Affect of Five Diets on Growth, Feed Conversion, Fin Quality, and Reproductive Efficiency of Erwin Strain Rainbow Trout (*Oncorhynchus mykiss)*.

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Abstract.- A two year study was conducted evaluating the performance of five diets on growth, feed conversion, fin quality and reproductive efficiency of Erwin strain broodstock rainbow trout (*Oncorhynchus mykiss*). The five diets evaluated in the study included a USFWS open diet, Silver Cup, Trout Grower (TG), a Skretting Nutra/ Vitalis diet, and Mix 1 diet. Fish fed the high protein Nutra/ Vitalis diet showed significantly better growth then fish fed three of the other four diets. All diet groups showed significantly lower mean Dorsal Fin Index (DFI) values then previously established wild steelhead (*Oncorhynchus mykiss)* DFI values. Fish fed the Silver Cup diet expressed the lowest number of silvers by proportion, producing 16.8 % more net eyed eggs at two years of age then the next 'best' diet type.

Introduction

Ennis National Fish Hatchery (Ennis NFH) is the largest rainbow trout *Oncorhynchus mykiss* broodstock facility in the National Broodstock Program. The facility currently manages six strains of rainbow trout, and produces over 20 million eggs annually. Historically, a Silver Cup closed formula trout diet and a Rangen-manufactured USFWS open feed formulation have been used almost exclusively. Growth rates, feed conversions, and fecundity have been good; however, fish quality measured by fin quality has been poor.

The first dry, nutritionally complete, practical fish diets were commercially fabricated in the United States about 1955 (Rumsey 1994). Since then, a lot of research has gone into improving trout diets. As a result, there are many commercial trout feeds available on the market today that differ in cost and quality.

Fish feed costs usually comprise a relatively small portion of a facilities annual budget; however, commercial feeds may vary dramatically in cost between manufacturers from United States Fish & Wildlife Service (USFWS) open formulas to standard and premium closed formulas. In the short term, cost may be the primary consideration in choosing a commercially manufactured fish feed given a limited hatchery budget. This is true in particular because trout feeds manufactured for United States government facilities must meet or exceed established USFWS minimum proximate analysis nutritional requirements.

Skretting (formerly MooreClark) is one of a number of commercial fish feed manufacturers on the market today. Recent favorable growth results from other hatcheries using Skretting's high-end premium and specialty feeds prompted the question as to whether, in the long run, the initial cost advantage of using a lower cost diet formula outweighed the purported long term advantages of using a higher cost, premium closed formula with regard to fish performance.

Five diets were evaluated for Erwin strain rainbow trout in the two year performance study. Performance was measured by growth, feed conversion, reproductive efficiency and fin quality.

Materials & Methods

The study was conducted for two years at Ennis NFH, MT using Erwin-strain rainbow trout. Blaine Spring Creek, a tributary of the Madison River, is the sole source of water for the facility. Water temperature remained constant at 54 degrees Fahrenheit throughout the course of the study. Dissolved Oxygen levels were maintained at or above 6.5 mg/L. The water has a pH of 7.8, total hardness of 216 ppm CaCO3, and total alkalinity of 150 ppm.

Ennis NFH Erwin strain rainbow trout were reared to two years of age and then spawned. Reproductive efficiency was evaluated over the entire two year period. Data used in the analysis for growth, feed conversion, and cost analysis was collected from Sept, 2002 through July, 2003. Fin quality data, as measured by Dorsal Fin Index, was collected from Oct, 2002 through Feb, 2003.

The USFWS study diet was comprised of Rangen Salmon Starter, and an open formula USFWS trout diet manufactured by Rangen (*see Table 1)*. This diet regime has been applied at Ennis NFH more often then any other over the past four years.

The Silver Cup study diet consisted of closed formula Silver Cup Salmon Fry, Silver Cup Trout, and Silver Cup Brood diets (*see Table 1)*. This diet regime has been used periodically, and in combination with the USFWS open diet at Ennis NFH in recent years.

Table 1. % *Protein/Fat analysis for five diet types*

Feed Size	**USFWS**	**Silver Cup**	**TG[5]**	**Nutra/ Vitalis**	**Mix1**
Mash			64/10	53/18[6]	53/18[9]
0	55/17[1]	52/14[3]	64/10	53/18[6]	53/18[9]
1	55/17[1]	52/14[3]	64/10		52/20[9]
2	55/17[1]	52/14[3]	64/10	50/23[6]	50/23[9]
3	42/13[2]	45/11[4]	64/10	50/23[6]	50/23[9]
4	42/13[2]	45/11[4]			
1.2					
1.5			64/10	50/23[7]	47/18[9]
2.0			64/10	50/23[7]	47/18[10]
1/8th	42/13[2]	40/10[4]	64/10		
2.5				50/24[7]	47/18[10]
4.0			64/10	48/27[7]	47/18[10]
3/16th	42/13[12]	45/10brood	64/10		45/10[11]
6.0	brood			48/14[8]	

[1] *Rangen Salmon starter diet.*
[2] *USFWS Open Diet*
[3] *Silver Cup Salmon Fry*
[4] *Silver Cup Trout*
[5] *Fish meal free diet.*
[6] *MC Nutra 2000.*
[7] *MC Nutra Fry*
[8] *MC VitalisBrood*
[9] *MC NutraPlus*
[10] *Apollo Fry*
[11] *Silver Cup Brood*
[12] *USFWS/ Brood*

The TG diet was made-up of two corn/wheat glutten & krill protein based diets -Trout Grower 2014 and Trout Grower 0230 (a lower krill protein based diet). The diet was formulated by Rick Barrows of the USFWS Region 6 Fish Technology Center (*see Table 1)*.

The Nutra/ Vitalis diet contained Skretting's Nutra 2000, Nutra Fry, and Vitalis Brood formulas- Skretting's premium trout feed formulas(*see Table 1)*.

The Mix1 diet included Skretting's Nutra Plus, Apollo Fry, and Silver Cup's Brood formulas(*see Table 1)*. Initially this diet was intended to be an exclusive Skretting Apollo formula however supply problems resulted in having to substitute the $3/16^{th}$ Skretting Apollo formula with the Silver Cup Brood diet formula.

Feed was distributed by way of twelve-hour belt feeders from swim-up to the week preceding the first week's spawn. During the four month spawning period fish were hand-fed once daily for five days per week.

The percent body weight fed daily was calculated using a hatchery constant (*see Table 2*). Fish were counted and weighed by circular tank at the beginning of each month and weight was averaged. Daily growth over the course of the month was projected. Fish growth was projected for Sept through Nov, 2003 & Jan through May 2004 in lieu of monthly total weights/ counts in order to reduce handling and consequent stress to fish during a period of low to moderate mortalities (see below).

The maximum density of fish encountered during the study period was 4.64 lbs/cu^3. This is well below the maximum carrying capacity established by Piper et al (1982) given the size of the fish (<8.8 lbs/cu^3).

Fish were anesthetized using180 ppm MS-222 (Tricaine Methanesulfonate). A 1% salt solution was added to the anesthetic solution to reduce stress to fish

Dorsal Fin index was calculated dividing the dorsal fin length by the total body length and then multiplying the ratio by one hundred. Dorsal fin lengths were measured by extending the fin rays perpendicular to the body and recording measurement from origin of fin to the tip of the longest ray.

Three replicates of each diet type were hatched and reared in fifteen, 70 cubic ft., flow-through rectangular, concrete tanks at 15 GPM flows on September 5, 2002. Fish began feed at swim-up on Sept 19, 2002. Tanks were cleaned twice daily. Mortalities were removed daily. Flows and depth were increased to 60 GPM and two feet respectively on Oct 29, 2002.

Feed Size (standard/ metric)	**Starting Length (in)**	**Hatchery Constant**	**Growth in Inches per month**
Starter/ Mash	0.65	7	0.7
#1/ Mash	1.1	7	0.7
#2/ #0, #1	1.8	10	1
#3/ #2, #3,1.2, 1.5, 2.0mm	2.8	10	1.1
#4/ 2.5, 4.0mm	4.5	12	1.2
$1\text{-}8^{th}$/ 6.0mm	6	13.8	1.2
$3\text{-}16^{th}$/ 6.0mm	12	13.8	1.2

Table-2 *Feed size, starting fish length, hatchery conversion and projected growth per month used in calculating daily feed percentage for five study diets*

Fish were total weighed/counted on Oct 29, 2002 to insure all tanks maintained equal numbers of fish (856/ tank). On Dec 10, 2002 fish were relocated to fifteen, 56 cubic foot, flow-through circular tanks at 35 gallons per minute flows for the remainder of the study period. At this time the number of fish per circular was reduced to 200. On May 29th, 2003 each circular was thinned to 160 fish and then thinned again to 70 fish per circular on Dec1, 2003 to maintain favorable load levels at circulars.

From March through Oct, 2003 we experienced low to moderate mortalities among all fifteen groups of fish (2% of all study fish over this period died). Up to ten-percent of fish were 'side-swimming'. In Aug 2003, staff from the Bozeman Fish Health Center examined the study fish for indications of a parasite and/ or bacterial infection; the results came back negative for either; however, due to ongoing mortalities, all fish received four, 20 minute exposure salt baths (10/10, 10/15, 10/28, and 11/18/03) and were placed on 3.6g AI Terramycin(TM-50)/ 100lbs fish/ day for ten days beginning October 15, 2003. A TM-50 prophylactic treatment was administered prior to spawning beginning March 30th, 2004 at the same dosage and duration prescribed above.

Fish were two years of age at time they were spawned. Fish were spawned from June 22nd, 2004 through Sept 22nd, 2004. Ripe females were sorted out weekly and spawned the following day. Females were weighed prior to air-spawning. Eggs were fertilized in a 0.75% saline solution with pre-collected milt from 5 to 10 males. Eggs were water hardened in a 50 ppm iodophor solution for thirty minutes immediately following fertilization then placed in designated incubators. Eggs were shocked on day fourteen following fertilization and picked on day 15 with Jensorter WB120 fish egg sorters. The good eggs from each week's circular tank were counted three times using a JenSorter Model BC egg counter; the three counts were then averaged.

DFI ratios of fish examined in this study were compared against a DFI ratio value established for wild steelhead trout by G.A. Kindschi et al 1991. Study fish DFI values were collected in February, 2003 when all fish were between 6.3 and 7.9 inches total length (the total length range of steelhead rainbow trout *Oncorhynchus mykiss* with a DFI value of 11.6 (G.A. Kindschi et al 1991)). DFI values were compared with an analysis of variance procedure (Dunnet). Growth, feed conversion and reproductive efficiency (as measured by net eye-up, and percent eye-up) data were analyzed using analysis of variance procedure with a multiple range test (Tukey).

Results & Discussion

Diet did affect growth performance in Erwin strain rainbow trout *(see Table 3)*. In general, diet Nutra/Vitalis showed the highest mean weight (0.949lbs) at the conclusion of the ten month study period though the difference was only statistically significant when compared against TG, USFWS and Silver Cup diets (ANOVA, $p<0.0001$). Diet Nutra/Vitalis had the highest fish-meal based crude protein level of all the study diets from size 3 feed, about 2.8" total fish length, through to the end of the study period.

Diet Mix 1 showed the second highest mean weight (0.901lbs), was statistically significant when compared against the USFWS and Silver Cup diets (ANOVA, $p<0.0001$), and had the second highest fish-meal based crude protein level.

Diet*	Weight Gain lbs/fish Mean + SD		Feed Conversion Mean + SD		Dorsal Fin Index Mean + SD		Percent Eye-up Mean + SD		Total Net-Eyed Eggs Mean + SD	
USFWS	0.704[d]	.035	1.29[a]	.046	7.18[c]	.67	64.08[a]	7.66	52170[a]	7353
Silver Cup	0.793[c]	.035	1.27[a]	.07	8.52[bc]	.87	62.93[a]	4.86	68393[a]	15598
TG	0.814[bc]	.051	1.19[a]	.04	8.62[b]	.34	68.2[a]	13.5	53733[a]	11890
Nutra/ Vitalis	0.949[a]	.005	1.25[a]	.029	8.11[bc]	.28	62.43[a]	7.66	55452[a]	13286
Mix1	0.901[ab]	.02	1.21[a]	.038	7.47[bc]	.57	65.07[a]	4.85	58528[a]	2910
P-Value	<.0001		.0713		<.0001		0.8999		.045	
F-value	24.9		4.23		23.36		0.26		1.00	
F –critical value	3.48		3.48		3.48		3.48		3.48	

Table 3. *The effect of diet on growth, feed conversion, fin quality, and reproductive fitness of rainbow trout.*
*****Diets with different superscripts are different, p<0.05.*

Fish fed the TG diet, a non-fish meal based protein diet comprised of corn(40.42%) /wheat(20.67%) glutten and krill(10%) , showed the third highest mean weight(0.814lbs), and was statistically significant when compared against the mean weight of fish fed the USFWS diet (0.704lbs).

Fish fed the Silver Cup diet(40%) showed a significantly higher mean growth then fish fed the USFWS diet (ANOVA, p<0.0001)

Fish fed the USFWS diet(42%), a USFWS open formula diet, showed a significantly lower mean growth then all other diets in the study (ANOVA, p<0.0001).

The Nutra/Vitalis(48%)* and Mix 1 (47%) diets had the highest fish meal based crude protein levels and generally grew faster then fish fed non-fish meal protein diets or diets with lower levels of crude fish-meal based protein (*see Table 1)*. G Reinitz (1983) compared high protein, high-fat diets (53/14) against low-protein, low-fat diets (34/7) for rainbow trout over different feeding rates and found the high-protein, high fat diets yielded the optimum combination of fish growth…with feed efficiency and unit cost.

Trout are very efficient at metabolizing digestible protein; however, protein is the most expensive component of fish feeds. The Nutra/Vitalis diet had the highest overall crude protein level of all the study diets and the highest cost per pound of gain ($0.81/ lb of gain(*see Table 4*)). In many circumstances using a diet such as the USFWS formula that provides a sufficient level of protein to maintain the health of farmed fish may be appropriate when maximum growth is not required and/or too cost-prohibitive.

Growth and feed conversion were evaluated in study fish through twelve inches total length. Though growth was statistically significant among fish fed different diets, there was no statistical difference in performance of feed conversions among the five diet groups (ANOVA, p= 0.0713(*see Table 3*)).

In general, all study DFI means were lower compared to the wild steelhead trout DFI value of 11.6 established in the G.A. Kindschi et al 1991 study. In comparing DFI values between study groups, fish fed the TG diet had significantly larger DFI means then fish fed the USFWS diet (ANOVA, p<.0001). Study group DFI mean values ranged from 7.18 for the USFWS diet to 8.62 for the TG diet.

Diet	USFWS	Silver Cup	TG	Nutra/ Vitalis	Mix1
$/ lb of gain	0.38	0.40	N/A	0.81	0.52

Table 5. Cost per pound of gain by diet type

Ennis NFH rainbow trout exhibit poor fin quality as measured by fin erosion and fin splitting. This is particularly true of fish greater then six inches in length. In a recent conversation with Rick Barrows, a USFWS Fish Nutritionalist and developer of the TG diet, copper is involved in the formation of connective tissue in fish and may have some beneficial effect in maintaining good fin quality. In addition, Lellis and Barrows (1997) found that rainbow trout fed fish meal diets had higher fin erosion then those fed a krill-based protein diet. Fish meal is high in calcium and phosphorous which compete with copper for absorption into the body.

Though nutrition may be a factor in poor fin quality, G.A. Kindschi et al (1991) found that steelhead trout held in isolation had excellent fins indicating that fin erosion was the result of aggressive interaction, stress, or some water quality factor correlated with density. Density indexes for all fish in the current diet study were 0.26 or below for the study duration, well below the suggested density index value threshold of 0.50 established by Piper et al (1982). Further studies are recommended in evaluating the cause(s) of poor fin quality in Ennis NFH rainbow trout.

There was no statistical difference in reproductive efficiency, as measured by percent eye-up (ANOVA, $p=0.899$) and total net-eyed eggs (ANOVA, $p<0.451$(*see Table 3)*). The cumulative number of net eyed eggs ranged from 156,128 (USFWS) to 205,178 (Silver Cup). Net eye-up between diet types ranged from 76.6% (Nutra/Vitalis) to 82.6% (TG).

In general, fish fed the Nutra/Vitalis diet produced 250 more eyed-eggs/ female then the next 'best' diet type (*see Table 5)*; however, 27.4% of the total female fish fed this diet didn't produce eggs at two years of age while fish fed the Silver Cup diet expressed the lowest percentage of silvers (13.5%(*see Table 5*)). Unfortunately, the three replicates of females by diet type were pooled before any statistical analysis of 'percent silvers' between diet types could be applied.

In general, unless specifically noted, below mentioned performance results express means that are not proven statistically different from one another but merit some consideration in evaluating the performance of the five diet types. Repeat trials are necessary to confirm/ dismiss differences.

Fish fed the Skretting Nutra/Vitalis diet yielded the best overall statistically significant growth and, in general, the highest number of eyed eggs per female; however, 27.4% of

Table 4. *Cumulative results of reproductive efficiency by diet type through entire study period.*

Diet	#Spawned/ Total Females	Total Eggs	Percent Eye-up	Eyed/ lbs of Fish	Eyed eggs/ female	Net # Eyed	Silvers (%female)
USFWS	67/ 93	194,812	80.1	894	2,336	156,128	28
Silver Cup	83/ 90	255,478	80.3	913	2,472	205,178	13.5
TG	72/ 96	205,053	82.6	860	2,352	169,316	20
Nutra/Vitalis	61/ 84	217,226	76.6	790	2,727	166,356	27.4
Mix 1	77/ 95	214,727	81.8	908	2,344	175,591	19

females fed the Nutra/Vitalis diet didn't produce eggs at two years of age which resulted in an overall mediocre number of cumulative total net eyed eggs for the study period. In addition, the Nutra/Vitalis diet showed the highest cost per pound gain ($0.81/ lb of gain) by a margin of over twice that of the USFWS and Silver Cup diets(*see Table 4)*. The overall average performance results of the Nutra/Vitalis diet didn't justify the cost per pound of gain.

Fish fed the Mix1 diet, a Skretting Nutra/Apollo/Silver Cup Brood diet, had the second highest mean growth (statistically significant when compared against Silver Cup and USFWS) and net-eyed per pound of fish as well as the second highest cost per pound of gain ($0.52/ lb of gain).

In general, fish fed the USFWS diet showed the worst growth, fin quality, feed conversion, fewest eyed eggs per female, greatest number of percent silvers (28%) and fewest cumulative total net eyed eggs of all other diets in the study period (although the only statistically significant differences were shown in growth and fin quality). The USFWS diet had the best cost per pound gain ($ 0.38/ lb of gain).

Fish fed the TG diet were median performers with the exception that they had the best overall fin quality (statistically better then the USFWS diet though statistically lower then the before mentioned assigned wild steelhead value) and, in general, showed the best feed conversion results. No cost analysis is available for this diet.

Fish fed the Silver Cup diet produced the highest number of cumulative net eyed eggs for the study period, the fewest number of silvers (13.5%), ranked second highest in eyed eggs per female, and were statistically better in mean growth then fish fed the USFWS diet. Furthermore, cost per pound gain was comparable to the USFWS diet at $0.40/ pound gain.

Conclusion

Ennis NFH is a broodstock facility and as such is concerned with the most cost effective, efficient means of egg production. Fish fed the Silver Cup diet performed comparatively well at all levels of the study evaluation but, more importantly, produced over 29,000 more cumulative net-eyed eggs (16.8%) then their closest competitor. Though cumulative net eyed eggs didn't prove to be to be statistically different among the five diets, we feel this difference warrants further investigation. If confirmed in further studies, a minimum16.8% increase in cumulative net-eyed egg production using the Silver Cup diet would result in a dramatic decrease in the number of females required to meet Ennis NFH egg production goals not to mention reduced load levels at raceways, lower feed costs, and an overall resultant less stressed, healthier population of hatchery reared rainbow trout broodstock.

All Ennis NFH 2005/6 replacement broodstock will be fed Silver Cup diet from swim-up to sexually maturity. Performance will be evaluated using this study's parameters and compared against previous USFWS diet reared broodstock results. Study results will be made available in 2009.

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