on results herein, additional impairments would be required to reach a point of nutritional deficiency (reduced growth, disease or death).

Chapter Summary

SBTIs had no effect on digesta dry matter content or apparent protein digestion, but had a slight inhibitory effect on TA in the small intestine. There was no indication of detrimental impacts on fingerling and smolting Atlantic salmon by SBTI concentrations up to 60% SBM equivalency. Moreover, results provide some evidence that Atlantic salmon are able to compensate for SBTI over the range of trypsin inhibitor tested.

In regards to SBM-based diets, research has shown that Atlantic salmon experience serious nutritional deficiencies when fed diets containing 10–30% SBM depending on various factors (Storebakken et al. 1998, Reftsie et al. 2000, Krogdahl et al. 2003). Data from this research strongly suggests that SBTIs alone may not be the main cause of these problems, but that other anti-nutritional components (e.g. soluble and non-soluble carbohydrate factions) must be contributing factors to effects observed by other researchers.

Chapter 5. Fixed Formulation Model Development of Practical High Energy SBM Diets for Atlantic Salmon (*Salmo salar*)

Introduction

SBM has received worldwide attention as a potential alternative for fish meal in formulated feeds for aquaculture. To date its use in formulated feeds for carnivorous fish has been limited. Extensive studies on trout and salmon have identified anti-nutritional factors associated with SBM and other plant-based protein sources (Refstie et al. 2000, Storebakken et al. 2000, Krogdahl et al. 2003). While research conducted in this area has shown varying results, the consensus among most researchers is that rainbow trout and Altantic salmon begin to show signs of nutritional deficiencies when fed diets containing 5% to 30% SBM (Hardy 2003, Krogdahl et al. 2003).

A current trend in commercial Atlantic salmon production appears to be a transition from the use of conventional salmonid diets to high nutrient dense (HND) diets (Einen and Roem 1997, Azevedo et al. 2002). Data obtained from the Fish Nutrition Research Laboratory in Canada indicated that HND salmonid starter diets require approximately 49% CP, 20% CF, DE 20 MJ/kg and DP:DE 22g/MJ. These values were in close agreement with Azevedo et al. (2002), who reported that an HND diet (46% CP, 25.6% CF, digestible energy (DE) 22 MJ/kg, DP:DE 20 g/MJ) fed to Atlantic salmon improved feed efficiency and lowered solid waste output over conventional diets. Einen and Roem (1997) recommended slightly lower DP:DE ratios of 19 g/MJ for 1 kg Atlantic salmon, and reported that carcass quality decreased with decreasing DP/DE ratios. Based on results from these researchers, optimal HND diets for young Atlantic salmon should contain between 46-52% CP, 19-25% CF, and 19-22 g/MJ DP:DE. Few studies have

analyzed elevated SBM levels in practical HND diets for Atlantic salmon.

The term "practical diet" refers to diets formulated with commercial ingredients, which are normally purchased in bulk from commodity markets. Formulated feeds used in commercial, State, Federal, and Provincial aquaculture facilities are typically purchased from commercial feed suppliers. In the US, commercial feed companies operate under a "closed formula" policy. Information on feed labels and data sheets usually include proximate analysis and feed ingredients, while ingredient quantities are considered proprietary information and not required to be listed (NRC 2003). Commercial feed manufactures also tend to use least-cost diet formulations, where ingredient quantities are routinely changed between feed lots based on the quality, price and availability of feed ingredients (Guillaume et al. 2001).

Non-proprietary "open formula" diets are those that ingredient quantities are available for public inspection. As apposed to least-cost formulations, fixed diets are those that ingredient quantities are fixed, or constant. Open formula, fixed diets are often selected for nutritional research purposes.

This study was part of an integrated research project funded by the United Soybean Board, Soy-in-Aquaculture Program. The project was designed to develop commercially acceptable SBM-based formulated feeds for Atlantic salmon and other species.

The goal of this portion of the study was to formulate potentially commercially viable, open formula practical diets for Atlantic salmon for use in subsequent feed trials designed to identify maximum SBM replacement levels for FM.

Study objectives included:

1) literature review in order to utilize to the extent possible the best available knowledge pertaining to
   - optimal commercial diets presently used in the Atlantic salmon industry,
   - practical ingredient nutrient utilization specific to Atlantic salmon,
   - related SBM nutritional studies conducted on salmonids,
2) maintain commercial viability through ingredient selection and cost minimization, and
3) minimize phosphorus output into the environment by maintaining feed phosphorous levels below 1.2 %.

Methods

Baseline Diet Formulation

The basal diet for this study was the open formula diet MNR-98HS (Table 5.1). MNR-98HS is an HND diet formulated by the Fish Nutrition Research Laboratory, University of Guelph and Ontario Ministry of Natural Resources, specifically for commercial Atlantic salmon fingerling production. The baseline properties of the basal diet are as follows: 49% CP, 20% CF, and a DP:DE ratio of 22 g/MJ.

Essential Amino Acid Requirements for Atlantic Salmon

Essential amino acid requirements for Atlantic salmon are provided in Table 5.2. The two most common limiting amino acids in formulated fish diets are Met and Lys (Craig and Helfrich 2002). Since FM contains more of both these amino acids than SBM, close attention was given to Met and Lys in the diet formulation process. Diets

Table 5.1 Dietary ingredients and base-line nutritional characteristics of the Ontario Ministry of Natural Resources open formula diet, MNR-98HS, used as a basal control diet to formulate soybean meal-based practical diets for Atlantic salmon.

Ingredient		Amount (g/kg)
Fish meal, anchovy		300
Blood meal		70
Poultry by-product meal		60
Whey		90
Brewers yeast		50
Corn gluten meal		250
Lysine.HCL		5
Vitamin premix		10
Mineral premix		5
Fish oil, menhaden		160
		1000
Crude protein (%)		49
Crude fat (%)		20
Digestible energy (MJ/kg)		20
DP:DE (g/MJ)		22
Ash (%)	<	8
Total phosphorus (%)	<	1

Table 5.2 Atlantic salmon essential amino acid requirements obtained from literature (% protein) and values selected for use in the diet formulation model (% as fed) based on 50% crude protein diets.

Amino Acid	Requirement (% protein)	Requirement (% as fed)	Reference
Arg	4.1 - 5.1	2.3	Lall et al. (1994), Berge et al. (1997), Rollin (1999)
His	1.8	0.9	Rollin (1999)
Ile	3.2	1.6	Rollin (1999)
Leu	5.2	2.6	Rollin (1999)
Lys	3.2 - 6.1	2.1	Anderson et al. (1993), Berge et al. (1998), Rollin (1999)
Met + Cys	3.1	1.55	Rollin (1999)
Phe + Tyr	5.8	2.9	Rollin (1999)
Thr	3.2	1.6	Rollin (1999)
Trp		0.17	NRC (1993, for Pacific salmon)
Val	3.9	1.95	Rollin (1999)

were also formulated to achieve a Lys:Arg ratio of 1:1, considered optimal for Atlantic salmon fed SBM diets (Davies et al. 1997).

Vitamin and Mineral Requirements for Atlantic Salmon

Vitamin and mineral requirements for Atlantic salmon and similar species are provided in Table 5.3. Values obtained for closely related species were used in the diet formulation model for cases where Atlantic salmon data were missing.

Ingredient Selection and Nutritional Properties

Basal diet ingredients (Table 5.1) were evaluated along with other practical fish diet ingredients for addition, reduction, or elimination to/from SBM treatment diet formulations. Wheat gluten meal (WGM) was added to the list of ingredients based on research by Storebakken et al. (2000), and Davies and Baker (1997), which showed high protein digestibility of WGM by Atlantic salmon and rainbow trout.

The NRC (1993) publication *The Nutrient Requirements of Fish* provided the majority of ingredient nutritional properties (Appendix 1) with a few exceptions. The fish meal used by feed processors to make test diets for the practical feed trial was Peruvian anchovy meal. The NRC (1993) publication reported a CP level of 65.4% for mechanically extracted anchovy meal. This value appeared to be somewhat lower than expected for high quality anchovy meal. Diet formulations instead were based on the more current Hertrampf and Piedad-Pascual (2000) values of CP 70.7%, CF 5.3%, and ash 16.9% for anchovy meal (true). Formulations, however, did include NRC (1993) amino acid profiles for anchovy meal because they were more conservative. Properties

Table 5.3 Atlantic salmon diet formulation vitamin and mineral requirements obtained from various sources.

Vitamin/ mineral	Dietary unit	Requirement	Species	Reference
Ca	(%)	1	Rainbow trout	NRC (1993)
Phos.	(%)	0.6	Atlantic salmon	Lall (2002)
Pot	(%)	0.8	Pacific salmon	NRC (1993)
Chlorine	(%)	0.9	Rainbow trout	NRC (1993)
Mg	(%)	0.04	Atlantic salmon	Lall (2002)
Na	(%)	0.6	Rainbow trout	NRC (1993)
Cu	(mg/kg)	5	Atlantic salmon	Lall (2002)
Fe	(mg/kg)	60	Atlantic salmon	Lall (2002)
Magn.	(mg/kg)	10	Atlantic salmon	Lall (2002)
Se	(mg/kg)	0.3	Rainbow trout	NRC (1993)
Zn	(mg/kg)	67	Atlantic salmon	Lall (2002)
Biotin	(mg/kg)	1.5	salmon	Halver (2002)
Choline	(mg/kg)	1000	Rainbow trout	NRC (1993)
Folacin	(mg/kg)	10	Atlantic salmon	Halver (1972), NRC (1973)
Niacin	(mg/kg)	200	salmon	Halver (2002)
Panta-Acid	(mg/kg)	50	Atlantic salmon	Halver (1972), NRC (1973)
Pyrdox	(mg/kg)	15	Atlantic salmon	Halver (1972), NRC (1973)
Ribo	(mg/kg)	25	salmon	NRC (1993)
Thia	(mg/kg)	15	Atlantic salmon	Halver (1972), NRC (1973)
B12	(mg/kg)	0.01	Rainbow trout	NRC (1993)
E	(IU/kg)	50	Pacific salmon	NRC (1993)
K	(mg/kg)	10	salmon	NRC (1993)

and compositions of WGM were obtained from Hertrampf and Piedad-Pascual (2000), and from the internet (www.nutritiondata.com, © 2007 CondéNet Inc), since this ingredient was not listed in the NRC publication. Menhaden fish oil was assumed to be 100% digestible with an energy value of 9019 kcal/kg (www.food-stats.com).

Vitamins and mineral supplements used in this study included US Fish and Wildlife Service (USFWS) vitamin premix #30 at 0.3% and USFWS mineral premix #3

at 0.2%, based on recommendations made by project collaborators (Steve Hart, Purdue University, personal communication; Rick Barrows, USDA ARS, personal communication). Vitamin and mineral premixes were consistent for all diet formulations unless otherwise noted. Di-calcium phosphate was selected as a phosphorus supplement and added to diet formulations as required in order to meet target phosphorous levels of 10.0–11.5 g P/kg feed.

Ingredient Digestibility Indices

Dietary ingredient DP and DE values obtained from literature review are provided in Table 5.4. Amino acid digestibility data has been published for various feed ingredients, but for relatively few individual species (NRC 1993, Hertrampf and Piedad-Pascual 2000). Data obtained for similar species (e.g. rainbow trout, Chinook or other Pacific salmon) were used where data for Atlantic salmon were lacking. The most conservative value was chosen for formulations when data from two or more similar species were available and Atlantic salmon data were absent.

SBM Content

SBM content in commercial salmonid diets most likely ranges between 1-10% (Hardy 2003). A median level of 5% was selected as a reasonable estimate of SBM typically included in standard commercial Atlantic salmon diets. The first treatment diet therefore was formulated to contain 5% SBM, which was considered equivalent to a typical commercial diet. Additional treatment diet formulations started at 20% SBM inclusion and increased at 5% intervals. FM was reduced by 20% in treatment diets

Table 5.4 Digestible protein (DP) and digestible energy (DE) values of practical ingredients used in diet formulations for Atlantic salmon.

Ingredient	DP (%)	DE (MJ/kg)
Wheat gluten meal	100 [1]	20.3 [1]
SBM	86.8 [2]	13.6 [6]
Fish meal, anchovy	88.5 [4]	20.2 [5,6]
Blood Meal	69.0 [3]	14.3 [7]
Poultry by-product meal	68.0 [3]	16.6 [7]
Corn gluten meal	89.7 [4]	17.8 [3]
Brewers yeast	78.8 [5]	14.7 [3]
Whey	87.8 [5]	11.6 [8]

[1] Glencross and Hawkins (2003) rainbow trout
[2] NRC (1993) Atlantic salmon
[3] NRC (1993) rainbow trout
[4] Hertrampf and Piedad-Pascual (2000) Atlantic salmon
[5] Hertrampf and Piedad-Pascual (2000) salmonids
[6] NRC (1993) Chinook salmon
[7] Hertrampf and Piedad-Pascual (2000) rainbow trout
[8] Hertrampf and Piedad-Pascual (2000) unspecified fish species

containing 20% and 30% SBM in order to assess further reductions in FM. Finally, SBM inclusion rates above 30% were evaluated based on nutritional requirements of Atlantic salmon and selected ingredients.

Diet Formulation Model

Three diet formulation methods were evaluated for potential use in this study: 1) a commercial diet formulation software program, 2) a livestock feed ration balancer program available through Department of Animal Science, Michigan State University, and 3) a new model construction using standard computer spreadsheet software such as Excel.

Ingredient nutritional property values were fixed inside the model (see "Ingredient Nutritional Properties" matrix in the Appendix. Ingredient inclusion levels (model input parameters), were adjusted to evaluate formulations containing baseline diet characteristics, graded levels of SBM, reductions in FM, and meet essential amino acid requirements of Atlantic salmon (model output variables). Amino acids Lys and Met, were synthetically added to test diet formulations as needed. Cost estimates were based on 2004 economic commodity values provided by project collaborators at Iowa State University and USDA ARS (Robert Summerfelt, Richard Barrows, personal communication, respectively). Model output values for the control MNR-98HS diet were compared with baseline characteristics reported by the University of Guelph Fish Nutrition Research Laboratory (Table 5.1), to assess relative accuracy of the diet formulation model.

Results

Diet Formulation Model

Of the three model methods evaluated, the two diet formulation software programs included least cost diet formulation options; however, neither contained nutritional characteristics for Atlantic salmon. No single method appeared to be more user friendly, and each method appeared near equally challenging to learn. This was compounded by the point that both diet software packages required modifications to ingredient selections, specific nutritional requirements, and digestibility characteristics for Atlantic salmon. Since the objective was to develop a simplified fixed cost diet formulation model, it was decided that building a model in Excel would provide

information on diet formulation model development not readily apparent with packaged software programs. Excel spreadsheet diet formulation model results are provided in the Appendix.

Practical SBM HND Diet Formulations for Atlantic Salmon

Atlantic salmon practical SBM diet formulations selected for commercial application feed trials are provided in Table 5.5. SBM treatment diets were identified according to the percent SBM and FM inclusion accordingly: SB5/F30, SB20/F30, SB20/F24, SB25/F30, SB30/F30, and SB30/F24. Diets containing 24% FM were formulated with 20% reduction in FM from the control. Differences between model output values and reported values (Table 5.1) for the control diet were within 1.5% for DP:DE ratios, 1% for CF, 0.5% for CP, DE and ash, and 0.1% for phosphorus.

All diet formulations met baseline characteristic criteria established for HND diets with a few exceptions. Diet formulation crude fat levels ranged between 18.1% and 19.27%. Crude fat levels of 4 out of 6 diets were slightly below the HND criteria of 19%.

All diet formulations met minimum amino acid requirements of Atlantic salmon (Table 5.2 and Appendix). Amino acids Arg and Thr typically fell within 20% of minimum requirements while all remaining essential amino acids ranged between 20–250% above minimum levels). The control diet was estimated to contain the lowest amount of Arg (2.31%). This value is equivalent to the minimum requirement reported for Atlantic salmon. All diet formulations were deficient in sodium by 0.1–0.25% and the control diet formulation was also potentially deficient in potassium. Phosphorus

content varied between 1.08–1.13%. Increasing levels of phosphorous supplementation were required in the form of di-calcium phosphate as dietary SBM levels were increased with concomitant reductions in FM (Table 5.5).

Formulations containing 35% SBM were achieved but had 55% greater crude fiber content than the control (Appendix). Fiber content for 40 and 45% SBM formulations were 1.71 and 1.79% respectively as compared to 1.05% for the control diet. Formulations containing 40% or more SBM required substantial increases in fish oil as well as methionine and phosphorus supplementations. In addition, diets over 35% SBM failed to meet one or more HND diet requirements and fell below a 20% safety margin for essential amino acids Iso, Thr and Trp.

Diet costs estimates ranged from $0.22/lb for SB5/F30, to $0.18/lb for SB20/F24, SB30/F30 and SB30/F24. The 30% SBM diets showed a cost reduction of $0.03/lb from that of the control.

Table 5.5 Atlantic salmon basal (MNR-98HS), and soybean meal-based diet formulations. Soybean meal treatment diets were identified according to percent soybean meal (SB) and fish meal (F) inclusion. Diets containing 24% fish meal were formulated with 20% reduction in fish meal from the control. Diet MNR-98HS is an open formula high nutrient dense diet developed by the Fish Nutrition Research Laboratory, University of Guelph and Ontario Ministry of Natural Resources.

	Diet						
Ingredients	MNR-98HS	SB5/F30	SB20/F30	SB20/F24	SB25/F30	SB30/F30	SB30/F24
Wheat gluten meal		70	70	70	70	70	100
Soybean meal[1]		50	200	200	250	300	300
Fish meal, anchovy	300	300	300	240	300	300	240
Blood meal	70	70	59.5	57	47.5	33	38.5
Poultry by-product meal	60	60	50	49	39.4	29	30.8
Whey	90	90	55	41	44	26.6	21
Brewers yeast	50	50	30	22.9	25	16	13
Corn gluten meal	250	143	59.2	135	43	39	60
Lysine.HCL	5		0.7	1.6	1	2	3
Vitamin premix[2]	10	3	3	3	3	3	3
Mineral premix[3]	5	2	2	2.4	2	2.2	2.4
Fish oil, menhanden	160	151.4	158	161.9	162	165	170
Methionine			1.1	0.8	1.3	1.5	1.5
Di-calcium Phosphate		1.6	2.5	6.4	2.8	3.7	7.8
Choline Chloride		6	6	6	6	6	6
Stay-C		3	3	3	3	3	3
Sum (g/kg)	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
Cost per lb ($/lb)[4]	0.21	0.22	0.20	0.18	0.20	0.18	0.18
DE (MJ/kg)	20.35	20.21	19.99	19.96	19.99	19.99	19.95
DP:DE (g/MJ)	20.54	21.67	21.89	21.80	21.83	21.90	21.97
Lys:Arg	1.40	1.14	1.13	1.11	1.12	1.12	1.11
Crude protein (%)	49.46	51.11	50.69	50.26	50.15	49.88	49.71
Crude fat (%)	19.01	18.11	18.57	18.75	18.82	18.99	19.27
Crude fiber (%)	1.05	1.09	1.38	1.40	1.47	1.56	1.55
Crude ash (%)	7.78	7.90	7.97	6.92	7.91	7.79	6.81
Met + Cys(%)	1.87	1.87	1.87	1.88	1.86	1.87	1.88
Carbohydrate (%)	23.74	22.89	22.77	24.08	23.12	23.34	24.21
Phosphorus (%)	1.10	1.13	1.13	1.08	1.13	1.13	1.08

[1] Purchased from Nelson's and Sons Inc. Murray, Utah
[2] USFWS vitamin premix #30, for MNR-98HS mixed at 3g/kg supplemented with wheat flour
[3] USFWS mineral premix #3, for MNR-98HS mixed at 2g/kg supplemented with wheat flour
[4] Cost estimates were based on 2004 economic commodity values

Discussion

The decision to develop the diet formulation model in Excel proved to be a worthwhile undertaking in that each step of the diet formulation modeling process was completed by the modeler. As the modeler builds the formulation model, he or she undertakes a series of exercises that ultimately require a thorough understanding of ingredient properties, ingredient bioavailability and fish nutritional requirements.

Finding suitable basal diets for diet formulation and development can be difficult due to the closed formula policies of commercial feed companies. In this study, we found a suitable HND open formula diet formulated specifically for Atlantic salmon. Not only did MNR-98HS provide an established base-line diet formulation, but published data on the basal diet also provided a means to compare results between our and previous experiments. The fixed formulation model from this study was within 1.5% of all proximate compositions for MNR-98HS reported by the Fish Nutrition Research Laboratory, University of Guelph.

Ingredient nutritional properties directly influence physiological and chemical interactions affecting fish health and growth. Ingredient selection also affects diet stability and cost of production. Study dietary ingredients were selected based on criteria of meeting HND specifications, Atlantic salmon nutritional requirements, and commercial viability (availability, costs, etc.). One commercial feed supplier (Todd Prowless, Zeigler Brothers, personal communication) strongly suggested avoiding animal by-product ingredients (e.g. blood meal and meat meal). While these have shown to have characteristically high nutritional value for fish, infectious diseases associated with dietary animal by-products have been reported over the past several years (CDC 2007,

Latouche et al. 1998). Diets made with animal by-products currently risk importation bans to various countries. For example, the European Union has banned the use of processed animal protein (notably meat and bone meal) in feeds for all farm animals since 2001 due to the threat of bovine spongiform encephalopathy (BSE or mad cow disease, EC Regulation 999/2001 of the European Parliament and of the Council). Meat meal and meat meal with bone also have relatively high phosphorus levels (4.1 and 4.6 % respectfully, NRC 1993).

Due to differences in protein content, replacement of FM with the addition of significant quantities of SBM in standard commercial salmonid diets required reductions of other main ingredients such as poultry and beef by-products. Atlantic salmon HND diets became increasingly more difficult to formulate when SBM levels exceeded 30%. Digestible energy, protein, and fiber content of primary dietary ingredients are provided in Figure 5.1. As the level of SBM increased, moderate increase in fish oil were required to maintain Atlantic salmon energy requirements. Few options were available to increase protein. The added ingredient, wheat gluten meal, has a relatively high nutritional value. WGM, however, was limited between 7-10% based on recommendations by USB project collaborators. Diets exceeding 10% WGM produced very hard diet pellets with poor palatability for rainbow trout (Richard Barrows, USDA ARS, personal communication). According to Figure 5.1 then, of the remaining ingredients, corn gluten appears to be the best available replacement for fish and blood meals.

Diet formulations showed increased dietary fiber levels with increased SBM. Certain types of fiber have been reported to result in poor digestion, faster gastric emptying rates and depressed growth in salmonids (Davies 1988, Hilton et al. 1983).

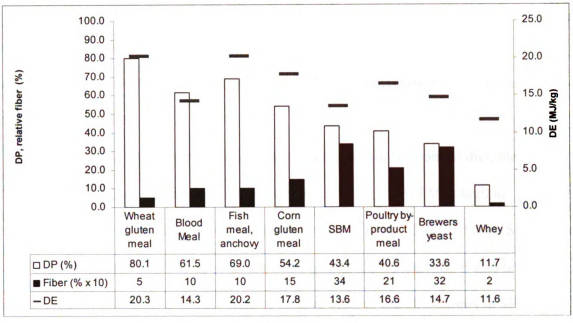

Figure 5.1 Digestible protein (DP), relative fiber and digestible energy (DE) contents of principle ingredients used to formulate high nutrient dense soybean meal diets for Atlantic salmon. Relative fiber is equal to % fiber x 10.0 as a scalar to the y-axis. Optimal ingredients would be high in DP and DE and low in fiber.

According to the National Research Council (NRC1993) most fish can tolerate up to 8% fiber in their diets; however, most types of fiber are indigestible for salmonids. Dietary fiber (non-starch polysaccharide), was identified as an additional parameter of concern over the course of this study. Attempts were therefore made to limit dietary fiber content to no more than 50% excess of the basal (control) level of 1.05% (Table 5.5). Replacing FM and blood meal with SBM and corn gluten could result in a substantial increase in dietary fiber (Figure 5.1).

Diet formulations met Atlantic salmon vitamin and mineral requirements with minor exceptions. The control diet resulted in a potassium content of 0.65%, which was slightly below the required 0.8% for Atlantic salmon. In addition, all diets showed slight

deficiencies in sodium content. According to De Silva and Anderson (1995), however, sodium, potassium and chlorine are extremely common in the environment, and among the most common elements found in fish. As such, supplementation of these elements is probably not required.

Phosphorus (P) supplementation was not required for the control diet, but was required for nearly all SBM diets. According to the NRC (1993) FM is high in phosphorus (2.43%) while SBM is comparatively low (0.64%). Low FM high SBM diets increased P supplementation requirements considerably. Supplemental P also helps ensure the presence of an available form of P since most of the phosphorus in SBM is tied up in the form of phytic acid (Hardy 2003).

Diet formulations generally met minimum essential amino acid requirements across all diets up to 35% SBM inclusion. However, higher level SBM diets required increased amounts of methionine supplementation. Diets containing 40 and 45% SBM required 2.5 and 3.5% supplementation respectively, while the control formulation did not require any supplemental methionine (see diets SB35/F20, SB40/F20, and SB45/F10 in Appendix).

Diet formulations above 30%SBM were difficult to maintain both HND diet protein and energy requirements. They also required substantial decreases in other (including more concentrated) protein sources, increased fish oil for energy and increased supplementation of methionine and phosphorus. Loss of protein sources would likely reduce the overall nutritional balance expected of a HND diet containing multiple protein sources. Due to these factors and elevated fiber content in high SBM diets, formulations

selected as having the best opportunity at being commercially viable were limited to 30% SBM or less (Table 5.5).

Cost comparisons showed a reduction of $0.03 US per pound ($0.014/kg) feed between the control diet and the high SBM diets. Based on 2004 economic commodity values, a 3% cost reduction in feed is conceivable provided Atlantic salmon can tolerate up to 30% SBM. At a feed conversion rate (FCR) of 1.0, this would equate to a 3% cost reduction per kg fish across a 1.5 million metric tonne (2004 value) Atlantic salmon industry.

Chapter Summary

The Atlantic salmon diet formulation modeling process undertaken in this study provided valuable information regarding diet formulation model development and Atlantic salmon nutritional requirements. Commercial and/or academic diet formulation software offer additional benefits including least cost diet formulations. A logical progression in future diet formulations for Atlantic salmon would be to incorporate the information summarized within the confines of this study into least cost, nutritional optimization diet formulation model(s).

High nutrient dense (HND) diets containing 5 - 30% SBM, 30%FM were formulated after the basal diet MNR-98HS (Ontario Ministry of Resources). All diets were based on specific nutrient requirements of Atlantic salmon, and formulated for 50% crude protein, 19% crude fat and DP:DE of 21 g/MJ. SBM treatment diets were identified according to percent SBM and FM inclusion accordingly: SB5/F30, SB20/F30, SB20/F24, SB25/F30, SB30/F30, and SB30/F24. Diets containing 24% FM were

formulated with 20% reduction in FM from the control. The resulting diet formulations will be used in a feed trial designed to identify maximum SBM replacement levels for FM in commercial diets for Atlantic salmon smolts.

Chapter 6 – Replacement of FM with SBM in High Energy Practical Diets of Smolting Atlantic Salmon (*Salmo salar*)

Introduction

In 2002, a collaborative project was funded by the United Soybean Board (USB), of the United States, to examine the extent of SBM anti-nutritional properties on salmonids. The goal of this collaboration was to determine how to overcome SBM associated nutritional problems in order to increase the potential use of SBM in formulated fish feeds. This particular study was the final feed trial of an integrated research project at Michigan State University, and was designed to develop a commercially viable practical diet SBM diet for Atlantic salmon. The main goal of this study was to determine maximum SBM replacement levels for FM in practical diets for Atlantic salmon.

Methods and Materials

Diet Formulations and Processing

The control diet (MNR-98HS) for the feed trial was an open formula high nutrient dense (HND) Atlantic salmon diet formulated by the Fish Nutrition Research Laboratory, University of Guelph and Ontario Ministry of Natural Resources. All test diets were formulated to contain 50% CP, 19%CF, with 20 MJ/kg digestible energy and DP:DE equal to 21g/MJ. The control and test diet formulations are provided in Table 5.5 (see Chapter 5). Additionally, all diets were formulated to contain a minimum of 1.87% Met + Cys, Lys:Arg ratio of 1.1, and phosphorus concentration between 1.0 and 1.15% (10.0 and 11.5 gP/kg feed). The control diet contained 0% SBM and 30% FM, and was

compared to six test diets containing 5 to 30% SBM. In two of the test diets, the amount of fish meal was reduced by 20% from that of the control diet. Test diets were identified according to percent SBM and FM inclusion accordingly: SB5/F30, SB20/F30, SB20/F24, SB25/F30, SB30/F30, and SB30/F24.

All diets were processed under the same conditions by project collaborators at USDA ARS and USFWS Bozeman Fish Technology Center (Richard Barrows, USDA ARS, personal communication). Diet processing methods were based on optimal processing parameters determined by Barrows et al. (2004) for practical SBM diets for rainbow trout. Ingredients were ground using an air-swept pulversizer (Jacobsen, JASP 18-H) and then mixed into a complete feed. The diets were manufactured using a twin-screw cooking extruder (DNDL-44, Buhler AG, Uzwil, Switzerland). The mix was introduced to the steam conditioning chamber with a volumetric feeder (K-tron, Inc., Pitman, NJ). Feed rate (1200 rpm) was set to provide 18 seconds of heat exposure in the extruder. The extruder had 6 barrel sections and temperature was controlled by heating section 2, 3, 4, and 5, with a maximum extruder barrel temperature of 262^{0}F, and an average die head pressure of 230 PSI. Sections 1 and 6 were cooled to insure smooth feeding and discharge. Upon discharge the diets were dried in a pulse bed drier (Buhler, Uzwil, Switzerland) for 25 minutes at 250^{o}F with a 10 minute cooling period which resulted in moisture levels less than 10%. After the diets had dried they were top-coated with the remaining oil (6%) at ambient pressures.

Experimental System and Animals

One-year old Atlantic salmon were obtained from the Michigan Department of Natural Resources, Thompson State Fish Hatchery, Manistique, Michigan, in November of 2004. The fish were transported to the Michigan State University Aquaculture Research Laboratory and were acclimated to water conditions in a 1,890-L flow-through tank culture system over a 30-day period. Fish were fed a commercial trout diet over the acclimation period. A total of 420 fish were randomly distributed in 21, 225-L tanks, at 20 fish per tank, and acclimated on the MNR-98HS control diet to feed trial rearing units for an additional 15-day period. Flow rates were maintained between 5.6–7.5 Lpm fresh well water based on target exchange rates of 1.5–2.0 water exchanges per hour. Water temperature for all tanks remained between 12.3 ± 1.0^{0}C for the duration of the study. Dissolved oxygen, total ammonia nitrogen, and all other water quality parameters fell within acceptable limits for salmonids (Piper et al. 1982).

At the start of the trial, parr marks were clearly visible on all fish. During the acclimation period several fish showed signs of bacterial infection. Prophylactic treatments of 10 ppt salt were administered once per week through the end of the feed trial. Thirty six mortalities occurred over the course of the 85-day feed trial. All mortalities showed external signs consistent with those observed in 2003 Atlantic salmon fingerling and smolt feed trials at the MSU Aquaculture Laboratory. Upon completion of the trial, nearly all fish had developed a silver sheen appearance and showed less noticeable parr marks.

Total weights of fish per replicate tank were recorded at the start of the feed trial. Average initial weight was 27.6 ± 1.0 grams/fish/tank. Triplicate groups of Atlantic

salmon were fed either the open formula MNR-98HS control diet, or one of six SBM treatment diets, 2 times daily (8:00–9:00 am, 4:30–5:30pm). Total weight samples were obtained every 2 weeks throughout the study. Daily feed levels were determined on a constant percent body weight basis (%BW) adjusted bi-weekly based on initial condition factor (k), average water temperature and total weight samples. For the trial, %BW was calculated as 90% of the theoretical optimal feed level for salmonids (Westers, 1987) Feed levels fell both above and below satiation levels of the fish across feeding times based on observations of excess feed in tank bottoms at various times through the feed trial.

The feed trial was conducted for a period of 12 weeks. At the end of the trial, total weights were recorded for each tank. Ten fish from each tank were randomly selected for length and weight measurements, collection of blood, whole liver, and intestinal digesta samples. Fish were euthanized in MS-222 at a concentration of 500mg/L (AVMA 2000). Blood samples were frozen at -20^{0}C until centrifuging at 4000 rpm, 4^{0}C for 10 minutes. Clear natant plasma was pipetted from the centrifuged blood samples and frozen at -80^{0}C for future analysis. Whole livers were removed and weighed. Intestinal digesta was scraped from the entire length of fish large intestines, pooled by tank, and frozen at -20^{0}C. Small intestines were excised from the first three fish sampled from each tank and fixed in 10% neutral buffered formalin for subsequent histological examination. Three fish from each replicate tank were pooled by tank and frozen at -20^{0}C for subsequent proximate body composition analysis.

At the end of the 12-week feed trial, 13 fish from the control group and 16 fish from the SB30/F24 group were available for further study after completion of the

sampling procedures described above. These fish were stocked into a two tanks, by dietary treatment group, and were fed their original treatment diet for an additional 10-week period. At the end of the second feeding period, all fish were sacrificed as described above and muscle tissues were collected from the mid-dorsal muscular regions. Tissue samples were then frozen at -80^{0}C for subsequent amino acid composition analysis.

Sample Analysis and Calculations

Feeding levels

Trial feed levels were calculated as 90% of the theoretical optimal %BW for salmonids as developed by Westers (1987) for standard commercial diets and a feed conversion rate (FCR) of 1.0:

$$\%BW = (2 \times {}^0C) / (W/k_i)^{1/3} \times 0.90$$

where:

^{0}C = temperature in Celsius

W = Wet weight of fish at time of sampling (g),

k_i = condition factor at the start of the trial.

Growth

Weight and length data were used to determine condition factor (k) and specific growth rate (SGR) over the course of the study:

$$k = W/L^3$$

$$SGR = (\ln W_f - \ln W_i)/d \times 100$$

where:

L = length (cm),

$W_{f \text{ or } i}$ = average final or initial wet weight (g),

d = time (days).

Feed conversion

Feed conversion rates were calculated as the standard apparent FCR with adjustment for mortalities:

$$FCR = \text{Cum Feed}/(\text{Net W Gained} + (W_{Morts} - (N_{Morts} \times W_i)))$$

where:

Net W Gained = net weight of fish gained over the feed trial (g),

Cum Feed = cumulative weight of feed (g),

W_{Morts} = Wet weight of mortalities removed (g),

N_{Morts} = Number of mortalities removed.

Protein efficiency

Protein efficiency ratios (PER) were based on the formula provided by De Silva and Anderson(1995) slightly modified to account for mortality:

$$PER = (\text{Net W Gained} + (W_{Morts} - (N_{Morts} \times W_i))/ TP_{Feed}$$

$$TP_{Feed} = \text{Cum Feed} \times \% P_{Feed} /100$$

where:

TP_{Feed} = total protein level in feed fed,

$\% P_{Feed}$ = % protein in diet as fed basis (%P/gfeed),

Whole body composition

Whole body composition analysis included lipid, protein, ash, dry matter and energy. Pooled, frozen whole body samples were thawed quickly under cool water and homogenized in a commercial-grade food processor. Samples were weighed, frozen and freeze-dried. All freeze-dried samples were finely ground and stored at -20^{0}C. Whole body lipid content was determined on duplicate 1.0–3.0 g samples by lipid extraction with diethyl ether (Soxtec System HT/1043 Extraction Unit, Tecator, Sweden). Nitrogen was determined on 0.5 g samples according to combustion method AOAC (2000) using a Leco nitrogen/protein analyzer (model FP-2000, Leco Corp., St. Joseph, MI). Protein was calculated as N x 6.25. Dry matter was obtained after oven drying 2.0 g samples at 105^{0}C for 18-24 hours. Ash content was determined after placing dry matter samples in muffle furnace at 500^{0}C for 18 hours. Gross energy was determined by bomb calorimetery on whole body samples from control and SB30/F24 replicates for comparison of the two most extreme treatment levels. All body composition samples were performed in duplicate, and repeated if differences between individual samples exceeded 3.0%.

Feed lipid, protein, ash and gross energy were determined for all diets using the methods described above for whole body samples. Feed percent total carbohydrate levels were determined using the difference method, by subtracting the sum of percent protein, lipid and ash from 100%.

Digesta dry matter

Large intestine digesta was lyophilized using a Tri-Philizer MP, FTS systems, for 48 hours minimum by laboratory personnel at MSU's Department of Animal Science (Dave Main, MSU, personal communication). Dry matter content was determined by measuring weight of digesta samples before and after freeze drying.

Trypsin activity

Lyophilized large intestine digesta samples were held at -20^{0}C until assayed for trypsin activity. Trypsin activities were determined colorimetrically using BAPA (Benzoyl-DL-arginine-p-nitranilide) as a substrate as described by Kakade et al. (1969) with slight modification. This procedure is described in detail in Chapter 4 of this dissertation. Trypsin activity (TA) in mg trypsin per gram intestinal fecal material was calculated as follows:

TA (mg/g sample) = Try (μg/vol) / wt (g)

Try (μg/vol) = Try (μg/ml from standard curve) x EVol (ml)

where:

Try = trypsin value; EVol = extract volume.

Plasma insulin

Plasma from three fish per tank were assayed for insulin concentrations at MSU's Diagnostic Center for Population and Animal Health Laboratory, using protocols (DSL Insulin RIA product insert, July 27, 1999) used for a 2004 Atlantic salmon feed trial conducted by fellow USB project collaborators, Purdue University.

Hepatic somatic indices

Excised whole liver weights were used to calculate and contrast hepatic somatic indices (HSI):

$$HSI = LW/W_f$$

where:

LW = Liver weight (g).

Intestinal histology

Fixed small intestine tissue samples were cut into multiple cross-sections, paraffin-embedded, sectioned at 5μm, and routinely processed for staining with hematoxylin and eosin (Prophet et al. 1992). All slides were evaluated microscopically by Dr. Scott Fitzgerald, an American College of Veterinary Pathologist, at MSU's Diagnostic Center for Population and Animal Health Laboratory. Sections of intestine were evaluated for villous to crypt ratio, whether or not the mucosal epithelium and microvillous brush border were intact, presence of inflammatory infiltrates, and for any other evidence of degeneration.

Muscle Tissue Amino Acids

Muscle amino acid composition analysis was performed on tissue samples from fish fed the two extreme diets of the feed trial (control and SB30/F24 diets), 5 samples per group. Amino acids were identified following HPLC separation (Waters 486 absorbance detector, Waters Corporation) at 254 nm by Julie Moore of MSU Department of Animal Science, according to the method described in Guay and Trottier (2006).

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) using SPSS (SPSS© Release 12.0, 2003) and SAS (SAS© Release 9.1, 2003) statistical software. Homogeneity of variance was confirmed by Levene's statistic analysis. Significant differences between means were compared by Duncan's multiple range test. Trend analyses were conducted using regression analysis. Treatment effects were considered significant at $P \leq 0.05$ unless otherwise noted.

Results from the amino acid assay were subjected to statistical analysis even though each sample group came from a single tank. Since these fish were fed their respective diets for a total of 22-weeks, it was assumed that tank effects would have little to no influence on muscle tissue amino acid compositions. Individual fish were therefore treated as replicates, for the amino acid analyses only.

Results

Feed Compositions

Diet formulation results (target levels) were compared to measured feed protein, lipid, ash and carbohydrate compositions (Table 6.1). Actual feed CP levels were within 0.8% from each other, but consistently 5–6% higher than the expected formulated values. Actual feed crude fat contents were about 1–2% higher than formulated values except for diet SB5/F30. Feed ash and carbohydrate compositions were 2–4% lower than formulated values. Both the OMNR control and the low SBM (5%) diets appeared to contain the greatest amounts of carbohydrates (20.4% and 21.7% respectively), while the 30% SBM diet contained the least (18.1–18.3%).

Table 6.1 Compositions of test diets (as fed basis). Measured crude protein (CP), crude fat (CF), ash, carbohydrate (CHO) and energy content (E). MNR-98HS is the open formula control diet. Test diets are identified according to percent soybean meal (SB) and percent fish meal (F). Formulated values are in parentheses.

Diet	%CP	%CF	%Ash	%CHO	E (MJ/Kg)
MNR-98HS	55.3 *(49.5)*	19.7 *(19.0)*	4.7 *(7.8)*	20.4 *(23.7)*	22.7
SB5/F30	56.0 *(51.1)*	17.7 *(18.1)*	4.6 *(7.9)*	21.7 *(22.9)*	23.2
SB20/F30	55.8 *(50.7)*	20.4 *(18.6)*	5.2 *(8.0)*	18.6 *(22.8)*	23.2
SB20/F24	55.0 *(50.3)*	20.3 *(18.7)*	5.0 *(6.9)*	19.7 *(24.1)*	23.6
SB25/F30	55.3 *(50.2)*	20.7 *(18.8)*	5.1 *(7.9)*	18.9 *(23.1)*	23.5
SB30/F30	55.3 *(49.9)*	21.3 *(19.0)*	5.3 *(7.8)*	18.1 *(23.3)*	23.3
SB30/F24	55.2 *(49.7)*	21.2 *(19.3)*	5.3 *(6.8)*	18.3 *(24.2)*	23.3

Growth Characteristics, Feed Conversion and Protein Retention

No differences were observed in final weight, growth (SGR), feed conversion (FCR), or protein retention (PER) of fish fed control and experimental diets (Table 6.2). Feed conversion rates ranged between 0.78–0.82. Only slight differences were observed in the condition factor (k). Condition factors for all diet groups ranged from 0.010–0.0107, and are well within optimal values for salmonids (Harry Westers, Aquaculture Bioengineering Corporation, personal communication).

Proximate Body Composition

Whole body composition analysis showed minor differences in DM, CP, and ash, but no trends were observed in these parameters (Table 6.3). Increased SBM in the diet, however, was inversely related to whole body fat content (Figure 6.1). A plot of body lipid composition with the amount of SBM in the diet produced a highly correlated negative linear functional response (linear, $P = 0.0001$) of:

Body Lipid Composition = -0.32 x %SBM +22.59 ($R^2 = 0.88$).

Table 6.2 Mean final weight, specific growth rate (SGR), condition factor (k), feed conversion rate (FCR), and protein efficiency ratio (PER) in smolting Atlantic salmon fed diets containing varying levels of soybean meal and fish meal. Mean standard errors are in parentheses. MNR-98HS is the open formula control diet. Test diets are identified according to percent soybean meal (SB) and percent fish meal (F). Values in each row awarded common superscripts are not significantly different (P > 0.05).

	MNR-98HS	SB5/F30	SB20/F30	SB20/F24	SB25/F30	SB30/F30	SB30/F24
Final weight (g)	81.39	81.13	76.32	82.24	82.03	82.53	82.50
	(2.64)	(0.50)	(0.56)	(0.90)	(2.28)	(3.07)	(1.18)
SGR (%/d)	1.94	1.94	1.88	1.92	1.93	1.94	1.93
	(0.02)	(0.01)	(0.02)	(0.02)	(0.03)	(0.02)	(0.03)
k	0.0107 [a]	0.0105 [ab]	0.0100 [b]	0.0106 [a]	0.0102 [ab]	0.0102 [ab]	0.0105 [ab]
	(0.0002)	(0.0003)	(0.0002)	(0.0001)	(0.0001)	(0.0002)	(0.0001)
FCR	0.823	0.789	0.797	0.788	0.789	0.780	0.803
	(0.027)	(0.028)	(0.032)	(0.007)	(0.002)	(0.013)	(0.04)
PER	2.20	2.27	2.26	2.31	2.29	2.32	2.27
	(0.07)	(0.08)	(0.09)	(0.02)	(0.01)	(0.04)	(0.11)

Table 6.3 Body composition analysis of smolting Atlantic salmon fed diets varying levels of soybean meal and fish meal. Mean standard errors are in parentheses. MNR-98HS is the open formula control diet. Test diets are identified according to percent soybean meal (SB) and percent fish meal (F). Values in each row awarded common superscripts are not significantly different (P > 0.05).

	MNR-98HS	SB5/F30	SB20/F30	SB20/F24	SB25/F30	SB30/F30	SB30/F24
Dry matter	28.82 ^{a}	27.89 ^{b}	28.47 ^{ab}	28.42 ^{ab}	28.48 ^{ab}	28.40 ^{ab}	28.32 ^{ab}
	(0.10)	(0.26)	(0.20)	(0.16)	(0.14)	(0.07)	(0.25)
Protein	49.45 ^{ab}	49.16 ^{b}	50.22 ^{ab}	50.82 ^{ab}	51.50 ^{a}	51.41 ^{a}	50.54 ^{ab}
	(0.83)	(0.36)	(0.45)	(1.03)	(0.12)	(0.84)	(0.31)
Fat	22.04 ^{a}	21.85 ^{a}	16.06 ^{b}	15.82 ^{bc}	15.04 ^{bcd}	12.95 ^{d}	13.20 ^{cd}
	(0.75)	(0.56)	(0.70)	(1.39)	(0.57)	(0.77)	(0.46)
Ash	6.73 ^{bc}	7.17 ^{a}	6.97 ^{abc}	6.94 ^{abc}	7.00 ^{ab}	6.57 ^{c}	6.79 ^{abc}
	(0.11)	(0.06)	(0.25)	(0.02)	(0.14)	(0.04)	(0.09)

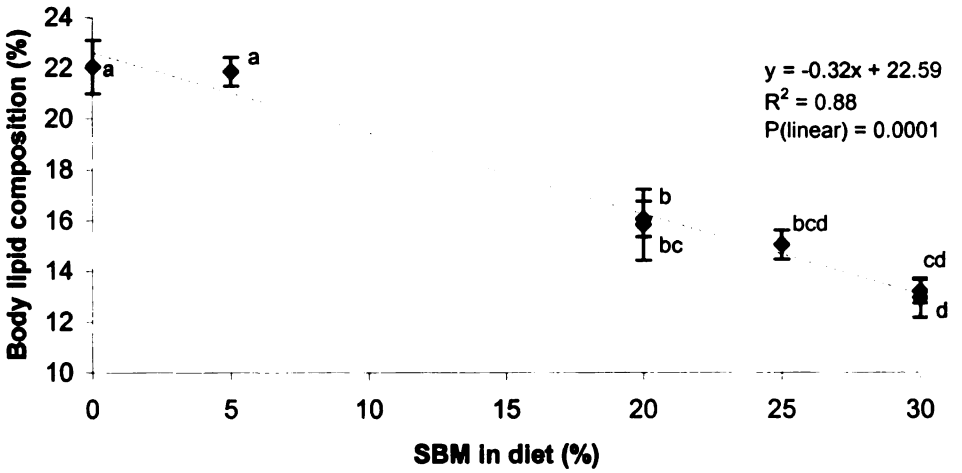

Figure 6.1 Percent whole body lipid compositions of smolting Atlantic salmon as a function of dietary soybean meal. P(linear) signifies P-value for linear regression analysis. Values in each row awarded common superscripts are not significantly different (P > 0.05).

Mean body fat content of fish fed the control (0% SBM) was approximately 9% higher than those of fish fed diets containing 30% SBM. This result was consistent for both 30% SBM diets regardless of the level of FM (30% and 24%).

Whole body gross energy values obtained by bomb calorimetery showed no significant differences between fish fed the control and fish fed the highest SBM /lowest FM diet (SB30/F24; Table 6.4).

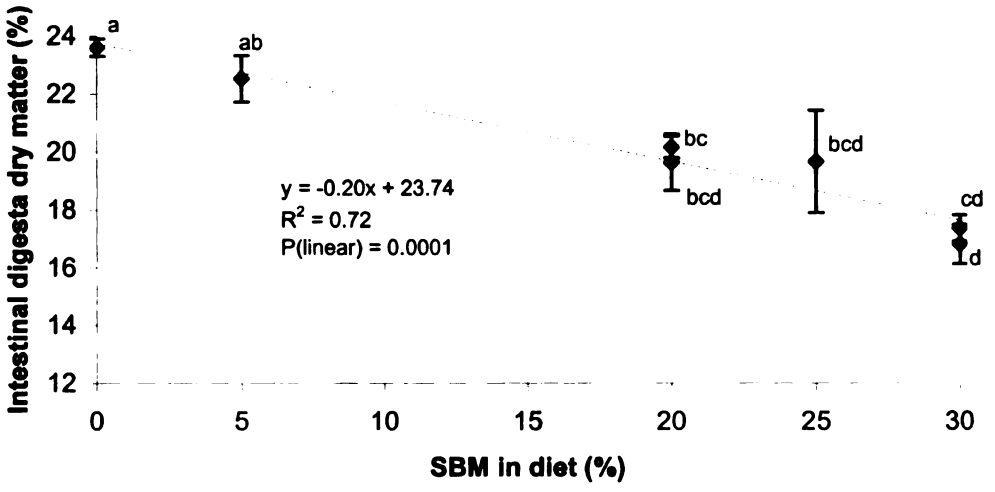

Figure 6.2 Large intestine digesta dry matter (lyophilized) of smolting Atlantic salmon as a function of dietary soybean meal. P(linear) signifies P-value for linear regression analysis. Values in each row awarded common superscripts are not significantly different (P > 0.05).

Digesta Dry Matter

Large intestine digesta dry matter content (lyophilized) showed differences across dietary treatments and provided evidence of a negative relationship between dry matter content and increased levels of dietary SBM (Figure 6.2). Digesta sampled from the 0%SBM control had a significantly higher dry matter content than dietary groups fed 20% SBM diets or greater. The observed negative linear response (linear, P = 0.0001) between intestinal digesta dry matter and SBM was as follows:

Large intestine digesta dry matter = -0.20 x %SBM + 23.74 (R^2 = 0.72).

Table 6.4 Hepatic somatic index (HSI), intestinal trypsin activity (TA) and whole body energy composition of smolting Atlantic salmon fed diets varying levels of soybean meal and fish meal. Mean standard errors are in parentheses. MNR-98HS is the open formula control diet. Test diets are identified according to percent soybean meal (SB) and percent fish meal (F). Values in each row awarded common superscripts are not significantly different (P > 0.05).

Diet	HSI	TA (mg/g)	E (MJ/Kg)
MNR-98HS	1.11 [a]	2.03	23.77
	(0.08)	(0.30)	(0.19)
SB5/F30	1.09 [ab]	1.49	----
	(0.02)	(0.08)	
SB20/F30	1.12 [a]	1.87	----
	(0.05)	(0.21)	
SB20/F24	1.03 [ab]	1.64	----
	(0.04)	(0.43)	
SB25/F30	1.09 [ab]	1.73	----
	(0.02)	(0.13)	
SB30/F30	1.02 [ab]	1.18	----
	(0.01)	(0.20)	
SB30/F24	0.96 [b]	1.37	23.95
	(0.04)	(0.23)	(0.09)

Trypsin Activity

Trypsin enzyme activity was slightly greater for the control group than all other dietary treatments (Table 6.4). TA was significantly greater for the control than all other treatment groups at $P \leq 0.10$ significance level, but no significant differences were detected at $P \leq 0.5$.

Hepatic Somatic Index

Hepatic somatic index was significantly greater for the control than the groups fed SB30/F24 at $P \leq 0.05$, and SB20/F24 and SB30/F30 groups at $P \leq 0.10$ (Table 6.4). No

significant differences in HSI were observed between the control, SB5/F30, SB20/F30, or SB25/F30 groups.

Plasma Insulin

Plasma insulin concentrations for all treatment groups fell below the minimum detection limit (30pmol/l) required by assay protocols (MSU's Diagnostic Center for Population and Animal Health Laboratory). As a result, no differences in plasma insulin could be attributed to dietary treatment effect.

Intestinal Histology

Histological sections of small intestine from representative fish in each study group were examined microscopically. The villous:crypt ratio was uniformly in the 2:1 to 3:1 range for all fish in all study groups. Neither the mucosal epithelium nor the microvillous brush border exhibited attenuation or discontinuity in any fish. Only one fish, from the SB30/F24 group, exhibited mild lymphocytic-plasmacytic infiltrates within the mucosal lamina propria. According to the histological examination report this type of defect was likely to be of an infectious cause rather than an effect of the diet. No significant microscopic alterations in the gastrointestinal tract were observed from any treatment group.

Muscle Tissue Amino Acids

Muscle tissue amino acid profiles (Table 6.5) were significantly different ($P \leq 0.05$) between the control (0%SBM) and SB30/F24 dietary groups for 14 of the 19 amino

Table 6.5 Muscle tissue amino acid compositions (umol/g) of smolting Atlantic salmon fed a control (MNR-98HS) and a 30% soybean meal (SB) 24% fish meal (F) diet for 22 weeks. Values with asterisk (*) were significantly different (P < 0.05).

	MNR-98HS	SB30/F24	
GLU	2.62	2.80	*
HYP	5.10	5.75	*
SER	3.73	3.89	
ASN	3.35	3.70	*
GLY	12.56	12.04	
TAU	3.41	3.71	
HIS	7.58	5.10	*
THR	3.95	3.98	
ALA	5.19	5.38	
PRO	3.05	3.28	*
TYR	2.86	3.07	*
VAL	2.91	3.14	*
MET	2.98	3.22	*
ILE	2.91	3.13	*
LEU	3.66	3.99	*
PHE	3.07	3.31	*
TRP	2.97	3.19	*
ORN	2.96	3.20	*
LYS	3.51	4.04	*

acids analyzed. Only one amino acid, HIS, was higher in fish fed the 0% SBM control. Fish fed the SB30/F24 diet had higher levels of 12 of the remaining 17 amino acids than fish fed the 0% SBM diet.

Mortalities

No correlations were found between average mortality per tank and level of SBM in the diet. Survival in each tank varied from 60% to 100%, with a 92% overall survival rate. The control diet had the highest number of mortalities with 8 (Figure 6.3). Mortalities did, however, decline considerably over time. Of the 36 total mortalities, 23 occurred over the first one-third of the trial (28 days), 10 occurred over the middle one-

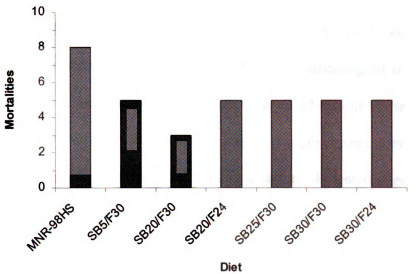

Figure 6.3 Mortalities by treatment (Diet) of smolting Atlantic salmon over the 12-week feed trial. MNR-98HS is the open formula control. Test diets are identified according to percent soybean meal (SB) and percent fish meal (F).

third of the trial (day 29 - 56), and only 3 mortalities were observed over the last one-third period of the trial (day 57 - 85). No mortalities were observed over the extended 10-week feeding trial.

Discussion

Feed Compositions

Differences in formulated versus measured diet compositions (Table 6.1) could be attributed to intrinsic and extrinsic factors as identified by Gizzi and Givens (2001). Intrinsic variability is that caused by real differences among feedstuffs (genetics,

processing techniques, etc). Extrinsic variability is caused by differences in sampling and analysis procedures. The diet formulation model (Chapter 5), slightly underestimated actual, or measured CP and CF levels. The model also slightly overestimated ash and carbohydrate compositions in the feed. Future applications of the diet formulation model used in this study should account for intrinsic variability to the extent possible and minimize variability arising from extrinsic factors. Potential improvements include testing individual diet ingredients and additional review of model input parameters.

Growth Characteristics, Feed Conversion and Protein Retention

Atlantic salmon smolts showed excellent growth characteristics across all dietary treatments. No differences were observed in final weight, SGR, FCR or PER. These results were encouraging since nearly all previous studies had shown depressed growth of Atlantic salmon fed SBM based diets with approximately equivalent FM replacement rates. For example, Refstie et al. (2000) reported a gain of 44% growth of Atlantic salmon fed 70%FM over those fed 32%FM/30%SBM. Those researchers also reported much lower SGR values than those observed in this study. Krogdahl et al. (2003) found a negative dose-dependent effect of SBM on growth parameters of Atlantic salmon fed extruded diets containing up to 27% SBM (35% of total protein). Differences in diet formulations, dietary ingredients and processing methods were likely causes for the differences observed between studies. Mean k values were slightly different, but this difference does not appear to be an effect of feed SBM content (Table 6.2)

Proximate Body Composition

Body lipid composition data provided strong evidence that SBM diets can have long term impacts on Atlantic salmon. Results from this trial showed a similar negative does-dependent response of lipid retention to that observed by other researchers (Storebakken et al. 1998, Krogdahl et al. 2000, Reftsie et al. 2000). Those authors theorized that salmon were unable to digest SBM oligosaccharides, which are alcohol soluble, and also non-starch polysaccharides (non soluble). A similar trend, however, was not observed by Carter and Hauler (2000) in a comparison of high energy diets containing 0%SBM 60%FM and 22%SBM 45%FM. Lipid utilization appears to be an important function affected by consumption of SBM diets by carnivorous fish.

No significant differences were detected in body dry matter, protein, ash content (Table 6.3) or energy (Table 6.4). These results agreed in part to those of Krogdahl et al. (2003) who found no differences in Atlantic salmon protein and ash body compositions fed up to 27% SBM (35% of total protein). The previous authors did, however, show a negative response of body dry matter compositions ($P = 0.007$) and body energy ($P = 0.004$) to increasing SBM levels. Neither body dry matter nor body energy was affected by SBM content in the study reported here.

Digesta Dry Matter

Figure 6.2 indicates a highly correlated functional response of decreasing digesta dry matter content to SBM level in diets for Atlantic salmon. In general, large intestine digesta increased in water content with increased levels of SBM. This finding agrees with results reported by others (Storebakken et al. 1998, Reftsie et al. 1999, 2000, 2001).

Reftsie et al. (2000) attributed reduced fecal dry matter and reduced lipid retention in Atlantic salmon to non-starch polysaccharides in SBM, while Arnesen et al. (1989) attributed a similar condition to alcohol soluble carbohydrates.

Trypsin Activity

Lack of differences in TA across diets suggests that soybean TIs were either not present in sufficient quantities to impair trypsin enzyme activity, or were destroyed by heat processes during diet manufacturing. This finding agrees with results from the previous TI studies (Chapters 3 and 4) which failed to show direct response of TA to SBTIs up to a level of 60%SBM equivalency. Our data also supports others who have reported that TIs are heat liable and can be destroyed or reduced by diet processing methods (Wilson 1992, Anderson and Wolf 1995).

Plasma Insulin and HSI

Increased levels of indigestible carbohydrates in diets of carnivorous fish have resulted in high glucose and insulin levels (Hemre et al. 1995, Cowley and Sheridan 1993), increased liver size and weight (Hilton and Atkinson 1982) and increased hepatic lipogenesis (Brauge et al. 1995, 1994). Conversely, increased levels of digestible carbohydrates in feeds for rainbow trout increased plasma glucose concentrations (Bergot 1979), and increased gelatinized starch intake has shown protein sparing effects for the same species (Kaushik and Oliva-Teles 1985).

Effects of the SBM practical diets on plasma insulin in this study were inconclusive. Plasma insulin concentrations for all treatment groups fell below the

minimum detection limit (30pmol/l) required by assay protocols (MSU's Diagnostic Center for Population and Animal Health Laboratory). Reserve plasma samples were destroyed when the cold storage unit holding the samples malfunctioned and the samples thawed for an extended period of time. Thus, plasma insulin tests could not be repeated after the initial assay.

Fish fed the control and SB20F30 diets had higher HSI values ($P \leq 0.05$) the fish fed the high SBM low FM diet (SB30/F24). No other differences were observed with HSI. Increased HSI (i.e. liver to body weight ratio) might be an indicator of lipid build up in the liver. Thus it does not appear that fish fed high SBM experienced high lipogenic activity. Rather, lower HSI values combined with decreased liver weight and body lipid suggested more of a protein sparing condition. According to Buhler and Halver (1961) the balance between lipid and starch affect protein sparing in Chinook salmon. Conceivably, use of HND diets aided carbohydrate utilization in the SBM diets.

Intestinal Histology

No diet effects were observed relating SBM to problems in the small intestine. Distal intestine (DI) sections, however, were not examined by histological methods. According to Baeverfjord and Krogdahl (1996), the DI is the primary region where SBM induced enteritis is likely to occur. In mammals, the vast majority of absorption of feed materials takes place in the anterior small intestine, while the hindgut is more for bacterial fermentation and fluid absorption (Dr. Scott Fitzgerald, Michigan State University, personal communication). Samples sent to MSU's Pathobiology and Diagnostics Center were analyzed using similar protocols to those previously done by

USB project collaborators at Purdue University. These protocols concentrated on the anterior intestine. Focusing on small intestinal histology instead of DI was an unfortunate oversight of this study.

Muscle Tissue Amino Acids

SBM induced changes were observed in muscle tissue amino acid profiles (Table 6.5), but not on growth, protein utilization efficiency (PER in Table 6.2), or protein composition (Table 6.3). Thus, it would appear that the SBM diets in this study had a direct impact on various metabolic pathways without impairing growth rates. Krogdahl et al. (2003), stated that lack of diet effects on nutrient and energy deposition suggested that the main effect of SBM on nutrient utilization can be attributed to digestive rather than metabolic processes. In that study, however, amino acid deposition was not assessed. Based on our findings, dietary induced changes in energy utilization as observed with changes to body lipid compositions could in turn cause alterations to physiological processes involved in tissue amino acid deposition.

Mortalities

Based on our results there is no evidence that mortality rates were correlated with increasing SBM content in feed. Rather, the declining mortality rates over time would suggest that factors such as initial transport stress, handling stress and/or smolting were more likely causes for the 36 mortalities observed over the course of the study.

Chapter Summary

This study was the final feed trial of an integrated research project at MSU designed to develop a commercially viable practical SBM diet for Atlantic salmon. The main goal of this study was to determine maximum SBM replacement levels for FM in practical diets for Atlantic salmon. High nutrient dense practical diets (55% CP, 20% CF), containing graded levels of SBM were fed in triplicate to one-year old Atlantic salmon for a period of 12 weeks. A control diet of 0% SBM and 30% FM was compared to test diets containing 5, 20, 25, 30% SBM and 30% FM. In two additional diets (20 and 30% SBM), the level of fish meal was reduced by 20% to that of the control. At the end of the trial, Atlantic salmon receiving the control and the 30% SBM/reduced FM diets were continued on their respective diets for 10 additional weeks to assess SBM effects on long term muscle tissue amino acid compositions.

Study results indicated no differences in growth, protein or energy retention, feed conversion or large intestinal TA for Atlantic salmon fed HND diets. Negative linear functional responses were observed between SBM content, body lipid composition and fecal dry matter. Changes were also observed in muscle amino acid composition but no detrimental affects of dietary SBM on Atlantic salmon were found. Results suggest that HND diets containing up to 30% SBM provided adequate energy to facilitate protein sparing mechanisms in Atlantic salmon; however, SBM induced changes occurred along various metabolic pathways and into muscle tissues.

Chapter 7 – Project Summary, Conclusions and Recommendations

Project Summary

This study was part of an integrated research project designed to develop a commercially acceptable SBM based formulated feed for Atlantic salmon. This research was conducted to test the following hypothesis:

> Based on literature review and recent unpublished study data the maximum level of SBM incorporation into formulated diets for Atlantic salmon is expected to be in the range of 20-30% wet ingredient weight without adverse affects on growth, feed efficiency, and/or other observable fish health characteristics.

Results from trypsin inhibitor feed trials indicated that SBTIs of 60% SBM equivalency had no significant affect on growth or feed efficiency. Slight changes were observed in short term trypsin concentrations and possibly long term body compositions. Data provided evidence of ability by Atlantic salmon to compensate for up to 60% SBM equivalency TI levels in semi-purified diets; however, questions remain as to whether such mechanisms would be indicative of signs of nutritional deficiencies.

Nutrient requirements of Atlantic salmon were reviewed and incorporated into a diet formulation model developed specifically for that species. Using this model optimally balanced HND diets containing SBM were formulated for Atlantic salmon using commercial-based, practical ingredients. Formulations above 30% SBM became increasingly more difficult to maintain proper balances between selected protein, energy, and amino acid requirements. Practical diets containing 0 to 30%SBM were processed

using methods determined optimal for SBM diets for a closely related salmonid species (Barrows et al. 2004). Based on results of the practical feed trial, no single parameter including mortality, growth, protein or energy retention, feed conversion nor TA provided evidence of nutritional induced stressors. Moreover, SGR was considered very good and slightly improved over the SBTI growth trial. Mortalities were highest for the 0%SBM control and regressed as the smolts appeared to complete physiological transformation. No mortalities were observed over the final 10 weeks of a 22 week combined study. Changes in fecal dry matter and body lipid retention were observed in direct response to the level of SBM in the diets. Modifications in long term amino acid compositions were also observed. Effects of HND SBM diets appeared to be a function of physiological protein sparing mechanisms, and results provided little evidence that the diets were nutritionally deficient.

Conclusions

Based on the results of this research, the following conclusions were made:

1. Purified soybean TIs in experimental diets for Atlantic salmon had no effect on growth to a TI level of 60% SBM equivalency.
2. Slight effects of dietary purified TIs were observed in body composition and trypsin enzyme activity. These effects may be due to physiological compensation factors by Atlantic salmon.

3. Trypsin inhibitors in practical Atlantic salmon diets containing 30% SBM are sufficiently reduced or destroyed by heating processes in SBM and diet manufacturing methods.
4. Atlantic salmon fed HND practical diets containing 30% SBM 24% FM had similar growth and feed efficiency rates as those fed the open formula control diet MNR-98HS.
5. High nutrient dense diets containing up to 30% SBM provided adequate energy to facilitate protein sparing mechanisms in Atlantic salmon, however, SBM induced changes occurred along various metabolic pathways and into muscle tissues.
6. Observations from this research suggest that, at least on a short term basis (example: last several weeks of grow-out), HND diets containing 30% SBM 24% FM may be commercially viable for Atlantic salmon production providing a slightly leaner fish is a desirable product by consumers.

Recommendations

Further testing of the practical diets developed within this study is recommended on Atlantic salmon and other salmonids prior to commercial use. Although no signs of detrimental health were observed at inclusions of 60% SBM equivalency TI (3.9 gTI/kg) in experimental diets, or 30% SBM in practical diets, additional feed trials should include distal intestine histology, plasma glucose and glycogen assays, and examine SBM effects on endocrine hormonal controls. Use of HND diets may be a key factor in overcoming SBM anti-nutritional factors observed on salmonids. Relationships between dietary

energy, lipid and carbohydrate utilization, and protein sparing mechanisms by Atlantic salmon should be further explored.

APPENDIX

APPENDIX - Diet Formulation Model Data

Ingredient Nutrient Content

Ingredients	DM (%)	DP (%)	DE (Kcal/kg)	CP (%)	CF (%)	C fiber (%)	C ash (%)
Wheat gluten meal	91	100	4853	80.10	1.50	0.50	0.70
SBM	93	88.3	3250	50.00	0.90	3.40	5.80
Anchovy meal	93	88.5	4828	70.70	5.30	1.00	16.90
Blood Meal	93	69	3406	89.20	0.74	1.00	2.30
Poultry by-product meal	93	68	3964	59.70	13.60	2.10	14.50
Whey	93	87.8	2784	13.30	0.60	0.20	9.20
Brewers yeast	93	78.8	3522	42.60	1.00	3.20	6.60
Corn gluten meal	91	89.7	4260	60.40	1.80	1.50	2.10
Lysine.HCL							
Vitamin premix (0.3%)							
Mineral premix (0.2%)							
Fish oil			9020		100		
Methionine							
DiCalcium Phosphate							
Choline Chloride							
Stay-C							

APPENDIX - Diet Formulation Model Data

Ingredient Nutrient Content

Ingredients	Arg (%)	His (%)	Ile (%)	Leu (%)	Lys (%)	Met (%)	Cys (%)	Phe (%)	Tyr (%)	Thr (%)	Trp (%)	Val (%)
Wheat gluten meal	3.04	1.60	2.96	5.05	1.20	1.28	2.08	3.60		1.28	0.84	3.20
SBM	3.67	1.22	2.14	3.63	3.08	0.68	0.75	2.44	1.76	1.89	0.69	2.55
Anchovy meal	3.85	1.61	3.17	5.05	5.04	1.99	0.6	2.78	2.24	2.82	0.75	3.5
Blood Meal	3.75	5.14	0.97	10.82	7.45	1.08	1.24	5.92	2.55	3.76	1.04	7.48
Poultry by-product meal	4.06	1.09	2.3	4.11	3.06	1.1	0.84	2.1	1.87	0.94	0.46	2.86
Whey	0.34		0.79	1.18	0.94	0.19	0.3	0.35	0.25	0.9	0.18	
Brewers yeast	2.25	1.09	1.98	2.85	2.97	0.67	0.49	1.62	1.5	2.04	0.52	2.36
Corn gluten meal	2.02	1.31	2.54	10.2	1.11	1.63	1.2	3.96	3.32	2.07	0.43	3.09
Lysine.HCL					100							
Vitamin premix (0.3%)												
Mineral premix (0.2%)												
Fish oil												
Methionine						100						
DiCalcium Phosphate												
Choline Chloride												
Stay-C												

APPENDIX - Diet Formulation Model Data

Ingredient Nutrient Content

Ingredients	Ca (%)	Phos. (%)	Pot (%)	Chlorine (%)	Mg (%)	Na (%)	Su (%)	Cop (mg/kg)	Fe (mg/kg)	Magn. (mg/kg)
Wheat gluten meal	0.22	0.1	1.25							
SBM	0.26	0.64	2.13	0.04	0.3	0.01	0.44	20.3	131	37.2
Anchovy meal	3.73	2.43	0.9	1	0.24	1.1	0.54	9.03	220	9.5
Blood Meal	0.41	0.3	0.15	0.25	0.15	0.38	0.34	8.2	2769	6.4
Poultry by-product meal	3.51	1.83	0.39	0.54	0.18	0.82	0.52	14.12	442	11
Whey	0.63	0.68	2.43	3.67	0.18	0.82		5	260	2.7
Brewers yeast	0.14	1.36	1.69	0.07	0.24	0.07	0.43	38.4	109	6.7
Corn gluten meal	0.07	0.44	0.19	0.07	0.07	0.05	0.57	26.1	229	6.3
Lysine.HCL										
Vitamin premix (0.3%)										
Mineral premix (0.2%)								2	3	70
Fish oil										
Methionine										
DiCalcium Phosphate		22								
Choline Chloride										
Stay-C										

APPENDIX - Diet Formulation Model Data

Ingredient Nutrient Content

Ingredients	Se (mg/kg)	Zn (mg/kg)	Biotin (mg/kg)	Choline (mg/kg)	Folacin (mg/kg)	Niacin (mg/kg)	Panta-Acid (mg/kg)	Pyrdox (mg/kg)	Ribo (mg/kg)	Thia (mg/kg)	B12 (mg/kg)	E (mg/kg)	K (mg/kg)
Wheat gluten meal													
SBM	0.1	57	0.32	2753	0.7	22	14.8	4.9	2.9	3.1		3.3	
Anchovy meal	1.36	103	0.23	4408	0.2	100	15	4.64	7.1	0.1	352	5	
Blood Meal		306	0.28	600	0.4	22	3.2	4.45	2.9	0.3	13		
Poultry by-product meal	0.78	121	0.09	6029	0.51	47	11.1	4.41	10.5	0.2	301.2	2.2	
Whey		6.5	0.24	1840			42.25				0.016		
Brewers yeast	0.91	39	1.04	3847	9.7	443	110.7	37.1	34.1	85.2	1	2.1	
Corn gluten meal	0.83	31	0.19	352	0.3	60	3.5	6.9	2	0.3		23.4	
Lysine.HCL													
Vitamin premix (0.3%)			1		13.2	330	158.4	46.2	79.2	52.8	30		16.5
Mineral premix (0.2%)		100											
Fish oil													
Methionine													
DiCalcium Phosphate													
Choline Chloride													
Stay-C													

APPENDIX - Diet Formulation Model Data

Diet Formulations

MNR-98HS

Ingredients	Amount g/kg	CP (%)	DP (%)	DE (kcal)	CF (%)	Cfiber (%)	Cash (%)
Wheat gluten meal	0	0	0	0	0	0	0
SBM	0	0	0	0	0	0	0
Fish meal	300	21.21	18.77	1448.40	1.59	0.30	5.07
Blood Meal	70	6.24	4.31	238.42	0.05	0.07	0.16
Poultry by-product meal	60	3.58	2.44	237.84	0.82	0.13	0.87
Whey	90	1.20	1.05	250.56	0.05	0.02	0.83
Brewers yeast	50	2.13	1.68	176.10	0.05	0.16	0.33
Corn gluten meal	250	15.10	13.54	1065.00	0.45	0.38	0.53
Lysine.HCL	5	0	0	0	0	0	0
Vitamin premix	10	0	0	0	0	0	0
Mineral premix	5	0	0	0	0	0	0
Fish oil	160	0	0	1443.12	16	0	0
Methionine	0						
DiCalcium Phosphate	0						
Choline Chloride	0						
Stay-C	0						
Sum	1000.00	49.46	41.79	4859.44	19.01	1.05	7.78
Req							

DE (MJ/kg)	20.35
DP:DE	20.54
Lys:Arg	1.40
Crude protein (%)	49.46
Crude fat (%)	19.01
Crude fiber (%)	1.05
Crude ash (%)	7.78
Met + Cys(%)	1.87

APPENDIX - Diet Formulation Model Data

MNR-98HS **Diet Formulation**

Ingredients	Arg (%)	His (%)	Ile (%)	Leu (%)	Lys (%)	Met (%)	Cys (%)	Phe (%)	Tyr (%)	Thr (%)	Trp (%)	Val (%)
Wheat gluten meal	0	0	0	0	0	0	0	0	0	0	0	0
SBM	0	0	0	0	0	0	0	0	0	0	0	0
Fish meal	1.16	0.48	0.95	1.52	1.51	0.60	0.18	0.83	0.67	0.85	0.23	1.05
Blood Meal	0.26	0.36	0.07	0.76	0.52	0.08	0.09	0.41	0.18	0.26	0.07	0.52
Poultry by-product meal	0.24	0.07	0.14	0.25	0.18	0.07	0.05	0.13	0.11	0.06	0.03	0.17
Whey	0.03	0.00	0.07	0.11	0.08	0.02	0.03	0.03	0.02	0.08	0.02	0.00
Brewers yeast	0.11	0.05	0.10	0.14	0.15	0.03	0.02	0.08	0.08	0.10	0.03	0.12
Corn gluten meal	0.51	0.33	0.64	2.55	0.28	0.41	0.30	0.99	0.83	0.52	0.11	0.77
Lysine.HCL	0	0	0	0	0.5	0	0	0	0	0	0	0
Vitamin premix	0	0	0	0	0	0	0	0	0	0	0	0
Mineral premix	0	0	0	0	0	0	0	0	0	0	0	0
Fish oil	0	0	0	0	0	0	0	0	0	0	0	0
Methionine						0						
DiCalcium Phosphate												
Choline Chloride												
Stay-C												
Sum	2.31	1.29	1.96	5.32	3.23	1.20	0.67	2.48	1.89	1.87	0.48	2.64
Req	2.3	0.9	1.6	2.6	2.1	1.55		2.9		1.6	0.204	1.95
x - met BM below min	x	x	x	x	x	x		x	x	x	x	x

Requirement for Met = Met + Cys
Requirement for Phe = Phe + Tyr

APPENDIX - Diet Formulation Model Data

MNR-98HS **Diet Formulation**

Ingredients	Ca (%)	Phos. (%)	Pot (%)	Chlorine (%)	Mg (%)	Na (%)	Su (%)	Cop (mg/kg)	Fe (mg/kg)	Magn. (mg/kg)
Wheat gluten meal	0	0	0	0	0	0	0	0	0	0
SBM	0	0	0	0	0	0	0	0	0	0
Fish meal	1.12	0.73	0.27	0.30	0.07	0.33	0.16	2.71	66.00	2.85
Blood Meal	0.03	0.02	0.01	0.02	0.01	0.03	0.02	0.57	193.83	0.45
Poultry by-product meal	0.21	0.11	0.02	0.03	0.01	0.05	0.03	0.85	26.52	0.66
Whey	0.06	0.06	0.22	0.33	0.02	0.07	0.00	0.45	23.40	0.24
Brewers yeast	0.01	0.07	0.08	0.00	0.01	0.00	0.02	1.92	5.45	0.34
Corn gluten meal	0.02	0.11	0.05	0.02	0.02	0.01	0.14	6.53	57.25	1.58
Lysine.HCL	0	0	0	0	0	0	0	0	0	0
Vitamin premix	0	0	0	0	0	0	0	0	0	0
Mineral premix	0	0	0	0	0	0	0	2	3	70
Fish oil	0	0	0	0	0	0	0	0	0	0
Methionine										
DiCalcium Phosphate		0								
Choline Chloride										
Stay-C										
Sum	1.44	1.10	0.65	0.70	0.14	0.50	0.38	15.03	375.45	76.11
Req	1	0.6	0.8	0	0.04	0.6	0	5	60	10
x - met BM below min	x	x	BM	x	x	BM	x	x	x	x

APPENDIX - Diet Formulation Model Data

MNR-98HS **Diet Formulation**

Ingredients	Se (mg/kg)	Zn (mg/kg)	Biotin (mg/kg)	Choline (mg/kg)	Folacin (mg/kg)	Niacin (mg/kg)	Panta-Acid (mg/kg)	Pyrdox (mg/kg)	Ribo (mg/kg)	Thia (mg/kg)	B12 (mg/kg)	E (mg/kg)	K (mg/kg)
Wheat gluten meal	0	0	0	0	0	0	0	0	0	0	0	0	0
SBM	0	0	0	0	0	0	0	0	0	0	0	0	0
Fish meal	0.41	30.90	0.07	1322.40	0.06	30.00	4.50	1.39	2.13	0.03	105.60	1.50	0
Blood Meal	0.00	21.42	0.02	42.00	0.03	1.54	0.22	0.31	0.20	0.02	0.91	0.00	0
Poultry by-product meal	0.05	7.26	0.01	361.74	0.03	2.82	0.67	0.26	0.63	0.01	18.07	0.13	0
Whey	0.00	0.59	0.02	165.60	0.00	0.00	3.80	0.00	0.00	0.00	0.00	0.00	0
Brewers yeast	0.05	1.95	0.05	192.35	0.49	22.15	5.54	1.86	1.71	4.26	0.05	0.11	0
Corn gluten meal	0.21	7.75	0.05	88.00	0.08	15.00	0.88	1.73	0.50	0.08	0.00	5.85	0
Lysine.HCL	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitamin premix	0.01	0	1	3.3	13.2	330	158.4	46.2	79.2	52.8	30	0	16.5
Mineral premix	0	100	0	0	0	0	0	0	0	0	0	0	0
Fish oil	0	0	0	0	0	0	0	0	0	0	0	0	0
Methionine													
DiCalcium Phosphate													
Choline Chloride													
Stay-C													
Sum	0.72	169.87	1.22	2175.39	13.88	401.51	174.00	51.75	84.37	57.20	154.63	7.59	16.50
Req	0.3	67	1.2	480	12	180	60	18	24	18	0.024	0	12
x - met BM below min	x	x	x	x	x	x	x	x	x	x	x	x	x

APPENDIX - Diet Formulation Model Data

SB5/F30 **Diet Formulation**

Ingredients	Amount g/kg	CP (%)	DP (%)	DE (kcal)	CF (%)	Cfiber (%)	Cash (%)
Wheat gluten meal	70	5.61	5.61	339.68	0.11	0.04	0.05
SBM	50	2.50	2.21	162.50	0.05	0.17	0.29
Fish meal	300	21.21	18.77	1448.40	1.59	0.30	5.07
Blood Meal	70	6.24	4.31	238.42	0.05	0.07	0.16
Poultry by-product meal	60	3.58	2.44	237.84	0.82	0.13	0.87
Whey	90	1.20	1.05	250.56	0.05	0.02	0.83
Brewers yeast	50	2.13	1.68	176.10	0.05	0.16	0.33
Corn gluten meal	143	8.64	7.75	609.18	0.26	0.21	0.30
Lysine.HCL	0	0	0	0	0	0	0
Vitamin premix	3	0	0	0	0	0	0
Mineral premix	2	0	0	0	0	0	0
Fish oil	151.4	0	0	1365.55	15.14	0	0
Methionine	0						
DiCalcium Phosphate	1.6						
Choline Chloride	6						
Stay-C	3						
Sum	1000.00	51.11	43.81	4828.24	18.11	1.09	7.90
Req							

DE (MJ/kg)	20.21
DP:DE	21.67
Lys:Arg	1.14
Crude protein (%)	51.11
Crude fat (%)	18.11
Crude fiber (%)	1.09
Crude ash (%)	7.90
Met + Cys(%)	1.87

APPENDIX - Diet Formulation Model Data

SB5/F30 **Diet Formulation**

Ingredients	Arg (%)	His (%)	Ile (%)	Leu (%)	Lys (%)	Met (%)	Cys (%)	Phe (%)	Tyr (%)	Thr (%)	Trp (%)	Val (%)
Wheat gluten meal	0.21	0.11	0.21	0.35	0.08	0.09	0.15	0.25	0.00	0.09	0.06	0.22
SBM	0.18	0.06	0.11	0.18	0.15	0.03	0.04	0.12	0.09	0.09	0.03	0.13
Fish meal	1.16	0.48	0.95	1.52	1.51	0.60	0.18	0.83	0.67	0.85	0.23	1.05
Blood Meal	0.26	0.36	0.07	0.76	0.52	0.08	0.09	0.41	0.18	0.26	0.07	0.52
Poultry by-product meal	0.24	0.07	0.14	0.25	0.18	0.07	0.05	0.13	0.11	0.06	0.03	0.17
Whey	0.03	0.00	0.07	0.11	0.08	0.02	0.03	0.03	0.02	0.08	0.02	0.00
Brewers yeast	0.11	0.05	0.10	0.14	0.15	0.03	0.02	0.08	0.08	0.10	0.03	0.12
Corn gluten meal	0.29	0.19	0.36	1.46	0.16	0.23	0.17	0.57	0.47	0.30	0.06	0.44
Lysine.HCL	0	0	0	0	0	0	0	0	0	0	0	0
Vitamin premix	0	0	0	0	0	0	0	0	0	0	0	0
Mineral premix	0	0	0	0	0	0	0	0	0	0	0	0
Fish oil	0	0	0	0	0	0	0	0	0	0	0	0
Methionine						0						
DiCalcium Phosphate												
Choline Chloride												
Stay-C												
Sum	2.49	1.32	2.00	4.76	2.85	1.15	0.72	2.43	1.62	1.83	0.52	2.66
Req	2.3	0.9	1.6	2.6	2.1	1.55		2.9		1.6	0.204	1.95
x - met BM below min	x	x	x	x	x	x		x	x	x	x	x

Requirement for Met = Met + Cys
Requirement for Phe = Phe + Tyr

APPENDIX - Diet Formulation Model Data

SB5/F30 **Diet Formulation**

Ingredients	Ca (%)	Phos. (%)	Pot (%)	Chlorine (%)	Mg (%)	Na (%)	Su (%)	Cop (mg/kg)	Fe (mg/kg)	Magn. (mg/kg)
Wheat gluten meal	0.02	0.01	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SBM	0.01	0.03	0.11	0.00	0.02	0.00	0.02	1.02	6.55	1.86
Fish meal	1.12	0.73	0.27	0.30	0.07	0.33	0.16	2.71	66.00	2.85
Blood Meal	0.03	0.02	0.01	0.02	0.01	0.03	0.02	0.57	193.83	0.45
Poultry by-product meal	0.21	0.11	0.02	0.03	0.01	0.05	0.03	0.85	26.52	0.66
Whey	0.06	0.06	0.22	0.33	0.02	0.07	0.00	0.45	23.40	0.24
Brewers yeast	0.01	0.07	0.08	0.00	0.01	0.00	0.02	1.92	5.45	0.34
Corn gluten meal	0.01	0.06	0.03	0.01	0.01	0.01	0.08	3.73	32.75	0.90
Lysine.HCL	0	0	0	0	0	0	0	0	0	0
Vitamin premix	0	0	0	0	0	0	0	0	0	0
Mineral premix	0	0	0	0	0	0	0	2	3	70
Fish oil	0	0	0	0	0	0	0	0	0	0
Methionine										
DiCalcium Phosphate		0.0352								
Choline Chloride										
Stay-C										
Sum	1.46	1.13	0.83	0.70	0.15	0.49	0.34	13.25	357.50	77.30
Req	1	0.6	0.8	0	0.04	0.6	0	5	60	10
x - met BM below min	x	x	x	x	x	BM	x	x	x	x

APPENDIX - Diet Formulation Model Data

SB5/F30 **Diet Formulation**

Ingredients	Se (mg/kg)	Zn (mg/kg)	Biotin (mg/kg)	Choline (mg/kg)	Folacin (mg/kg)	Niacin (mg/kg)	Panta-Acid (mg/kg)	Pyrdox (mg/kg)	Ribo (mg/kg)	Thia (mg/kg)	B12 (mg/kg)	E (mg/kg)	K (mg/kg)
Wheat gluten meal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
SBM	0.01	2.85	0.02	137.65	0.04	1.10	0.74	0.25	0.15	0.16	0.00	0.17	0
Fish meal	0.41	30.90	0.07	1322.40	0.06	30.00	4.50	1.39	2.13	0.03	105.60	1.50	0
Blood Meal	0.00	21.42	0.02	42.00	0.03	1.54	0.22	0.31	0.20	0.02	0.91	0.00	0
Poultry by-product meal	0.05	7.26	0.01	361.74	0.03	2.82	0.67	0.26	0.63	0.01	18.07	0.13	0
Whey	0.00	0.59	0.02	165.60	0.00	0.00	3.80	0.00	0.00	0.00	0.00	0.00	0
Brewers yeast	0.05	1.95	0.05	192.35	0.49	22.15	5.54	1.86	1.71	4.26	0.05	0.11	0
Corn gluten meal	0.12	4.43	0.03	50.34	0.04	8.58	0.50	0.99	0.29	0.04	0.00	3.35	0
Lysine.HCL	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitamin premix	0.01	0	1	3.3	13.2	330	158.4	46.2	79.2	52.8	30	0	16.5
Mineral premix	0	100	0	0	0	0	0	0	0	0	0	0	0
Fish oil	0	0	0	0	0	0	0	0	0	0	0	0	0
Methionine													
DiCalcium Phosphate													
Choline Chloride													
Stay-C													
Sum	0.63	169.40	1.21	2275.38	13.88	396.19	174.37	51.25	84.30	57.32	154.63	5.25	16.50
Req	0.3	67	1.2	480	12	180	60	18	24	18	0.024	0	12
x - met BM below min	x	x	x	x	x	x	x	x	x	x	x	x	x

APPENDIX - Diet Formulation Model Data

SB20/F30 **Diet Formulation**

Ingredients	Amount g/kg	CP (%)	DP (%)	DE (kcal)	CF (%)	Cfiber (%)	Cash (%)
Wheat gluten meal	70	5.61	5.61	339.68	0.11	0.04	0.05
SBM	200	10.00	8.83	650.00	0.18	0.68	1.16
Fish meal	300	21.21	18.77	1448.40	1.59	0.30	5.07
Blood Meal	59.5	5.31	3.66	202.66	0.04	0.06	0.14
Poultry by-product meal	50	2.99	2.03	198.20	0.68	0.11	0.73
Whey	55	0.73	0.64	153.12	0.03	0.01	0.51
Brewers yeast	30	1.28	1.01	105.66	0.03	0.10	0.20
Corn gluten meal	59.2	3.58	3.21	252.19	0.11	0.09	0.12
Lysine.HCL	0.7	0	0	0	0	0	0
Vitamin premix	3	0	0	0	0	0	0
Mineral premix	2	0	0	0	0	0	0
Fish oil	158	0	0	1425.08	15.8	0	0
Methionine	1.1						
DiCalcium Phosphate	2.5						
Choline Chloride	6						
Stay-C	3						
Sum	1000.00	50.69	43.76	4774.99	18.57	1.38	7.97
Req							

DE (MJ/kg)	19.99
DP:DE	21.89
Lys:Arg	1.13
Crude protein (%)	50.69
Crude fat (%)	18.57
Crude fiber (%)	1.38
Crude ash (%)	7.97
Met + Cys(%)	1.87

APPENDIX - Diet Formulation Model Data

SB20/F30 **Diet Formulation**

Ingredients	Arg (%)	His (%)	Ile (%)	Leu (%)	Lys (%)	Met (%)	Cys (%)	Phe (%)	Tyr (%)	Thr (%)	Trp (%)	Val (%)
Wheat gluten meal	0.21	0.11	0.21	0.35	0.08	0.09	0.15	0.25	0.00	0.09	0.06	0.22
SBM	0.73	0.24	0.43	0.73	0.62	0.14	0.15	0.49	0.35	0.38	0.14	0.51
Fish meal	1.16	0.48	0.95	1.52	1.51	0.60	0.18	0.83	0.67	0.85	0.23	1.05
Blood Meal	0.22	0.31	0.06	0.64	0.44	0.06	0.07	0.35	0.15	0.22	0.06	0.45
Poultry by-product meal	0.20	0.05	0.12	0.21	0.15	0.06	0.04	0.11	0.09	0.05	0.02	0.14
Whey	0.02	0.00	0.04	0.06	0.05	0.01	0.02	0.02	0.01	0.05	0.01	0.00
Brewers yeast	0.07	0.03	0.06	0.09	0.09	0.02	0.01	0.05	0.05	0.06	0.02	0.07
Corn gluten meal	0.12	0.08	0.15	0.60	0.07	0.10	0.07	0.23	0.20	0.12	0.03	0.18
Lysine.HCL	0	0	0	0	0.07	0	0	0	0	0	0	0
Vitamin premix	0	0	0	0	0	0	0	0	0	0	0	0
Mineral premix	0	0	0	0	0	0	0	0	0	0	0	0
Fish oil	0	0	0	0	0	0	0	0	0	0	0	0
Methionine						0.11						
DiCalcium Phosphate												
Choline Chloride												
Stay-C												
Sum	2.73	1.31	2.01	4.20	3.08	1.18	0.69	2.33	1.52	1.82	0.56	2.63
Req	2.3	0.9	1.6	2.6	2.1	1.55		2.9		1.6	0.204	1.95
x - met BM below min	x	x	x	x	x	x	x	x	x	x	x	x

Requirement for Met = Met + Cys
Requirement for Phe = Phe + Tyr

APPENDIX - Diet Formulation Model Data

SB20/F30 **Diet Formulation**

Ingredients	Ca (%)	Phos. (%)	Pot (%)	Chlorine (%)	Mg (%)	Na (%)	Su (%)	Cop (mg/kg)	Fe (mg/kg)	Magn. (mg/kg)
Wheat gluten meal	0.02	0.01	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SBM	0.05	0.13	0.43	0.01	0.06	0.00	0.09	4.06	26.20	7.44
Fish meal	1.12	0.73	0.27	0.30	0.07	0.33	0.16	2.71	66.00	2.85
Blood Meal	0.02	0.02	0.01	0.01	0.01	0.02	0.02	0.49	164.76	0.38
Poultry by-product meal	0.18	0.09	0.02	0.03	0.01	0.04	0.03	0.71	22.10	0.55
Whey	0.03	0.04	0.13	0.20	0.01	0.05	0.00	0.28	14.30	0.15
Brewers yeast	0.00	0.04	0.05	0.00	0.01	0.00	0.01	1.15	3.27	0.20
Corn gluten meal	0.00	0.03	0.01	0.00	0.00	0.00	0.03	1.55	13.56	0.37
Lysine.HCL	0	0	0	0	0	0	0	0	0	0
Vitamin premix	0	0	0	0	0	0	0	0	0	0
Mineral premix	0	0	0	0	0	0	0	2	3	70
Fish oil	0	0	0	0	0	0	0	0	0	0
Methionine										
DiCalcium Phosphate		0.055								
Choline Chloride										
Stay-C										
Sum	1.43	1.13	1.01	0.56	0.17	0.45	0.34	12.94	313.18	81.94
Req	1	0.6	0.8	0	0.04	0.6	0	5	60	10
x - met BM below min	x	x	x	x	x	BM	x	x	x	x

APPENDIX - Diet Formulation Model Data

SB20/F30 **Diet Formulation**

Ingredients	Se (mg/kg)	Zn (mg/kg)	Biotin (mg/kg)	Choline (mg/kg)	Folacin (mg/kg)	Niacin (mg/kg)	Panta-Acid (mg/kg)	Pyrdox (mg/kg)	Ribo (mg/kg)	Thia (mg/kg)	B12 (mg/kg)	E (mg/kg)	K (mg/kg)
Wheat gluten meal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
SBM	0.02	11.40	0.06	550.60	0.14	4.40	2.96	0.98	0.58	0.62	0.00	0.66	0
Fish meal	0.41	30.90	0.07	1322.40	0.06	30.00	4.50	1.39	2.13	0.03	105.60	1.50	0
Blood Meal	0.00	18.21	0.02	35.70	0.02	1.31	0.19	0.26	0.17	0.02	0.77	0.00	0
Poultry by-product meal	0.04	6.05	0.00	301.45	0.03	2.35	0.56	0.22	0.53	0.01	15.06	0.11	0
Whey	0.00	0.36	0.01	101.20	0.00	0.00	2.32	0.00	0.00	0.00	0.00	0.00	0
Brewers yeast	0.03	1.17	0.03	115.41	0.29	13.29	3.32	1.11	1.02	2.56	0.03	0.06	0
Corn gluten meal	0.05	1.84	0.01	20.84	0.02	3.55	0.21	0.41	0.12	0.02	0.00	1.39	0
Lysine.HCL	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitamin premix	0.01	0	1	3.3	13.2	330	158.4	46.2	79.2	52.8	30	0	16.5
Mineral premix	0	100	0	0	0	0	0	0	0	0	0	0	0
Fish oil	0	0	0	0	0	0	0	0	0	0	0	0	0
Methionine													
DiCalcium Phosphate													
Choline Chloride													
Stay-C													
Sum	0.55	169.92	1.21	2450.90	13.76	384.90	172.46	50.58	83.75	56.05	151.46	3.72	16.50
Req	0.3	67	1.2	480	12	180	60	18	24	18	0.024	0	12
x - met BM below min	x	x	x	x	x	x	x	x	x	x	x	x	x

APPENDIX - Diet Formulation Model Data

SB20/F24 **Diet Formulation**

Ingredients	Amount g/kg	CP (%)	DP (%)	DE (kcal)	CF (%)	Cfiber (%)	Cash (%)
Wheat gluten meal	70	5.61	5.61	339.68	0.11	0.04	0.05
SBM	200	10.00	8.83	650.00	0.18	0.68	1.16
Fish meal	240	16.97	15.02	1158.72	1.27	0.24	4.06
Blood Meal	57	5.08	3.51	194.14	0.04	0.06	0.13
Poultry by-product meal	49	2.93	1.99	194.24	0.67	0.10	0.71
Whey	41	0.55	0.48	114.14	0.02	0.01	0.38
Brewers yeast	22.9	0.98	0.77	80.65	0.02	0.07	0.15
Corn gluten meal	135	8.15	7.31	575.10	0.24	0.20	0.28
Lysine.HCL	1.6	0	0	0	0	0	0
Vitamin premix	3	0	0	0	0	0	0
Mineral premix	2.4	0	0	0	0	0	0
Fish oil	161.9	0	0	1460.26	16.19	0	0
Methionine	0.8						
DiCalcium Phosphate	6.4						
Choline Chloride	6						
Stay-C	3						
Sum	1000.00	50.26	43.51	4766.94	18.75	1.40	6.92
Req							

DE (MJ/kg)	19.96
DP:DE	21.80
Lys:Arg	1.11
Crude protein (%)	50.26
Crude fat (%)	18.75
Crude fiber (%)	1.40
Crude ash (%)	6.92
Met + Cys(%)	1.88

APPENDIX - Diet Formulation Model Data

SB20/F24 **Diet Formulation**

Ingredients	Arg (%)	His (%)	Ile (%)	Leu (%)	Lys (%)	Met (%)	Cys (%)	Phe (%)	Tyr (%)	Thr (%)	Trp (%)	Val (%)
Wheat gluten meal	0.21	0.11	0.21	0.35	0.08	0.09	0.15	0.25	0.00	0.09	0.06	0.22
SBM	0.73	0.24	0.43	0.73	0.62	0.14	0.15	0.49	0.35	0.38	0.14	0.51
Fish meal	0.92	0.39	0.76	1.21	1.21	0.48	0.14	0.67	0.54	0.68	0.18	0.84
Blood Meal	0.21	0.29	0.06	0.62	0.42	0.06	0.07	0.34	0.15	0.21	0.06	0.43
Poultry by-product meal	0.20	0.05	0.11	0.20	0.15	0.05	0.04	0.10	0.09	0.05	0.02	0.14
Whey	0.01	0.00	0.03	0.05	0.04	0.01	0.01	0.01	0.01	0.04	0.01	0.00
Brewers yeast	0.05	0.02	0.05	0.07	0.07	0.02	0.01	0.04	0.03	0.05	0.01	0.05
Corn gluten meal	0.27	0.18	0.34	1.38	0.15	0.22	0.16	0.53	0.45	0.28	0.06	0.42
Lysine.HCL	0	0	0	0	0.16	0	0	0	0	0	0	0
Vitamin premix	0	0	0	0	0	0	0	0	0	0	0	0
Mineral premix	0	0	0	0	0	0	0	0	0	0	0	0
Fish oil	0	0	0	0	0	0	0	0	0	0	0	0
Methionine						0.08						
DiCalcium Phosphate												
Choline Chloride												
Stay-C												
Sum	2.62	1.29	1.98	4.60	2.90	1.14	0.74	2.43	1.62	1.77	0.54	2.61
Req	2.3	0.9	1.6	2.6	2.1	1.55		2.9		1.6	0.204	1.95
x - met BM below min	x	x	x	x	x	x	x	x	x	x	x	x

Requirement for Met = Met + Cys
Requirement for Phe = Phe + Tyr

APPENDIX - Diet Formulation Model Data

SB20/F24 **Diet Formulation**

Ingredients	Ca (%)	Phos. (%)	Pot (%)	Chlorine (%)	Mg (%)	Na (%)	Su (%)	Cop (mg/kg)	Fe (mg/kg)	Magn. (mg/kg)
Wheat gluten meal	0.02	0.01	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SBM	0.05	0.13	0.43	0.01	0.06	0.00	0.09	4.06	26.20	7.44
Fish meal	0.90	0.58	0.22	0.24	0.06	0.26	0.13	2.17	52.80	2.28
Blood Meal	0.02	0.02	0.01	0.01	0.01	0.02	0.02	0.47	157.83	0.36
Poultry by-product meal	0.17	0.09	0.02	0.03	0.01	0.04	0.03	0.69	21.66	0.54
Whey	0.03	0.03	0.10	0.15	0.01	0.03	0.00	0.21	10.66	0.11
Brewers yeast	0.00	0.03	0.04	0.00	0.01	0.00	0.01	0.88	2.50	0.15
Corn gluten meal	0.01	0.06	0.03	0.01	0.01	0.01	0.08	3.52	30.92	0.85
Lysine.HCL	0	0	0	0	0	0	0	0	0	0
Vitamin premix	0	0	0	0	0	0	0	0	0	0
Mineral premix	0	0	0	0	0	0	0	2.4	3.6	84
Fish oil	0	0	0	0	0	0	0	0	0	0
Methionine										
DiCalcium Phosphate		0.1408								
Choline Chloride										
Stay-C										
Sum	1.20	1.08	0.92	0.45	0.16	0.37	0.35	14.39	306.16	95.74
Req	1	0.6	0.8	0	0.04	0.6	0	5	60	10
x - met BM below min	x	x	x	x	x	BM	x	x	x	x

APPENDIX - Diet Formulation Model Data

SB20/F24 **Diet Formulation**

Ingredients	Se (mg/kg)	Zn (mg/kg)	Biotin (mg/kg)	Choline (mg/kg)	Folacin (mg/kg)	Niacin (mg/kg)	Panta-Acid (mg/kg)	Pyrdox (mg/kg)	Ribo (mg/kg)	Thia (mg/kg)	B12 (mg/kg)	E (mg/kg)	K (mg/kg)
Wheat gluten meal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
SBM	0.02	11.40	0.06	550.60	0.14	4.40	2.96	0.98	0.58	0.62	0.00	0.66	0
Fish meal	0.33	24.72	0.06	1057.92	0.05	24.00	3.60	1.11	1.70	0.02	84.48	1.20	0
Blood Meal	0.00	17.44	0.02	34.20	0.02	1.25	0.18	0.25	0.17	0.02	0.74	0.00	0
Poultry by-product meal	0.04	5.93	0.00	295.42	0.02	2.30	0.54	0.22	0.51	0.01	14.76	0.11	0
Whey	0.00	0.27	0.01	75.44	0.00	0.00	1.73	0.00	0.00	0.00	0.00	0.00	0
Brewers yeast	0.02	0.89	0.02	88.10	0.22	10.14	2.54	0.85	0.78	1.95	0.02	0.05	0
Corn gluten meal	0.11	4.19	0.03	47.52	0.04	8.10	0.47	0.93	0.27	0.04	0.00	3.16	0
Lysine.HCL	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitamin premix	0.01	0	1	3.3	13.2	330	158.4	46.2	79.2	52.8	30	0	16.5
Mineral premix	0	120	0	0	0	0	0	0	0	0	0	0	0
Fish oil	0	0	0	0	0	0	0	0	0	0	0	0	0
Methionine													
DiCalcium Phosphate													
Choline Chloride													
Stay-C													
Sum	0.53	184.84	1.20	2152.50	13.70	380.20	170.43	50.54	83.21	55.46	130.00	5.17	16.50
Req	0.3	67	1.2	480	12	180	60	18	24	18	0.024	0	12
x - met BM below min	x	x	BM	x	x	x	x	x	x	x	x	x	x

APPENDIX - Diet Formulation Model Data

SB25/F30 **Diet Formulation**

Ingredients	Amount g/kg	CP (%)	DP (%)	DE (kcal)	CF (%)	Cfiber (%)	Cash (%)
Wheat gluten meal	70	5.61	5.61	339.68	0.11	0.04	0.05
SBM	250	12.50	11.04	812.50	0.23	0.85	1.45
Fish meal	300	21.21	18.77	1448.40	1.59	0.30	5.07
Blood Meal	47.5	4.24	2.92	161.79	0.04	0.05	0.11
Poultry by-product meal	39.4	2.35	1.60	156.18	0.54	0.08	0.57
Whey	44	0.59	0.51	122.50	0.03	0.01	0.40
Brewers yeast	25	1.07	0.84	88.05	0.03	0.08	0.17
Corn gluten meal	43	2.60	2.33	183.18	0.08	0.06	0.09
Lysine.HCL	1	0	0	0	0	0	0
Vitamin premix	3	0	0	0	0	0	0
Mineral premix	2	0	0	0	0	0	0
Fish oil	162	0	0	1461.16	16.2	0	0
Methionine	1.3						
DiCalcium Phosphate	2.8						
Choline Chloride	6						
Stay-C	3						
Sum	1000.00	50.15	43.62	4773.43	18.82	1.47	7.91
Req							

DE (MJ/kg)	19.99
DP:DE	21.83
Lys:Arg	1.12
Crude protein (%)	50.15
Crude fat (%)	18.82
Crude fiber (%)	1.47
Crude ash (%)	7.91
Met + Cys(%)	1.86

APPENDIX - Diet Formulation Model Data

SB25/F30 **Diet Formulation**

Ingredients	Arg (%)	His (%)	Ile (%)	Leu (%)	Lys (%)	Met (%)	Cys (%)	Phe (%)	Tyr (%)	Thr (%)	Trp (%)	Val (%)
Wheat gluten meal	0.21	0.11	0.21	0.35	0.08	0.09	0.15	0.25	0.00	0.09	0.06	0.22
SBM	0.92	0.31	0.54	0.91	0.77	0.17	0.19	0.61	0.44	0.47	0.17	0.64
Fish meal	1.16	0.48	0.95	1.52	1.51	0.60	0.18	0.83	0.67	0.85	0.23	1.05
Blood Meal	0.18	0.24	0.05	0.51	0.35	0.05	0.06	0.28	0.12	0.18	0.05	0.36
Poultry by-product meal	0.16	0.04	0.09	0.16	0.12	0.04	0.03	0.08	0.07	0.04	0.02	0.11
Whey	0.01	0.00	0.03	0.05	0.04	0.01	0.01	0.02	0.01	0.04	0.01	0.00
Brewers yeast	0.06	0.03	0.05	0.07	0.07	0.02	0.01	0.04	0.04	0.05	0.01	0.06
Corn gluten meal	0.09	0.06	0.11	0.44	0.05	0.07	0.05	0.17	0.14	0.09	0.02	0.13
Lysine.HCL	0	0	0	0	0.1	0	0	0	0	0	0	0
Vitamin premix	0	0	0	0	0	0	0	0	0	0	0	0
Mineral premix	0	0	0	0	0	0	0	0	0	0	0	0
Fish oil	0	0	0	0	0	0	0	0	0	0	0	0
Methionine						0.13						
DiCalcium Phosphate												
Choline Chloride												
Stay-C												
Sum	2.78	1.27	2.02	4.01	3.10	1.18	0.68	2.29	1.50	1.80	0.56	2.57
Req	2.3	0.9	1.6	2.6	2.1	1.55		2.9		1.6	0.204	1.95
x - met BM below min	x	x	x	x	x	x	x	x	x	x	x	x

Requirement for Met = Met + Cys
Requirement for Phe = Phe + Tyr

APPENDIX - Diet Formulation Model Data

SB25/F30 **Diet Formulation**

Ingredients	Ca (%)	Phos. (%)	Pot (%)	Chlorine (%)	Mg (%)	Na (%)	Su (%)	Cop (mg/kg)	Fe (mg/kg)	Magn. (mg/kg)
Wheat gluten meal	0.02	0.01	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SBM	0.07	0.16	0.53	0.01	0.08	0.00	0.11	5.08	32.75	9.30
Fish meal	1.12	0.73	0.27	0.30	0.07	0.33	0.16	2.71	66.00	2.85
Blood Meal	0.02	0.01	0.01	0.01	0.01	0.02	0.02	0.39	131.53	0.30
Poultry by-product meal	0.14	0.07	0.02	0.02	0.01	0.03	0.02	0.56	17.41	0.43
Whey	0.03	0.03	0.11	0.16	0.01	0.04	0.00	0.22	11.44	0.12
Brewers yeast	0.00	0.03	0.04	0.00	0.01	0.00	0.01	0.96	2.73	0.17
Corn gluten meal	0.00	0.02	0.01	0.00	0.00	0.00	0.02	1.12	9.85	0.27
Lysine.HCL	0	0	0	0	0	0	0	0	0	0
Vitamin premix	0	0	0	0	0	0	0	0	0	0
Mineral premix	0	0	0	0	0	0	0	2	3	70
Fish oil	0	0	0	0	0	0	0	0	0	0
Methionine										
DiCalcium Phosphate		0.0616								
Choline Chloride										
Stay-C										
Sum	1.39	1.13	1.07	0.51	0.18	0.42	0.34	13.03	274.70	83.44
Req	1	0.6	0.8	0	0.04	0.6	0	5	60	10
x - met BM below min	x	x	x	x	x	BM	x	x	x	x

APPENDIX - Diet Formulation Model Data

SB25/F30 **Diet Formulation**

Ingredients	Se (mg/kg)	Zn (mg/kg)	Biotin (mg/kg)	Choline (mg/kg)	Folacin (mg/kg)	Niacin (mg/kg)	Panta-Acid (mg/kg)	Pyrdox (mg/kg)	Ribo (mg/kg)	Thia (mg/kg)	B12 (mg/kg)	E (mg/kg)	K (mg/kg)
Wheat gluten meal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
SBM	0.03	14.25	0.08	688.25	0.18	5.50	3.70	1.23	0.73	0.78	0.00	0.83	0
Fish meal	0.41	30.90	0.07	1322.40	0.06	30.00	4.50	1.39	2.13	0.03	105.60	1.50	0
Blood Meal	0.00	14.54	0.01	28.50	0.02	1.05	0.15	0.21	0.14	0.01	0.62	0.00	0
Poultry by-product meal	0.03	4.77	0.00	237.54	0.02	1.85	0.44	0.17	0.41	0.01	11.87	0.09	0
Whey	0.00	0.29	0.01	80.96	0.00	0.00	1.86	0.00	0.00	0.00	0.00	0.00	0
Brewers yeast	0.02	0.98	0.03	96.18	0.24	11.08	2.77	0.93	0.85	2.13	0.03	0.05	0
Corn gluten meal	0.04	1.33	0.01	15.14	0.01	2.58	0.15	0.30	0.09	0.01	0.00	1.01	0
Lysine.HCL	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitamin premix	0.01	0	1	3.3	13.2	330	158.4	46.2	79.2	52.8	30	0	16.5
Mineral premix	0	100	0	0	0	0	0	0	0	0	0	0	0
Fish oil	0	0	0	0	0	0	0	0	0	0	0	0	0
Methionine													
DiCalcium Phosphate													
Choline Chloride													
Stay-C													
Sum	0.53	167.05	1.21	2472.26	13.73	382.05	171.97	50.43	83.54	55.77	148.11	3.47	16.50
Req	0.3	67	1.2	480	12	180	60	18	24	18	0.024	0	12
x - met BM below min	x	x	x	x	x	x	x	x	x	x	x	x	x

APPENDIX - Diet Formulation Model Data

SB30/F30 **Diet Formulation**

Ingredients	Amount g/kg	CP (%)	DP (%)	DE (kcal)	CF (%)	Cfiber (%)	Cash (%)
Wheat gluten meal	70	5.61	5.61	339.68	0.11	0.04	0.05
SBM	300	15.00	13.25	975.00	0.27	1.02	1.74
Fish meal	300	21.21	18.77	1448.40	1.59	0.30	5.07
Blood Meal	33	2.94	2.03	112.40	0.02	0.03	0.08
Poultry by-product meal	29	1.73	1.18	114.96	0.39	0.06	0.42
Whey	26.6	0.35	0.31	74.05	0.02	0.01	0.24
Brewers yeast	16	0.68	0.54	56.35	0.02	0.05	0.11
Corn gluten meal	39	2.36	2.11	166.14	0.07	0.06	0.08
Lysine.HCL	2	0	0	0	0	0	0
Vitamin premix	3	0	0	0	0	0	0
Mineral premix	2.2	0	0	0	0	0	0
Fish oil	165	0	0	1488.22	16.5	0	0
Methionine	1.5						
DiCalcium Phosphate	3.7						
Choline Chloride	6						
Stay-C	3						
Sum	1000.00	49.88	43.79	4775.20	18.99	1.56	7.79
Req							

DE (MJ/kg)	19.99
DP:DE	21.90
Lys:Arg	1.12
Crude protein (%)	49.88
Crude fat (%)	18.99
Crude fiber (%)	1.56
Crude ash (%)	7.79
Met + Cys(%)	1.87

APPENDIX - Diet Formulation Model Data

SB30/F30 **Diet Formulation**

Ingredients	Arg (%)	His (%)	Ile (%)	Leu (%)	Lys (%)	Met (%)	Cys (%)	Phe (%)	Tyr (%)	Thr (%)	Trp (%)	Val (%)
Wheat gluten meal	0.21	0.11	0.21	0.35	0.08	0.09	0.15	0.25	0.00	0.09	0.06	0.22
SBM	1.10	0.37	0.64	1.09	0.92	0.20	0.23	0.73	0.53	0.57	0.21	0.77
Fish meal	1.16	0.48	0.95	1.52	1.51	0.60	0.18	0.83	0.67	0.85	0.23	1.05
Blood Meal	0.12	0.17	0.03	0.36	0.25	0.04	0.04	0.20	0.08	0.12	0.03	0.25
Poultry by-product meal	0.12	0.03	0.07	0.12	0.09	0.03	0.02	0.06	0.05	0.03	0.01	0.08
Whey	0.01	0.00	0.02	0.03	0.03	0.01	0.01	0.01	0.01	0.02	0.00	0.00
Brewers yeast	0.04	0.02	0.03	0.05	0.05	0.01	0.01	0.03	0.02	0.03	0.01	0.04
Corn gluten meal	0.08	0.05	0.10	0.40	0.04	0.06	0.05	0.15	0.13	0.08	0.02	0.12
Lysine.HCL	0	0	0	0	0.2	0	0	0	0	0	0	0
Vitamin premix	0	0	0	0	0	0	0	0	0	0	0	0
Mineral premix	0	0	0	0	0	0	0	0	0	0	0	0
Fish oil	0	0	0	0	0	0	0	0	0	0	0	0
Methionine						0.15						
DiCalcium Phosphate												
Choline Chloride												
Stay-C												
Sum	2.83	1.23	2.05	3.91	3.17	1.19	0.68	2.26	1.50	1.79	0.57	2.53
Req	2.3	0.9	1.6	2.6	2.1	1.55		2.9		1.6	0.204	1.95
x - met BM below min	x	x	x	x	x	x	x	x	x	x	x	x

Requirement for Met = Met + Cys
Requirement for Phe = Phe + Tyr

APPENDIX - Diet Formulation Model Data

SB30/F30 **Diet Formulation**

Ingredients	Ca (%)	Phos. (%)	Pot (%)	Chlorine (%)	Mg (%)	Na (%)	Su (%)	Cop (mg/kg)	Fe (mg/kg)	Magn. (mg/kg)
Wheat gluten meal	0.02	0.01	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SBM	0.08	0.19	0.64	0.01	0.09	0.00	0.13	6.09	39.30	11.16
Fish meal	1.12	0.73	0.27	0.30	0.07	0.33	0.16	2.71	66.00	2.85
Blood Meal	0.01	0.01	0.00	0.01	0.00	0.01	0.01	0.27	91.38	0.21
Poultry by-product meal	0.10	0.05	0.01	0.02	0.01	0.02	0.02	0.41	12.82	0.32
Whey	0.02	0.02	0.06	0.10	0.00	0.02	0.00	0.13	6.92	0.07
Brewers yeast	0.00	0.02	0.03	0.00	0.00	0.00	0.01	0.61	1.74	0.11
Corn gluten meal	0.00	0.02	0.01	0.00	0.00	0.00	0.02	1.02	8.93	0.25
Lysine.HCL	0	0	0	0	0	0	0	0	0	0
Vitamin premix	0	0	0	0	0	0	0	0	0	0
Mineral premix	0	0	0	0	0	0	0	2.2	3.3	77
Fish oil	0	0	0	0	0	0	0	0	0	0
Methionine										
DiCalcium Phosphate		0.0814								
Choline Chloride										
Stay-C										
Sum	1.35	1.13	1.11	0.44	0.18	0.39	0.35	13.44	230.39	91.96
Req	1	0.6	0.8	0	0.04	0.6	0	5	60	10
x - met BM below min	x	x	x	x	x	BM	x	x	x	x

APPENDIX - Diet Formulation Model Data

SB30/F30

Diet Formulation

Ingredients	Se (mg/kg)	Zn (mg/kg)	Biotin (mg/kg)	Choline (mg/kg)	Folacin (mg/kg)	Niacin (mg/kg)	Panta-Acid (mg/kg)	Pyrdox (mg/kg)	Ribo (mg/kg)	Thia (mg/kg)	B12 (mg/kg)	E (mg/kg)	K (mg/kg)
Wheat gluten meal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
SBM	0.03	17.10	0.10	825.90	0.21	6.60	4.44	1.47	0.87	0.93	0.00	0.99	0
Fish meal	0.41	30.90	0.07	1322.40	0.06	30.00	4.50	1.39	2.13	0.03	105.60	1.50	0
Blood Meal	0.00	10.10	0.01	19.80	0.01	0.73	0.11	0.15	0.10	0.01	0.43	0.00	0
Poultry by-product meal	0.02	3.51	0.00	174.84	0.01	1.36	0.32	0.13	0.30	0.01	8.73	0.06	0
Whey	0.00	0.17	0.01	48.94	0.00	0.00	1.12	0.00	0.00	0.00	0.00	0.00	0
Brewers yeast	0.01	0.62	0.02	61.55	0.16	7.09	1.77	0.59	0.55	1.36	0.02	0.03	0
Corn gluten meal	0.03	1.21	0.01	13.73	0.01	2.34	0.14	0.27	0.08	0.01	0.00	0.91	0
Lysine.HCL	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitamin premix	0.01	0	1	3.3	13.2	330	158.4	46.2	79.2	52.8	30	0	16.5
Mineral premix	0	110	0	0	0	0	0	0	0	0	0	0	0
Fish oil	0	0	0	0	0	0	0	0	0	0	0	0	0
Methionine													
DiCalcium Phosphate													
Choline Chloride													
Stay-C													
Sum	0.52	173.61	1.21	2470.47	13.66	378.12	170.80	50.20	83.22	55.15	144.78	3.50	16.50
Req	0.3	67	1.2	480	12	180	60	18	24	18	0.024	0	12
x - met BM below min	x	x	x	x	x	x	x	x	x	x	x	x	x

APPENDIX - Diet Formulation Model Data

SB30/F24 **Diet Formulation**

Ingredients	Amount g/kg	CP (%)	DP (%)	DE (kcal)	CF (%)	Cfiber (%)	Cash (%)
Wheat gluten meal	100	8.01	8.01	485.26	0.15	0.05	0.07
SBM	300	15.00	13.25	975.00	0.27	1.02	1.74
Fish meal	240	16.97	15.02	1158.72	1.27	0.24	4.06
Blood Meal	38.5	3.43	2.37	131.13	0.03	0.04	0.09
Poultry by-product meal	30.8	1.84	1.25	122.09	0.42	0.06	0.45
Whey	21	0.28	0.25	58.46	0.01	0.00	0.19
Brewers yeast	13	0.55	0.44	45.79	0.01	0.04	0.09
Corn gluten meal	60	3.62	3.25	255.60	0.11	0.09	0.13
Lysine.HCL	3	0	0	0	0	0	0
Vitamin premix	3	0	0	0	0	0	0
Mineral premix	2.4	0	0	0	0	0	0
Fish oil	170	0	0	1533.32	17	0	0
Methionine	1.5						
DiCalcium Phosphate	7.8						
Choline Chloride	6						
Stay-C	3						
Sum	1000.00	49.71	43.82	4765.37	19.27	1.55	6.81
Req							

DE (MJ/kg)	19.95
DP:DE	21.97
Lys:Arg	1.11
Crude protein (%)	49.71
Crude fat (%)	19.27
Crude fiber (%)	1.55
Crude ash (%)	6.81
Met + Cys(%)	1.88

APPENDIX - Diet Formulation Model Data

SB30/F24 **Diet Formulation**

Ingredients	Arg (%)	His (%)	Ile (%)	Leu (%)	Lys (%)	Met (%)	Cys (%)	Phe (%)	Tyr (%)	Thr (%)	Trp (%)	Val (%)
Wheat gluten meal	0.30	0.16	0.30	0.50	0.12	0.13	0.21	0.36	0.00	0.13	0.08	0.32
SBM	1.10	0.37	0.64	1.09	0.92	0.20	0.23	0.73	0.53	0.57	0.21	0.77
Fish meal	0.92	0.39	0.76	1.21	1.21	0.48	0.14	0.67	0.54	0.68	0.18	0.84
Blood Meal	0.14	0.20	0.04	0.42	0.29	0.04	0.05	0.23	0.10	0.14	0.04	0.29
Poultry by-product meal	0.13	0.03	0.07	0.13	0.09	0.03	0.03	0.06	0.06	0.03	0.01	0.09
Whey	0.01	0.00	0.02	0.02	0.02	0.00	0.01	0.01	0.01	0.02	0.00	0.00
Brewers yeast	0.03	0.01	0.03	0.04	0.04	0.01	0.01	0.02	0.02	0.03	0.01	0.03
Corn gluten meal	0.12	0.08	0.15	0.61	0.07	0.10	0.07	0.24	0.20	0.12	0.03	0.19
Lysine.HCL	0	0	0	0	0.3	0	0	0	0	0	0	0
Vitamin premix	0	0	0	0	0	0	0	0	0	0	0	0
Mineral premix	0	0	0	0	0	0	0	0	0	0	0	0
Fish oil	0	0	0	0	0	0	0	0	0	0	0	0
Methionine						0.15						
DiCalcium Phosphate												
Choline Chloride												
Stay-C												
Sum	2.76	1.24	2.00	4.02	3.06	1.15	0.74	2.32	1.45	1.72	0.56	2.52
Req	2.3	0.9	1.6	2.6	2.1	1.55		2.9		1.6	0.204	1.95
x - met BM below min	x	x	x	x	x	x	x	x	x	x	x	x

Requirement for Met = Met + Cys
Requirement for Phe = Phe + Tyr

APPENDIX - Diet Formulation Model Data

SB30/F24 **Diet Formulation**

Ingredients	Ca (%)	Phos. (%)	Pot (%)	Chlorine (%)	Mg (%)	Na (%)	Su (%)	Cop (mg/kg)	Fe (mg/kg)	Magn. (mg/kg)
Wheat gluten meal	0.02	0.01	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SBM	0.08	0.19	0.64	0.01	0.09	0.00	0.13	6.09	39.30	11.16
Fish meal	0.90	0.58	0.22	0.24	0.06	0.26	0.13	2.17	52.80	2.28
Blood Meal	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.32	106.61	0.25
Poultry by-product meal	0.11	0.06	0.01	0.02	0.01	0.03	0.02	0.43	13.61	0.34
Whey	0.01	0.01	0.05	0.08	0.00	0.02	0.00	0.11	5.46	0.06
Brewers yeast	0.00	0.02	0.02	0.00	0.00	0.00	0.01	0.50	1.42	0.09
Corn gluten meal	0.00	0.03	0.01	0.00	0.00	0.00	0.03	1.57	13.74	0.38
Lysine.HCL	0	0	0	0	0	0	0	0	0	0
Vitamin premix	0	0	0	0	0	0	0	0	0	0
Mineral premix	0	0	0	0	0	0	0	2.4	3.6	84
Fish oil	0	0	0	0	0	0	0	0	0	0
Methionine										
DiCalcium Phosphate		0.1716								
Choline Chloride										
Stay-C										
Sum	1.14	1.08	1.08	0.36	0.17	0.33	0.33	13.58	236.54	98.55
Req	1	0.6	0.8	0	0.04	0.6	0	5	60	10
x - met BM below min	x	x	x	x	x	BM	x	x	x	x

APPENDIX - Diet Formulation Model Data

SB30/F24 **Diet Formulation**

Ingredients	Se (mg/kg)	Zn (mg/kg)	Biotin (mg/kg)	Choline (mg/kg)	Folacin (mg/kg)	Niacin (mg/kg)	Panta-Acid (mg/kg)	Pyrdox (mg/kg)	Ribo (mg/kg)	Thia (mg/kg)	B12 (mg/kg)	E (mg/kg)	K (mg/kg)
Wheat gluten meal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
SBM	0.03	17.10	0.10	825.90	0.21	6.60	4.44	1.47	0.87	0.93	0.00	0.99	0
Fish meal	0.33	24.72	0.06	1057.92	0.05	24.00	3.60	1.11	1.70	0.02	84.48	1.20	0
Blood Meal	0.00	11.78	0.01	23.10	0.02	0.85	0.12	0.17	0.11	0.01	0.50	0.00	0
Poultry by-product meal	0.02	3.73	0.00	185.69	0.02	1.45	0.34	0.14	0.32	0.01	9.28	0.07	0
Whey	0.00	0.14	0.01	38.64	0.00	0.00	0.89	0.00	0.00	0.00	0.00	0.00	0
Brewers yeast	0.01	0.51	0.01	50.01	0.13	5.76	1.44	0.48	0.44	1.11	0.01	0.03	0
Corn gluten meal	0.05	1.86	0.01	21.12	0.02	3.60	0.21	0.41	0.12	0.02	0.00	1.40	0
Lysine.HCL	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitamin premix	0.01	0	1	3.3	13.2	330	158.4	46.2	79.2	52.8	30	0	16.5
Mineral premix	0	120	0	0	0	0	0	0	0	0	0	0	0
Fish oil	0	0	0	0	0	0	0	0	0	0	0	0	0
Methionine													
DiCalcium Phosphate													
Choline Chloride													
Stay-C													
Sum	0.45	179.83	1.19	2205.68	13.63	372.25	169.44	49.99	82.77	54.90	124.27	3.69	16.50
Req	0.3	67	1.2	480	12	180	60	18	24	18	0.024	0	12
x - met BM below min	x	x	BM	x	x	x	x	x	x	x	x	x	x

APPENDIX - Diet Formulation Model Data

SB35/F20 **Diet Formulation**

Ingredients	Amount g/kg	CP (%)	DP (%)	DE (kcal)	CF (%)	Cfiber (%)	Cash (%)
Wheat gluten meal	100	8.01	8.01	485.26	0.15	0.05	0.07
SBM	350	17.50	15.45	1137.50	0.32	1.19	2.03
Fish meal	200	14.14	12.51	965.60	1.06	0.20	3.38
Blood Meal	70	6.24	4.31	238.42	0.05	0.07	0.16
Poultry by-product meal	10.5	0.63	0.43	41.62	0.14	0.02	0.15
Whey	5	0.07	0.06	13.92	0.00	0.00	0.05
Brewers yeast	5	0.21	0.17	17.61	0.01	0.02	0.03
Corn gluten meal	48.3	2.92	2.62	205.76	0.09	0.07	0.10
Lysine.HCL	3	0	0	0	0	0	0
Vitamin premix	3	0	0	0	0	0	0
Mineral premix	2.4	0	0	0	0	0	0
Fish oil	182	0	0	1641.55	18.2	0	0
Methionine	1.8						
DiCalcium Phosphate	10						
Choline Chloride	6						
Stay-C	3						
Sum	1000.00	49.72	43.55	4747.24	20.01	1.62	5.97
Req							

DE (MJ/kg)	19.88
DP:DE	21.91
Lys:Arg	1.13
Crude protein (%)	49.72
Crude fat (%)	20.01
Crude fiber (%)	1.62
Crude ash (%)	5.97
Met + Cys(%)	1.86

APPENDIX - Diet Formulation Model Data

SB35/F20 **Diet Formulation**

Ingredients	Arg (%)	His (%)	Ile (%)	Leu (%)	Lys (%)	Met (%)	Cys (%)	Phe (%)	Tyr (%)	Thr (%)	Trp (%)	Val (%)
Wheat gluten meal	0.30	0.16	0.30	0.50	0.12	0.13	0.21	0.36	0.00	0.13	0.08	0.32
SBM	1.28	0.43	0.75	1.27	1.08	0.24	0.26	0.85	0.62	0.66	0.24	0.89
Fish meal	0.77	0.32	0.63	1.01	1.01	0.40	0.12	0.56	0.45	0.56	0.15	0.70
Blood Meal	0.26	0.36	0.07	0.76	0.52	0.08	0.09	0.41	0.18	0.26	0.07	0.52
Poultry by-product meal	0.04	0.01	0.02	0.04	0.03	0.01	0.01	0.02	0.02	0.01	0.00	0.03
Whey	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Brewers yeast	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.00	0.01
Corn gluten meal	0.10	0.06	0.12	0.49	0.05	0.08	0.06	0.19	0.16	0.10	0.02	0.15
Lysine.HCL	0	0	0	0	0.3	0	0	0	0	0	0	0
Vitamin premix	0	0	0	0	0	0	0	0	0	0	0	0
Mineral premix	0	0	0	0	0	0	0	0	0	0	0	0
Fish oil	0	0	0	0	0	0	0	0	0	0	0	0
Methionine						0.18						
DiCalcium Phosphate												
Choline Chloride												
Stay-C												
Sum	2.77	1.35	1.91	4.10	3.13	1.11	0.75	2.41	1.43	1.74	0.58	2.63
Req	2.3	0.9	1.6	2.6	2.1	1.55		2.9		1.6	0.204	1.95
x - met BM below min	x	x	x	x	x	x	x	x	x	x	x	x

Requirement for Met = Met + Cys
Requirement for Phe = Phe + Tyr

APPENDIX - Diet Formulation Model Data

SB35/F20 **Diet Formulation**

Ingredients	Ca (%)	Phos. (%)	Pot (%)	Chlorine (%)	Mg (%)	Na (%)	Su (%)	Cop (mg/kg)	Fe (mg/kg)	Magn. (mg/kg)
Wheat gluten meal	0.02	0.01	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SBM	0.09	0.22	0.75	0.01	0.11	0.00	0.15	7.11	45.85	13.02
Fish meal	0.75	0.49	0.18	0.20	0.05	0.22	0.11	1.81	44.00	1.90
Blood Meal	0.03	0.02	0.01	0.02	0.01	0.03	0.02	0.57	193.83	0.45
Poultry by-product meal	0.04	0.02	0.00	0.01	0.00	0.01	0.01	0.15	4.64	0.12
Whey	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.03	1.30	0.01
Brewers yeast	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.19	0.55	0.03
Corn gluten meal	0.00	0.02	0.01	0.00	0.00	0.00	0.03	1.26	11.06	0.30
Lysine.HCL	0	0	0	0	0	0	0	0	0	0
Vitamin premix	0	0	0	0	0	0	0	0	0	0
Mineral premix	0	0	0	0	0	0	0	2	3	70
Fish oil	0	0	0	0	0	0	0	0	0	0
Methionine										
DiCalcium Phosphate		0.22								
Choline Chloride										
Stay-C										
Sum	0.93	1.01	1.09	0.26	0.17	0.27	0.32	13.11	304.23	85.83
Req	1	0.6	0.8	0	0.04	0.6	0	5	60	10
x - met BM below min	BM	x	x	x	x	BM	x	x	x	x

APPENDIX - Diet Formulation Model Data

SB35/F20 **Diet Formulation**

Ingredients	Se (mg/kg)	Zn (mg/kg)	Biotin (mg/kg)	Choline (mg/kg)	Folacin (mg/kg)	Niacin (mg/kg)	Panta-Acid (mg/kg)	Pyrdox (mg/kg)	Ribo (mg/kg)	Thia (mg/kg)	B12 (mg/kg)	E (mg/kg)	K (mg/kg)
Wheat gluten meal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
SBM	0.04	19.95	0.11	963.55	0.25	7.70	5.18	1.72	1.02	1.09	0.00	1.16	0
Fish meal	0.27	20.60	0.05	881.60	0.04	20.00	3.00	0.93	1.42	0.02	70.40	1.00	0
Blood Meal	0.00	21.42	0.02	42.00	0.03	1.54	0.22	0.31	0.20	0.02	0.91	0.00	0
Poultry by-product meal	0.01	1.27	0.00	63.30	0.01	0.49	0.12	0.05	0.11	0.00	3.16	0.02	0
Whey	0.00	0.03	0.00	9.20	0.00	0.00	0.21	0.00	0.00	0.00	0.00	0.00	0
Brewers yeast	0.00	0.20	0.01	19.24	0.05	2.22	0.55	0.19	0.17	0.43	0.01	0.01	0
Corn gluten meal	0.04	1.50	0.01	17.00	0.01	2.90	0.17	0.33	0.10	0.01	0.00	1.13	0
Lysine.HCL	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitamin premix	0.01	0	1	3.3	13.2	330	158.4	46.2	79.2	52.8	30	0	16.5
Mineral premix	0	100	0	0	0	0	0	0	0	0	0	0	0
Fish oil	0	0	0	0	0	0	0	0	0	0	0	0	0
Methionine													
DiCalcium Phosphate													
Choline Chloride													
Stay-C													
Sum	0.37	164.97	1.19	1999.19	13.58	364.85	167.85	49.72	82.22	54.37	104.48	3.32	16.50
Req	0.3	67	1.2	480	12	180	60	18	24	18	0.024	0	12
x - met BM below min	x	x	BM	x	x	x	x	x	x	x	x	x	x

APPENDIX - Diet Formulation Model Data

SB40/F20 **Diet Formulation**

Ingredients	Amount g/kg	CP (%)	DP (%)	DE (kcal)	CF (%)	Cfiber (%)	Cash (%)
Wheat gluten meal	100	8.01	8.01	485.26	0.15	0.05	0.07
SBM	400	20.00	17.66	1300.00	0.36	1.36	2.32
Fish meal	200	14.14	12.51	965.60	1.06	0.20	3.38
Blood Meal	64.9	5.79	3.99	221.05	0.05	0.06	0.15
Poultry by-product meal	5	0.30	0.20	19.82	0.07	0.01	0.07
Whey	3	0.04	0.04	8.35	0.00	0.00	0.03
Brewers yeast	2	0.09	0.07	7.04	0.00	0.01	0.01
Corn gluten meal	10	0.60	0.54	42.60	0.02	0.02	0.02
Lysine.HCL	3	0	0	0	0	0	0
Vitamin premix	3	0	0	0	0	0	0
Mineral premix	2.6	0	0	0	0	0	0
Fish oil	185	0	0	1668.61	18.5	0	0
Methionine	2.5						
DiCalcium Phosphate	10						
Choline Chloride	6						
Stay-C	3						
Sum	1000.00	48.97	43.03	4718.33	20.21	1.71	6.05
Req							

DE (MJ/kg)	19.75
DP:DE	21.78
Lys:Arg	1.12
Crude protein (%)	48.97
Crude fat (%)	20.21
Crude fiber (%)	1.71
Crude ash (%)	6.05
Met + Cys(%)	1.87

APPENDIX - Diet Formulation Model Data

SB40/F20 **Diet Formulation**

Ingredients	Arg (%)	His (%)	Ile (%)	Leu (%)	Lys (%)	Met (%)	Cys (%)	Phe (%)	Tyr (%)	Thr (%)	Trp (%)	Val (%)
Wheat gluten meal	0.30	0.16	0.30	0.50	0.12	0.13	0.21	0.36	0.00	0.13	0.08	0.32
SBM	1.47	0.49	0.86	1.45	1.23	0.27	0.30	0.98	0.70	0.76	0.28	1.02
Fish meal	0.77	0.32	0.63	1.01	1.01	0.40	0.12	0.56	0.45	0.56	0.15	0.70
Blood Meal	0.24	0.33	0.06	0.70	0.48	0.07	0.08	0.38	0.17	0.24	0.07	0.49
Poultry by-product meal	0.02	0.01	0.01	0.02	0.02	0.01	0.00	0.01	0.01	0.00	0.00	0.01
Whey	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Brewers yeast	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Corn gluten meal	0.02	0.01	0.03	0.10	0.01	0.02	0.01	0.04	0.03	0.02	0.00	0.03
Lysine.HCL	0	0	0	0	0.3	0	0	0	0	0	0	0
Vitamin premix	0	0	0	0	0	0	0	0	0	0	0	0
Mineral premix	0	0	0	0	0	0	0	0	0	0	0	0
Fish oil	0	0	0	0	0	0	0	0	0	0	0	0
Methionine						0.25						
DiCalcium Phosphate												
Choline Chloride												
Stay-C												
Sum	2.83	1.32	1.89	3.80	3.18	1.14	0.73	2.33	1.36	1.72	0.59	2.58
Req	2.3	0.9	1.6	2.6	2.1	1.55		2.9		1.6	0.204	1.95
x - met BM below min	x	x	x	x	x	x	x	x	x	x	x	x

Requirement for Met = Met + Cys
Requirement for Phe = Phe + Tyr

APPENDIX - Diet Formulation Model Data

SB40/F20 **Diet Formulation**

Ingredients	Ca (%)	Phos. (%)	Pot (%)	Chlorine (%)	Mg (%)	Na (%)	Su (%)	Cop (mg/kg)	Fe (mg/kg)	Magn. (mg/kg)
Wheat gluten meal	0.02	0.01	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SBM	0.10	0.26	0.85	0.02	0.12	0.00	0.18	8.12	52.40	14.88
Fish meal	0.75	0.49	0.18	0.20	0.05	0.22	0.11	1.81	44.00	1.90
Blood Meal	0.03	0.02	0.01	0.02	0.01	0.02	0.02	0.53	179.71	0.42
Poultry by-product meal	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.07	2.21	0.06
Whey	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.02	0.78	0.01
Brewers yeast	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.22	0.01
Corn gluten meal	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.26	2.29	0.06
Lysine.HCL	0	0	0	0	0	0	0	0	0	0
Vitamin premix	0	0	0	0	0	0	0	0	0	0
Mineral premix	0	0	0	0	0	0	0	2.2	3.3	77
Fish oil	0	0	0	0	0	0	0	0	0	0
Methionine										
DiCalcium Phosphate		0.22								
Choline Chloride										
Stay-C										
Sum	0.92	1.01	1.18	0.25	0.18	0.26	0.32	13.08	284.91	94.33
Req	1	0.6	0.8	0	0.04	0.6	0	5	60	10
x - met BM below min	BM	x	x	x	x	BM	x	x	x	x

APPENDIX - Diet Formulation Model Data

SB40/F20 **Diet Formulation**

Ingredients	Se (mg/kg)	Zn (mg/kg)	Biotin (mg/kg)	Choline (mg/kg)	Folacin (mg/kg)	Niacin (mg/kg)	Panta-Acid (mg/kg)	Pyrdox (mg/kg)	Ribo (mg/kg)	Thia (mg/kg)	B12 (mg/kg)	E (mg/kg)	K (mg/kg)
Wheat gluten meal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
SBM	0.04	22.80	0.13	1101.20	0.28	8.80	5.92	1.96	1.16	1.24	0.00	1.32	0
Fish meal	0.27	20.60	0.05	881.60	0.04	20.00	3.00	0.93	1.42	0.02	70.40	1.00	0
Blood Meal	0.00	19.86	0.02	38.94	0.03	1.43	0.21	0.29	0.19	0.02	0.84	0.00	0
Poultry by-product meal	0.00	0.61	0.00	30.15	0.00	0.24	0.06	0.02	0.05	0.00	1.51	0.01	0
Whey	0.00	0.02	0.00	5.52	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0
Brewers yeast	0.00	0.08	0.00	7.69	0.02	0.89	0.22	0.07	0.07	0.17	0.00	0.00	0
Corn gluten meal	0.01	0.31	0.00	3.52	0.00	0.60	0.04	0.07	0.02	0.00	0.00	0.23	0
Lysine.HCL	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitamin premix	0.01	0	1	3.3	13.2	330	158.4	46.2	79.2	52.8	30	0	16.5
Mineral premix	0	110	0	0	0	0	0	0	0	0	0	0	0
Fish oil	0	0	0	0	0	0	0	0	0	0	0	0	0
Methionine													
DiCalcium Phosphate													
Choline Chloride													
Stay-C													
Sum	0.34	174.27	1.20	2071.92	13.57	361.95	167.97	49.54	82.11	54.25	102.75	2.57	16.50
Req	0.3	67	1.2	480	12	180	60	18	24	18	0.024	0	12
x - met BM below min	x	x	BM	x	x	x	x	x	x	x	x	x	x

APPENDIX - Diet Formulation Model Data

SB45/F10 **Diet Formulation**

Ingredients	Amount g/kg	CP (%)	DP (%)	DE (kcal)	CF (%)	Cfiber (%)	Cash (%)
Wheat gluten meal	100	8.01	8.01	485.26	0.15	0.05	0.07
SBM	450	22.50	19.87	1462.50	0.41	1.53	2.61
Fish meal	149.1	10.54	9.33	719.85	0.79	0.15	2.52
Blood Meal	65	5.80	4.00	221.39	0.05	0.07	0.15
Poultry by-product meal	0	0.00	0.00	0.00	0.00	0.00	0.00
Whey	0	0.00	0.00	0.00	0.00	0.00	0.00
Brewers yeast	0	0.00	0.00	0.00	0.00	0.00	0.00
Corn gluten meal	0	0.00	0.00	0.00	0.00	0.00	0.00
Lysine.HCL	3	0	0	0	0	0	0
Vitamin premix	3	0	0	0	0	0	0
Mineral premix	2.4	0	0	0	0	0	0
Fish oil	200	0	0	1803.90	20	0	0
Methionine	3.5						
DiCalcium Phosphate	15						
Choline Chloride	6						
Stay-C	3						
Sum	1000.00	46.85	41.21	4692.91	21.39	1.79	5.35
Req							

DE (MJ/kg)	19.65
DP:DE	20.97
Lys:Arg	1.10
Crude protein (%)	46.85
Crude fat (%)	21.39
Crude fiber (%)	1.79
Crude ash (%)	5.35
Met + Cys(%)	1.87

APPENDIX - Diet Formulation Model Data

SB45/F10 **Diet Formulation**

Ingredients	Arg (%)	His (%)	Ile (%)	Leu (%)	Lys (%)	Met (%)	Cys (%)	Phe (%)	Tyr (%)	Thr (%)	Trp (%)	Val (%)
Wheat gluten meal	0.30	0.16	0.30	0.50	0.12	0.13	0.21	0.36	0.00	0.13	0.08	0.32
SBM	1.65	0.55	0.96	1.63	1.39	0.31	0.34	1.10	0.79	0.85	0.31	1.15
Fish meal	0.57	0.24	0.47	0.75	0.75	0.30	0.09	0.41	0.33	0.42	0.11	0.52
Blood Meal	0.24	0.33	0.06	0.70	0.48	0.07	0.08	0.38	0.17	0.24	0.07	0.49
Poultry by-product meal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Whey	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Brewers yeast	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Corn gluten meal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lysine.HCL	0	0	0	0	0.3	0	0	0	0	0	0	0
Vitamin premix	0	0	0	0	0	0	0	0	0	0	0	0
Mineral premix	0	0	0	0	0	0	0	0	0	0	0	0
Fish oil	0	0	0	0	0	0	0	0	0	0	0	0
Methionine						0.35						
DiCalcium Phosphate												
Choline Chloride												
Stay-C												
Sum	2.77	1.28	1.80	3.59	3.04	1.15	0.72	2.26	1.29	1.64	0.57	2.48
Req	2.3	0.9	1.6	2.6	2.1	1.55		2.9		1.6	0.204	1.95
x - met BM below min	x	x	x	x	x	x	x	x	x	x	x	x

Requirement for Met = Met + Cys
Requirement for Phe = Phe + Tyr

APPENDIX - Diet Formulation Model Data

SB45/F10 **Diet Formulation**

Ingredients	Ca (%)	Phos. (%)	Pot (%)	Chlorine (%)	Mg (%)	Na (%)	Su (%)	Cop (mg/kg)	Fe (mg/kg)	Magn. (mg/kg)
Wheat gluten meal	0.02	0.01	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SBM	0.12	0.29	0.96	0.02	0.14	0.00	0.20	9.14	58.95	16.74
Fish meal	0.56	0.36	0.13	0.15	0.04	0.16	0.08	1.35	32.80	1.42
Blood Meal	0.03	0.02	0.01	0.02	0.01	0.02	0.02	0.53	179.99	0.42
Poultry by-product meal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Whey	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Brewers yeast	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Corn gluten meal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lysine.HCL	0	0	0	0	0	0	0	0	0	0
Vitamin premix	0	0	0	0	0	0	0	0	0	0
Mineral premix	0	0	0	0	0	0	0	2.4	3.6	84
Fish oil	0	0	0	0	0	0	0	0	0	0
Methionine										
DiCalcium Phosphate		0.33								
Choline Chloride										
Stay-C										
Sum	0.72	1.01	1.23	0.18	0.18	0.19	0.30	13.41	275.34	102.57
Req	1	0.6	0.8	0	0.04	0.6	0	5	60	10
x - met BM below min	BM	x	x	x	x	BM	x	x	x	x

APPENDIX - Diet Formulation Model Data

SB45/F10 **Diet Formulation**

Ingredients	Se (mg/kg)	Zn (mg/kg)	Biotin (mg/kg)	Choline (mg/kg)	Folacin (mg/kg)	Niacin (mg/kg)	Panta-Acid (mg/kg)	Pyrdox (mg/kg)	Ribo (mg/kg)	Thia (mg/kg)	B12 (mg/kg)	E (mg/kg)	K (mg/kg)
Wheat gluten meal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
SBM	0.05	25.65	0.14	1238.85	0.32	9.90	6.66	2.21	1.31	1.40	0.00	1.49	0
Fish meal	0.20	15.36	0.03	657.23	0.03	14.91	2.24	0.69	1.06	0.01	52.48	0.75	0
Blood Meal	0.00	19.89	0.02	39.00	0.03	1.43	0.21	0.29	0.19	0.02	0.85	0.00	0
Poultry by-product meal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
Whey	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
Brewers yeast	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
Corn gluten meal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
Lysine.HCL	0	0	0	0	0	0	0	0	0	0	0	0	0
Vitamin premix	0.01	0	1	3.3	13.2	330	158.4	46.2	79.2	52.8	30	0	16.5
Mineral premix	0	120	0	0	0	0	0	0	0	0	0	0	0
Fish oil	0	0	0	0	0	0	0	0	0	0	0	0	0
Methionine													
DiCalcium Phosphate													
Choline Chloride													
Stay-C													
Sum	0.26	180.90	1.20	1938.38	13.57	356.24	167.50	49.39	81.75	54.23	83.33	2.23	16.50
Req	0.3	67	1.2	480	12	180	60	18	24	18	0.024	0	12
x - met BM below min	BM	x	BM	x	x	x	x	x	x	x	x	x	x

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