

Effects of trypsin inhibitors on growth, enzyme activity, intestinal histology and body composition in purified and practical soybean meal based diets for young Atlantic salmon (2004)

*Report to the United Soybean Board
January 13, 2005*

Project Summary

In compliance with the 2003-2004 USB Managed Aquaculture Program, researchers at Michigan State University (MSU) developed a 2-phase experimental design with the goal of examining the effect of trypsin inhibitors in SBM based diets on Atlantic salmon. Phase I 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. Two feed trials were completed in 2003 under phase I of this project.

The second phase of MSU's research was initially designed to examine effects of trypsin inhibitors in SBM based diets containing practical feed ingredients under different processing conditions. Based on results obtained from other researchers in the Managed Aquaculture Program in 2003, and an extensive literature review, MSU expanded the focus of this research towards the development of an open formula practical diet, containing 5-30% SBM, that is nutritionally acceptable for young Atlantic salmon. Our objective is to develop a practical diet containing the highest level of SBM possible, based on best available knowledge, which would be available for incorporation by commercial feed suppliers for use with not only Atlantic salmon, but other piscivorous fish as well (e.g. salmonids).

Phase II research was initiated in spring of 2004. Diets were formulated to contain 0-30% SBM, which according to literature, is the range in which SBM diets appear to have anti-nutritional characteristics affecting Atlantic salmon. An open formula practical diet developed by the Ontario Ministry of Natural Resources (OMNR) for Atlantic salmon was used as a baseline diet formulation. In late May of 2004, a setback was encountered. The well pumps which supply water to the MSU Aquaculture Lab had a variable frequency drive malfunction. The repair was rather extensive and wasn't completed in September.

The Atlantic salmon available for the study were donated from the Michigan Department of Natural Resources. Prior to pick up, we learned that these fish had Bacterial Kidney Disease (BKD). The fish were transported to MSU in late October, 2004, and were treated with erythromycin for BKD under the direction of Dr. Mohammad Faisal, MSU faculty member and State Fish Health Specialist. An INAD was not required since these fish are to be used solely for research and not human consumption.

The phase II feed trial began after the erythromycin treatments were completed on 1/11/05. The trial is designed to evaluate the potential effects of long term feeding (3-4 months) of SBM diets on young Atlantic salmon.

Methods

Feed Trial 1 (Phase I)

In the first trial, 440 one-year old Atlantic salmon, average weight 99grams, were randomly distributed in eighteen 110 liter tanks, 22 fish per tank, receiving approximately 2.75 lpm fresh water at 11.8-12.2⁰C. Fish were fed a commercial Atlantic salmon diet over a 10-day acclimation period. Just prior to initiating the feed trial, 1-2 fish were removed from each tank in order to begin the study with 20 fish per tank, depending whether any mortalities occurred over the acclimation period. Whole body samples from 15 fish were frozen for subsequent body composition analysis. Beginning 10/1/03 fish were fed, in triplicate, either an Atlantic salmon commercial diet, or one of 5 experimental test diets containing graded levels of TI. Test diet TI inclusion rates were equivalent to diets containing 0, 15, 30, 45, and 60% soybean meal. Fish were fed three times daily, approximately 1.1% body weight per day, for a period of 21 days.

On day 10 and day 21 of the feed trial, fecal and digestive samples were collected separately from portions of the small intestine, proximal large intestine, and distal large intestine from 10 fish from each tank. Samples were pooled by tank, freeze dried, and kept frozen until they could be analyzed by the Animal Science Laboratory at MSU. Analyses included trypsin activity levels and feed digestibility. At the completion of the first trial, 3 fish from each tank were randomly selected for whole body composition analysis including protein, lipids, ash, and energy.

Feed Trial 2 (Phase I)

The second feed trial was designed to examine the effects of trypsin inhibitors, associated with SBM based diets, on growth, feed conversion, and body composition of juvenile Atlantic salmon. A total of 440 Atlantic salmon, mean weight 17.5 grams, were randomly distributed in eighteen 110 liter tanks, 22 fish per tank, receiving approximately 2.75 lpm fresh water at 11.8-12.2⁰C. Fish were fed a commercial Atlantic salmon diet over a 7-day acclimation period. Just prior to initiating the feed trial, 1-2 fish were removed from each tank in order to begin the study with 20 fish per tank, depending whether any mortalities occurred over the acclimation period. Weight and length measurements were taken from whole body samples of 15 fish, which were then frozen for subsequent body composition analysis. The average condition factor (k) was obtained from weight-length samples described above. Total weight samples were taken from all tanks on 10/1/03. Mean weights were calculated from the total weight samples of 20 fish per tank.

Beginning 10/2/03, fish were fed, in triplicate, a commercial control and experimental test diets containing the same TI inclusion rates fed in the preceding trial (equivalent to feeds containing 0, 15, 30, 45, and 60% soybean meal). Fish were fed three times daily for a period of 8 weeks (56 days), based on a theoretical feed level (percent body weight) for salmonids determined by weight samples taken every 14 days, initial condition factor, and water temperature. At the completion of the study, weight samples were taken on all fish, and 5 fish per tanks were randomly selected for body composition analysis. These samples have been freeze dried and will be analyzed for protein, lipids, ash, and energy at MSU Animal Science and Aquaculture laboratories.

The feeding levels for the juvenile Atlantic salmon feed trial are presented in table 1. Feed levels were determined based on the equation:

$$\%BW = (2 \times {}^0C)/(Wt/k)^{1/3} \times 80\%$$

We used 80% of a theoretical feed level equation developed by Westers (personal communication) for salmonids. As it turns out, selected feed levels were both above and below satiation levels of the fish across feeding times.

Table 1. Feed levels for feed trial 2

juvenile Atlantic salmon

Week	%BW
1-2	1.60
3-4	1.42
5-6	1.31
7-8	1.19

Feed Trial 3(Phase II)

Diets were formulated containing 0-30% SBM using the open formula MNR-98HS as the baseline diet. Diet formulations are presented in table 2.

Table 2. Diet formulations for the SBM practical diet study at MSU

Ingredients	Test Diets						
	MNR-98HS	Control	SBM20A	SBM20B	SBM25	SBM30A	SBM30B
Ingredient amount (g/kg)							
Wheat gluten meal		70	70	70	70	70	100
SBM		50	200	200	250	300	300
Fish meal	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
USFWS vitamin premix (# 30)	10*	3	3	3	3	3	3
USFWS mineral premix (# 3)	5*	2	2	2.4	2	2.2	2.4
Fish oil	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	1000	1000	1000	1000	1000	1000	1000
(* - MNR Premixes)							
DE (MJ/kg)	20.41	20.28	20.05	20.01	20.05	20.05	20.00
DP:DE	20.90	22.01	22.18	22.02	22.10	22.16	22.14
Lys:Arg	1.35	1.13	1.11	1.10	1.10	1.10	1.10
Crude protein (%)	49.85	51.50	51.08	50.57	50.54	50.27	50.02
Crude fat (%)	19.94	19.04	19.50	19.49	19.75	19.92	20.02
Crude fiber (%)	0.93	0.97	1.26	1.30	1.35	1.44	1.45
Crude ash (%)	5.83	5.95	6.02	5.36	5.96	5.84	5.25
Met + Cys(%)	1.93	1.94	1.94	1.93	1.93	1.94	1.94
Phosphorus (%)	0.87	0.90	0.90	0.90	0.90	0.90	0.90
% FM reduction		0.00	0.0	20.0	0.0	0.0	20.0
% BM reduction		0.00	15.0	18.6	32.1	52.9	45.0
% PBM reduction		0.00	16.7	18.3	34.3	51.7	48.7
% Whey reduction		0.00	38.9	54.4	51.1	70.4	76.7
% B. yeast reduction		0.00	40.0	54.2	50.0	68.0	74.0

Diets were extruded under the supervision of Rick Barrows, University of Idaho, based on the best available knowledge obtained from research of SBM-based diet processing for rainbow trout (USB Managed Aquaculture Program). Pellets were 3mm in size, slow sinking, and appeared to be of good to high quality.

A total of 420 young-of-year Atlantic salmon (average weight 27.5g) have been randomly distributed in twenty one 227 liter tanks receiving a flow rate of approximately 5.6 lpm (exchange rate = 2/hr). Fish will be fed the control (MNR-98HS) and the 6 test diets in triplicate. Feed levels will be based on average weight and condition factor of the entire lot using the equation: $\%BW = (2 \times 0C)/(Wt/k)^{1/3}$, adjusted every 2-4 weeks based on weight and length samples. MSU researchers will examine effects of SBM test diets on growth (weight gain), feed utilization, body composition, protein enzyme factors and intestinal pathology of fingerling Atlantic salmon

Preliminary results

Feed Trial 1

Residual trypsin concentrations from intestinal samples for the one-plus year-old Atlantic salmon are shown graphically in figure 1. Our results show a severe difference in trypsin concentrations between the commercial control and 0% TI inclusion (TI0) diet in the small intestine. This is an interesting and unexpected finding and suggests that practical feed diets and purified diets contain properties which affect the digestion processes of Atlantic salmon in widely different ways. While no significant differences may exist between the TI0 and higher trypsin level diets, there appears to be negative trend above a SBM equivalency of 15%. Since differences are not necessarily significant, trypsin inhibitor levels equal to 30-60% SBM inclusion does not appear be the main inhibitory factor, but rather, a potential contributing factor, effecting nutrient absorption in the intestine of Atlantic salmon.

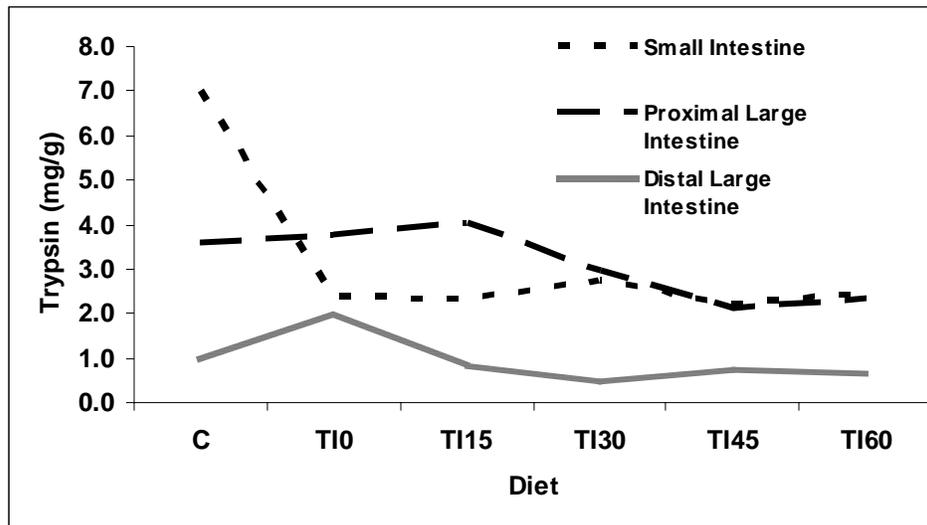


Figure 1. Residual trypsin concentrations from intestinal samples. C = commercial control TI0 = 0% eq TI, TI15 = 15% eq TI, TI30 = 30% eq TI, TI45 = 45% eq TI, TI60 = 60% eq TI.

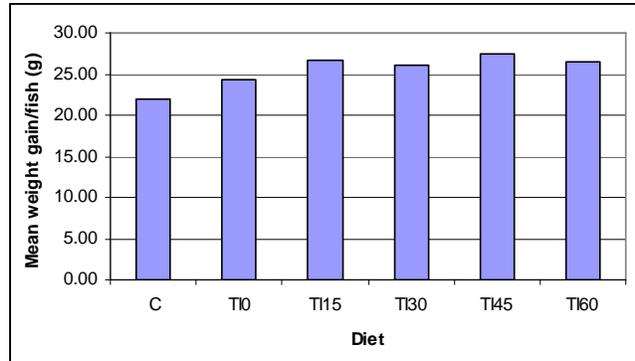
Feed Trial 2

Mean weight gain per treatment are provided in table 3 and graphically in figure 2. All test diets showed increased weight gain over the commercial control. This may be due in part that the control diet obtained by MSU contained 50% protein and 15% fat, while test diets manufactured by Purdue University were formulated for 50% protein and 19% fat. Mean weight gain does not appear to be affected by the level of TI in the test diets.

Table 3. Mean weight gain per fish for treatments of commercial control (C) and TI test diets equivalent to diets containing 0, 15, 30, 45, and 60% SBM

Diet	Mean wt gain (g/fish)
C	21.92
TI0	24.24
TI15	26.76
TI30	26.09
TI45	27.37
TI60	26.55

Figure 2. Mean weight gain per fish of juvenile Atlantic salmon in feed trial 2.

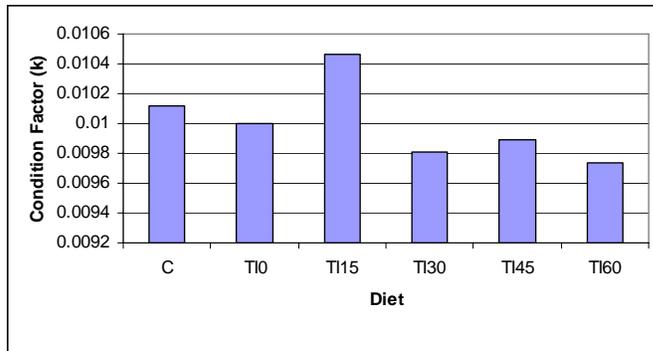


Condition factor (k) represents the relationship between weight and length:

$$k = Wt / L^3$$

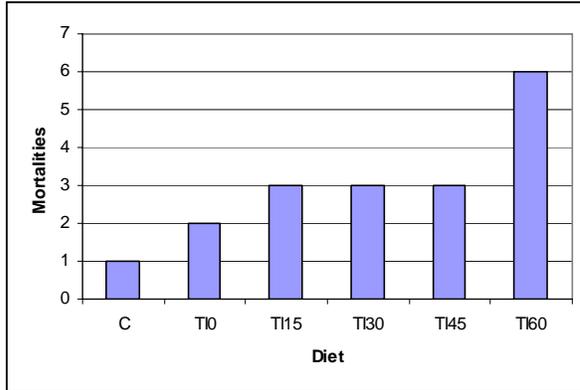
The sample mean for k at the beginning of the feed trial was 0.0088. Final k values for test diets are shown graphically in figure 3. The control group and diets containing 0% and 15% SBM equivalent TI had greater k values than those fed diets containing 30% to 60% SBM equivalent TI diets. Fish fed the TI60 diet had lower k values than all other treatments.

Figure 3. Condition factor (k) for juvenile Atlantic salmon in feed trial 2.



Mortalities through the course of the feed trial were relatively low until the last week of the study. It is unknown whether stress related issues pertaining to tank confinement contributed to the mortalities, however, water quality parameters, oxygen, nitrogen, and suspended solids, were well within known tolerances for salmonid culture. Fish fed diets containing higher levels of TI (figure 4) experienced greater mortality than fish fed the control or 0% TI.

Figure 4. Mortalities of juvenile Atlantic salmon in feed trial 2.



Feed Trial 3.

Results from the third and final feed trial for MSU are pending upon completion of the project which is anticipated to end in April or May 2005.