Colorado Hatchery Feed Experiments

Eric R. Fetherman
Aquatic Wildlife Research Section, Fort Collins, Colorado

Brad Neuschwanger, Tracy Davis, Chris Praamsma, and David Karr
Aquatic Wildlife Research Section, Bellvue Fish Research Hatchery, Bellvue, Colorado

The following describes two experiments conducted at the Colorado Parks and Wildlife Bellvue Fish Research Hatchery (BFRH) examining the growth and health of, and angler preference for fish reared on the basic feeds from four different feed manufacturers. In the first experiment, the manufacturer's recommendations for feeding rates (percent body weight per day) were followed. In the second experiment, feeding rates were standardized across the four feeds. A third experiment was conducted in the second year to examine the effects of size-at-stocking and feed on post-stocking survival.

Citation: Fetherman, E. R., B. Neuschwanger, T. Davis, C. Praamsma, and D. Karr. 2019. Colorado hatchery feed experiments. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado

Experiment 1 – Manufacturer's Recommendations for Feeding Rates

Motivation

The purchase of commercial feeds for large scale hatchery production often dominates the operating budget for many state-run hatchery facilities. In general, feed costs are the largest expenditure for finfish producers (Trushenski et al. 2006), with feed costs covering up to 60-70% of the total expense of trout farming (Kim 1997). As such, hatcheries strive to minimize feed loss to improve profitability and reduce environmental impacts through changing portion sizes (Bailey and Alanärä 2006), assessing delivery methods (Wagner et al. 1996; Noble et al. 2007), and improving feed efficiency (Silverstein 2006). Selecting diets formulated and well suited for a target species can also help overcome financial challenges (Trushenski et al. 2006). Growing larger fish from the same amount of feed (i.e., improving feed efficiency) could not only reduce production costs, but also the environmental impacts of fish farming (Silverstein 2006).

Costs for diet formulations vary widely depending on ingredients, with protein representing the largest single and most costly component of finfish diets. Although fish meal is the "ideal protein" because the amino acid profile of the feed mimics the whole-body amino acid profile of the animal being fed, alternative sources of protein have been sought to reduce cost (Trushenski et al. 2006). The use of alternative protein sources, as well as variety in other ingredients such as lipids/fats, micronutrients, and fillers, may result in reduced cost, but also reduced feed intake and increased feed conversion ratios. Therefore, lower cost feeds may not always produce the best growth, potentially resulting in more food waste and higher long-term costs than more expensive feeds when evaluated on a cost-per-fish basis. The objective of the 2016 hatchery feed experiment was to assess differences in growth, condition, appearance, taste, and production cost per rainbow trout using four commercial trout feeds to determine if statewide annual and long-term production costs could be reduced using different commercial diets.

Feeds Evaluated

Table 1.1. Feed size and corresponding percent (%) crude protein, % crude fat, and description of the feed from the catalogs provided by EWOS, Skretting, Bio Oregon, and Rangen.

Feed Size	% Crude Protein	% Crude Fat	Description
			EWOS
#0 and #1 #2 and 1.2 mm	54 53	16 20	Premium fish oil and low temperature fishmeal give fry the best start in order to maximize survival and growth throughout the production cycle; premium ingredients and a high quality final product ensure high digestibility and excellent water quality. Particle size has been designed to match the ability of fish to consume feed.
1.5, 2, 3, 4, 5, 7, and 9 mm	43	14	Proven formulation with moderately high protein and low fat levels that delivers good growth potential while minimizing cost. Premium fishmeal and a blend of highly digestible alternative raw materials ensure good feed conversion.
			SKRETTING
#0, #1, and #2	52	16	Nutrient-rich, crumbled starter feed suitable for trout, steelhead, and a range of other cold and warm water species, produced from a highly digestible extruded pellet.
1 and 2 mm	45	16	Medium-energy, extruded sinking or floating fry diet, specifically formulated for good growth and healthy fry.
3, 4, 6, and 8 mm	40	12	Proven low-cost, low-energy trout feed.
			BIO OREGON
#0 #1 and #2	53 52	18 20	Combines traditional dietary values with an increased level of alternative ingredients to reduce cost; contains an enhanced vitamin pack and natural pigment to promote healthy fish and natural coloration; natural palatability enhancers ensure an active first feeding response.
1.2, 1.5, 2, 2.5, and 3 mm	47	18	Mid-level energy fish feed for moderate or controlled growth; includes alternative ingredients to reduce cost; contains enhanced vitamin pack and pigment to promote healthy fish and natural coloration.
4 mm 6 mm 9 mm	45 43 40	24 24 24	High-energy trout and steelhead diet designed to give maximum growth and the lowest feed conversion rates. Pigment is included in sizes 6 and 9 mm to promote natural coloration.
			RANGEN
#0, #1, and #2	52	16	Especially formulated for first feeding fry and fingerling; nearly free of dust and fines; high levels of quality fish and animal protein; utilizes marine oils, beta glucans, pigments, and high vitamin levels, including stabilized vitamin C.
#3 and #4	45	15	Nutritionally complete and balanced; main components are high quality fish and animal proteins; finely ground to ensure superior digestibility and that fish receive full complement of nutrients.
3/32”, 1/8”, and 5/32”	40	12	Sinking pellets manufactured by steam pelleting; suitable for fish from 15 grams to 900 grams or more.
3/16” and 1/4”	40	10	Includes high levels of vitamins, minerals, and antioxidants; unique ingredients and balanced formulation proven to aid in the well being of brood fish and viable egg production; extrusion process allows slow sinking or floating pellets.

Feeds from four commercial trout feed companies were evaluated in this experiment, EWOS, Skretting, Bio Oregon, and Rangen. EWOS is a Canada based company that produces feeds for a variety of aquaculture species including salmon, trout, bass, sturgeon, flounder, and shrimp, among others. EWOS is a group of aquaculture feed producers that conduct research on nutrition and feeding, and manufacture and ship feeds globally. Skretting is a multinational feed corporation, and Skretting USA is a Utah based subsidiary whose mission is to deliver outstanding nutrition to produce sustainable, healthy, and delicious food, and produces a wide range of feeds for both coldwater and warmwater species. Bio-Oregon is an Oregon based company that commits itself to producing top quality fish feeds backed by extensive research and highly developed logistics, and is focused on trout and salmon feed production. Rangen, Inc. is an Idaho based company with specialty operations in aquaculture, general feeds, and commodities. The Aquaculture Division at Rangen, Inc. produces and markets specialized high quality aquaculture feeds for trout, salmon, and shrimp. Rangen, Inc. is the current provider of feed to Colorado's hatcheries.

To maintain low cost and consistency among the four feed companies, the basic feeds from each company were used in this experiment. Each company uses slightly different proportions of crude protein and crude fat in their diets, and proportions change with a change in feed size (Table 1.1). Though proportions are similar among diets produced by the four companies, the type and source of ingredients (often proprietary) used to produce the diets result in differences in cost and proposed feed conversion ratios. Each company also has their own recommendations for feeding rates (Tables 1.2, 1.3, 1.4, and 1.5), and these recommendations were followed to ensure that estimates of feed conversion and growth were obtained in a manner consistent with the expectations developed by each company for their feeds.

Table 1.2. EWOS suggested feeding rate (percent body weight per day [% BW/d]) by feed size, fish size, and at a temperature of 52-54°F. Note that EWOS's guidelines are for a moderate growth rate and about 5-6 feedings per day for starter sizes #0 and #1.

Feed Size	Count per Pound	Weight (g)	Feeding Rate
#0	5000-1500	0.09-0.30	2.41
#1	1500-1000	0.30-0.45	2.40
#1	1000-800	0.45-0.57	1.94
#1	800-600	0.57-0.76	1.73
#2	600-500	0.76-0.91	1.62
#2	500-300	0.91-1.5	1.5
1.2 mm	300-175	1.5-2.6	1.44
1.2 mm	175-100	2.6-4.5	1.41
1.5 mm	100-50	4.5-9.1	1.28
2.0 mm	50-20	9.1-22.7	1.14
3.0 mm	20-10	22.7-45	0.88
4.0 mm	10-5	45.4-91	0.78
5.0 mm	5-0.5	90.8-908	0.72

Table 1.3. Skretting suggested feeding rate (% BW/d) by feed size, fish size, and at a temperature of 54°F.

Feed Size	Count per Pound	Length (in)	Weight (g)	Feeding Rate
#0	3000-570	Hatch-1.7	0.15-0.8	3.3
#1	570-300	1.7-2.1	0.8-1.5	3.1
#2	300-150	2.1-2.6	1.5-3.0	3.0
1.0 mm	150-60	2.6-3.1	3-8	2.9
2.0 mm	60-11	3.1-4.6	8-40	2.4
3.0 mm	11-6	4.6-7.4	40-80	1.4
4.0 mm	6-1.5	7.4-12.5	80-300	1.0

Table 1.4. Bio Oregon suggested feeding rate (% BW/d) by feed size, fish size, and at a temperature of 54°F.

Feed Size	Count per Pound	Length (in)	Weight (g)	Feeding Rate
#0	3000-570	Hatch-1.7	0.15-0.8	3.3
#1	570-300	1.7-2.1	0.8-1.5	3.1
#2	300-150	2.1-2.6	1.5-3.0	3.0
1.2 mm	150-90	2.6-3.1	3.0-5.0	2.9
1.5 mm	90-60	3.1-3.5	5.0-8.0	2.7
2 mm	60-25	3.5-4.6	8.0-18	2.4
2.5 mm	25-11	4.6-6.0	18-40	1.9
3 mm	11-6	6.0-7.4	40-75	1.4
4 mm	> 6	> 7.4	> 75	1.0

Table 1.5. Rangen suggested feeding rate (% BW/d) by feed size, fish size, and at a temperature of 53°F.

Feed Size	Count per Pound	Length (in)	Weight (g)	Feeding Rate
#0	< 1,200	< 1.3	< 0.4	5.4
#1	1,200	1.3	0.4-0.8	5.4
#2	600	1.5	0.8-1.5	4.5
#2	300	2.0	1.5-2.3	3.9
#3	200	2.3	2.3-4.5	3.5
#3	100	2.8	4.5-6.0	2.9
#4	80	3.0	6.0-8.0	2.7
#4	60	3.5	8.0-11.0	2.5
3/32”	40	4.0	11.0-15.0	2.3
3/32”	30	4.5	15.0-21.0	2.2
3/32”	22	4.8	21.0-30.0	2.0
1/8”	15	5.5	30.0-38.0	1.8
1/8”	12	6.0	38.0-50.0	1.7
5/32”	9	6.5	50.0-76.0	1.6
5/32”	6	7.5	76.0-114.0	1.4
3/16”	4	8.5	114.0-151.0	1.3

Two samples of 125 grams each were retained from each feed size from each feed company. An analysis is being conducted by the U.S. Fish and Wildlife Service Bozeman Fish Technology Center (Bozeman, Montana) to ensure that the protein and lipid ratios are the same as those stated on the label for each feed. At the time of writing this report, the results of this analysis were not yet available. In addition, samples were dried and weighed to calculate amount of dry matter in each feed size and diet. The feed conversion ratio (see below) is dependent upon the weight of dry matter consumed in relation to the average individual weight gained, not total weight of the feed provided.

Hatchery Feed Experiment Methods

Rainbow trout (pure Hofer [GR]) used for this experiment were spawned at the BFRH in December 2015. A single male-female pair was used to create all of the eggs needed for this experiment as relationships among feed intake, growth, and feed efficiency are easier to determine using full-sib families (Silverstein 2006). Eggs were distributed to egg cups contained within four, 20-gallon experimental tanks. Eggs were sized using a von Bayer trough (Piper et al. 1982), and initially counted by hand to determine the volume of eggs (mL) needed for each egg cup. This known volume was used to distribute eggs to each of the four egg cups. Egg mortality was monitored and recorded throughout the egg rearing process. After hatching, dead eggs and cripples were removed from the egg cups and recorded. Upon 50% swim up, which occurred on January 29, 2016, fish were released into their tanks to begin feeding. Each tank initially contained between 812 and 817 swim-up fry.

Fish may take to feed better on different diets depending on attraction and palatability of the feed. Therefore, fish were fed the starter diet for the feed company to which each tank had been assigned. Feed companies were assigned to starter tank using a random number generator. Prior to feeding, a subset of 20 fish was removed from the tank and individually measured (total length; TL) and weighed to provide a baseline for estimation of feed conversion and growth in the first week post-swim-up. The average weight per fish and the number of fish per tank were used to set the daily feed amounts based on the recommended rate (percent body weight per day [%BW/d]) for each feed company (Tables 1.2, 1.3, 1.4, and 1.5). Fish were fed eight times daily. Twenty fish were similarly measured and weighed to adjust feed amounts after the first week. Mortality was monitored and recorded to determine the percentage of fish that did not take to feed in each tank. At the end of the second week, another 20 fish were measured and weighed to estimate feed conversion and growth in the second week post-swim-up. Feeding fish for two weeks post-swim-up helped ensure that all fish included in the hatchery feed experiment were actively feeding prior to the start of the experiment. Data from the first two weeks was used to compare initial growth rates and feed conversion rates among the feed companies.

Table 1.6. Assignment of feed company to tank, assigned using a random number generator.

Feed Company	Tank
EWOS	1
Skretting	2
Bio Oregon	3
Rangen	4
Skretting	5
EWOS	6
Rangen	7
Bio Oregon	8
Rangen	9
Skretting	10
Bio Oregon	11
EWOS	12

The hatchery feed experiment was started at two weeks post-swim-up, at which time 150 fish each were counted out of the starter tank and distributed into three replicate, 10-gallon glass tanks for each feed company in FR1 (see Table 1.6 for tank assignments). Any remaining fish in the starter tank were counted, euthanized, and retained for proximate analyses. Counts and mortality records were used to determine the starting number of fish per tank at swim-up and to back-calculate the mortality rate of fish that did not take to feed. An initial sample weight was taken for each tank by placing all 150 fish for a given replicate tank in a tarred water bucket on a scale, obtaining individual weights by dividing the total weight by the known number of fish, and calculating the number of fish per pound. This known weight was used to assign a feeding rate (% BW/d) and calculate total amount of feed per day (g) for each tank. In addition, a subset of 20 fish were individually measured and weighed to calculate a Fulton's condition factor (K; Ney 1999) at the onset of the experiment.

Feeding occurred six times daily while fish remained in FR1, with one sixth of the day's total ration delivered to the tank at each feeding. It was assumed that all feed given to the fish was consumed for the purpose of calculating the feed conversion ratios. Given the GR's voracious appetite and ability to consume a large portion of the food presented to them, this assumption was likely met during this experiment. Throughout the entirety of the experiment, tanks and raceways were fed in a clockwise or counterclockwise direction, alternating rotations between the two directions, and the tank with which feeding began advanced by one tank daily. For example, on day one, tank 1 was fed first, and feeding occurred in a clockwise direction. On day two, tank 2 was fed first, and feeding occurred in a counterclockwise direction. This prevented an anticipated feeding response resulting from feeding in the same order every day that could have increased pre-feeding energy use and affected consumption efficiency.

Table 1.7. Summary of sampling times and feed delivery methods by fish size and Colorado State Fish Hatchery.

Hatchery	Sample	Fish Size	Feeding Method
Pueblo (PUE)	Once a week	Less than 5”	Hand
	Once a month	Greater than 5”	Hand
Monte Vista (MVU)	Once a month	Less than 3”	Automatic Feeders
	Once a month	Greater than 3”	Hand
Spicer Facility (SLS)	Once a month	All	Hand
Durango (DUR)	Once a week	Subcatchables	Hand
Pitkin (PKN)	2-4 times per month	Less than 3”	Hand
	1-2 times per month	3-5”	Hand
	Once a month	Greater than 5”	Blower
Finger Rock (FRO)	Bi-weekly	All	Hand Blower in raceways
Roaring Judy (ROJ)	Once a week	Subcatchables	Hand Belt Feeder
	Once a month	Catchables	Hand up to 15/lb Blower
Chalk Cliffs (CCL)	Once a month	Subcatchables	Hand
		Larger than 20/lb	Truck
Glenwood Springs (GSU)	Once a month	Subcatchables	Hand Automatic Feeders
Bellvue-Watson (BWT)	Once a week	Less than 3”	Hand
	Bi-weekly	Greater than 3”	Hand
		Greater than 5”	Demand Feeders (supplemental)
Mount Shavano (MSO)	Biweekly	Less than 5”	Sweeny Vibratory Feeders Hand (3-8 times/day)
		Greater than 5”	Belt Feeders Hand (2 times/day)
Rifle (RIF)	Every 2-3 months (Rely on growth charts)	Subcatchables	Hand
		Catchables	Blowers

Two to three batch weights of 20 fish each were obtained from each tank on a weekly basis and amount of feed fed per day was adjusted based on these weights. This sampling schedule was similar to that used by other state hatcheries which sample smaller fish once a week, with time between samples increasing as fish get larger (Table 1.7). Daily feed amounts were adjusted for mortalities based on the average weight of an individual fish from the previous weekly sampling event. Once a given tank reached the maximum average individual weight of the range for a given feed size, the tank was switched to the next size of feed and/or to a different feeding rate (e.g., Rangen suggests multiple changes in feeding rate within each feed size [Table 1.5]). A subset of 20 fish were individually measured and weighed on the day that feed size was changed (note that a subset of fish were not processed when feeding rate changed within a feed size, as with Rangen). Fifteen of the 20 fish were returned to the tank after being processed. The remaining five fish were euthanized, dissected to obtain liver and viscera weights, and retained for proximate analysis. Fin condition was also accessed on all 20 fish. Fin condition can be assessed to determine differences in fish appearance when using different feeds and feed delivery methods using the Health/Condition Profile system (HCP; Goede and Barton 1990), which uses a rating scale between 0 and 3 and is based on the degree of hemorrhaging. Wagner et al. (1996) modified the HCP fin index to base scores on fin length, with 0 = perfect fin, 1 = slight erosion, and 2 = severe erosion. Fins were visually assessed for fin length using the scale developed by Wagner et al. (1996).

To maintain suggested density indices of pounds per cubic foot less than or equal to half of the fish length in inches (Piper et al. 1982), fish started in the 10 gallon glass tanks in FR1. Upon reaching an average of 5 grams per fish, fish were moved to 20 gallon aluminum tanks within FR1, and the number of fish was counted and confirmed. Once fish reached an average of 18 grams per fish, they were moved from the tanks in FR1 to the BFRH fiberglass hatchery troughs. Again, the number of fish was counted and confirmed upon moving fish to the hatchery. Twelve hatchery troughs were used to rear the fish inside the hatchery to maintain replication. Fish in the hatchery were fed four times daily. Fish were held in one half of the trough until they reached an average of 75 grams per fish, at which point the divider was removed and the fish were allowed to use the entire trough for the remainder of the growth experiment. The experiment was concluded once fish reached an average of 200 grams of fish, or on November 1, 2016, whichever came first. At the end of the experiment, all fish remaining in a hatchery trough tank were measured and weighed, and 20 fish were euthanized, dissected to obtain liver and viscera weights, and retained for proximate analyses. Fifteen fish from each tank were then moved to round tanks where they continued to be fed on the same size and ration of feed until they were used in the fish preference experiment (described below).

There are a number of standard metrics used to evaluate growth performance in feed comparison experiments, including weight gain (%), feed conversion ratio (FCR), specific growth rate (SGR; percent body weight per day [% BW/d]), feed intake (% BW/d), hepatosomatic index (HSI), and viscerosomatic index (VSI; Trushenski et al. 2011; Gause and Trushenski 2013),calculated using the following formulas:

$$Weight\ gain = 100 \times \frac{average\ final\ weight - average\ initial\ weight}{average\ initial\ weight}$$

$$Feed\ conversion\ ratio = 100 \times \frac{average\ initial\ feed\ consumption\ (dry\ matter)}{average\ individual\ weight\ gain}$$

$$Specific\ growth\ rate = 100 \times \frac{\log_e(average\ final\ weight) - \log_e(average\ initial\ weight)}{days\ of\ feeding}$$

$$Feed\ intake = 100 \times \frac{total\ dry\ matter\ intake\ /(initial\ individual\ weight \times final\ individual\ weight)^{0.5}}{days\ of\ feeding}$$

$$HSI = 100 \times \frac{liver\ weight}{BW}$$

$$VSI = 100 \times \frac{total\ viscera\ weight}{BW}$$

Average individual values were calculated by dividing tank values by the number of fish in the tank at the time. Parameters associated with feed consumption were based on average individual values calculated on a daily basis (i.e., average consumption values were calculated daily and summed over the course of the trial; Gause and Trushenski 2013). Weight gain, FCR, SGR, and feed intake were also calculated for each size of feed for each company. HSI and VSI were computed for fish that were ≥ 2 grams; HSI and VSI were not calculated for feed sizes in which the average weight per fish was < 2 grams due to difficulty of dissection. The HSI and VSI indicate the amount of energy reserves stored in the liver and as fat in the viscera, excess energy that could be used during periods of low food availability after being stocked. The higher the HSI and VSI, the higher the amount of stored energy that can be utilized at a later date.

In addition to the growth metrics listed above, the coefficient of variation in length and weight was used to determine if certain feeds produce a wider range in variation in size than others (Wagner et al. 1996). The coefficient of variation (CV) is calculated as $CV = \frac{s}{\bar{y}}$, where s is the standard deviation in length or weight, and $\bar{y}$ is the mean. The CV was calculated for each size of feed from each company and used to determine when size variation began to occur during the experiment, if at all. Mortality, an important metric for assessing feed quality, especially at smaller sizes while fish are taking to feed (Kientz et al. 2012), was calculated for each size of feed for each company, as well as for the entire growth period from hatch to the end of the experiment.

An analysis of variance (ANOVA) implemented in SAS PROC GLM (SAS Institute 2016) was used to determine if there were differences in mortality, length, and weight between the feed companies following the first two weeks of feeding. Similarly, an ANOVA was used to determine if there were differences in overall feed conversion ratios, HSI, VSI, fin condition, CV length, CV weight, and mortality among the feed companies at the end of the experiment. Overall growth differences were not comparable since all feed companies did not reach the target weight of 200 grams per fish by the end of the experiment.

Because feed size changes occurred at different fish sizes within each of the feed companies, growth and health metrics were not comparable among the feed companies throughout the majority of the experiment. Within a feed company, an ANOVA (n = 4) was used to compare differences in CV length, CV weight, HSI, VSI, and fin condition among the feed sizes. Summary statistics are provided for mortality, FCR, SGR, weight gain, feed intake, and K for each feed size within each feed company. Colorado hatcheries stock fish at various sizes including *Myxobolus cerebralis*-negative subcatchables at 75 mm, *M. cerebralis*-positive subcatchables at 150 mm, and *M. cerebralis*-negative and –positive catchables at 250 mm. Results from the various feed sizes are discussed in relation to these fish sizes at stocking.

Fish Preference Methods

Colorado stocks over 2.5 million catchable rainbow trout annually. Often, these fish are caught by anglers and taken for consumption shortly after being stocked. As a continuation of the hatchery feed experiment, thirty people, consisting of CPW employees and the general public, participated in two preference tests pertaining to the appearance and taste of fish reared on the feeds from three feed companies, Skretting, Bio Oregon, and Rangen. EWOS was not included in this portion of the experiment because fish had not attained a large enough size to be comparable to the other three feed companies (see results section below). Preference tests were conducted at the Salud! Cooking School attached to the Whole Foods Market in Fort Collins, Colorado. The objective of these preference tests was to determine if there were differences in appearance and taste based on the feed company used to rear catchable rainbow trout in Colorado hatcheries.

For the appearance test, two fish from each of the three feed companies were randomly placed in clear, 20 gallon tanks. Feed company was not known to those participating in the test to reduce bias. Participants were asked to rate a number of metrics for the fish in each tank including fish color, fin quality, total length, body depth, head shape, body shape, and overall satisfaction. The rating scale ranged from 1 to 10, with 1 being completely unsatisfied with the appearance and 10 being completely satisfied with the appearance for each metric.

Two volunteer chefs prepared fish for the taste test. Chefs were asked to choose their favorite preparation style for the test. One chef pan seared the fish, and the fish were served with green beans and a white wine cream sauce. The other chef brined and smoked the fish prior to the event, and served the fish over a corn cake with cilantro lime sour cream. Because the chefs were the only impartial observers to work with the fish in their raw form, they were asked to rate several variables relating to fish appearance, workability, and overall satisfaction. Ratings were based on the chef's previous experience working with and preparing fish. The rating scale ranged from 1 to 10 and varied based on the characteristics being rated. Fillet color rating scale ranged from light (1) to dark (10). Aroma ranged from not fishy (1) to fishy (10). Texture ranged from not firm (1) to firm (10). Moisture content ranged from dry (1) to juicy (10). Tenderness ranged from tough (1) to tender (10). Workability ranged from falling apart and hard to work with (1) to staying together and easy to work with (10). Lastly, chef's were asked to rate the overall acceptability of the fish ranging from not acceptable (1) to acceptable (10).

All three feed companies included in the test were prepared in the same manner by each chef. In addition, Whole Foods Market (WFM) rainbow trout were included as a store-purchased control to see how rainbow trout reared by Colorado hatcheries compared to those sold by Whole Foods. Participants were provided fish from each of the four groups, prepared in the two different styles (eight total fish to rate), throughout the evening. The order in which fish were presented to the participants was randomized to prevent association with a feed group between preparation styles, and participants were asked to independently rate each fish rather than try to do a comparison among the fish. Similar to the rating scale for appearance, the taste test rating scale ranged from 1 to 10, with 1 being completely unsatisfied with a given quality and 10 being completely satisfied with a given quality. Participants rated the fillet color, fishiness, fish texture, palatability, overall flavor (of the fish, not the cooking style) and overall satisfaction with taste. Participants were also asked to rate satisfaction with style of preparation. An ANOVA was used to compare differences in satisfaction for each quality included for appearance, chef preparation, and taste tests among the three feed companies (and the WFM fish included in the chef preparation and taste test).

Hatchery Feed Experiment Results

Table 1.8. Comparisons of overall survival (%), feed conversion (grams of feed per gram of fish [g feed/g fish]), weight gain (%), specific growth rate (SGR; % body weight per day [% BW/d]), feed intake (% BW/d), length (mm; CV length in parentheses), weight (g; CV weight in parentheses), and average fin rating among the four feed companies (EWOS, Skretting, Bio Oregon, and Rangen) within the first two weeks post-swim-up. Different letters within the same row for a given metric represent significant differences among the feed companies.

Metric	**EWOS**	**Skretting**	**Bio Oregon**	**Rangen**
Overall Survival	99.27^{z}	99.88^{z}	99.75^{z}	99.51^{z}
Week 1	99.39	99.88	99.88	99.63
Week 2	99.87	99.87	99.87	99.87
Feed Conversion	0.41	0.42	0.33	0.56
Weight Gain	131.06	176.14	128.96	227.74
SGR	4.93	5.97	6.90	6.98
Feed Intake	2.08	2.64	2.31	4.17
Length (CV)				
Start	22.10^{z} (0.03)	22.55^{z} (0.04)	22.50^{z} (0.04)	22.05^{z} (0.05)
Week1	27.40^{z} (0.04)	27.35^{z} (0.05)	27.75^{z} (0.04)	27.55^{z} (0.05)
Week2	28.65^{z} (0.08)	28.95^{z} (0.09)	29.80^{z} (0.07)	31.90^{y} (0.04)
Weight (CV)				
Start	0.09^{z} (0.15)	0.09^{z} (0.10)	0.09^{z} (0.12)	0.08^{z} (0.12)
Week1	0.18^{z} (0.21)	0.20^{z} (0.16)	0.21^{z} (0.12)	0.20^{z} (0.17)
Week2	0.21^{x} (0.25)	0.24^{xy} (0.28)	0.27^{yz} (0.20)	0.30^{z} (0.12)
Average Fin Rating	0.00	0.00	0.00	0.00

There were no significant differences in overall survival in the first two weeks among the feed companies (Table 1.8), indicating that fish took to feed equally on all four feed types. Feed conversion (grams of feed needed to produce one gram of mass) varied among the feed companies, with Bio Oregon having the lowest feed conversion rate and Rangen having the highest feed conversion rate. The lower the feed conversion rate, the more efficiently fish were able to convert feed to mass. Weight gain, specific growth rate, and feed intake also varied among the feed companies, and all three metrics were highly dependent upon the feeding rate used for each feed company (Tables 1.2, 1.3, 1.4, and 1.5). Length and weight did not differ at the start of the experiment, nor after one week of feeding. However, by the end of week two fish fed Rangen were significantly longer than fish fed the other three feed companies, and significantly heavier than fish fed EWOS or Skretting. Although fish fed EWOS, Skretting, and Bio Oregon did not differ in length at the end of the first two weeks, fish fed EWOS were significantly smaller than fish fed Bio Oregon; the weight of fish fed Skretting and Bio Oregon did not differ. No fin wear was observed in the first two weeks, with fish from all the feed companies having a fin rating of 0 (Table 1.8).

Table 1.9. Comparison of survival (%), feed conversion (g feed/g fish), weight gain (%), specific growth rate (SGR; % BW/d), feed intake (% BW/d), length (mm), CV length, weight (g), CV weight, Fulton's condition factor (K), hepatosomatic index (HSI), viscerosomatic index (VSI), and fin rating among the seven feed sizes used from EWOS. Different letters within the same row for a given metric represent significant differences among the feed sizes.

Metric	Size 0	Size 1	Size 2	1.2 mm	1.5 mm	2.0 mm	3.0 mm
Survival	100	98.83	100	99.75	99.19	98.05	99.39
Feed Conversion	0.48	0.48	0.45	0.55	0.62	0.67	0.70
Weight Gain	69.18	142.00	108.54	188.60	96.92	139.86	73.07
SGR	4.04	3.48	2.87	2.29	1.71	1.49	1.14
Feed Intake	1.97	1.73	1.29	1.31	1.07	1.03	0.81
Length	32.25	41.75	53.15	73.33	92.95	123.15	151.48
CV Length	0.08^{b}	0.08^{bc}	0.12^{a}	0.11^{ac}	0.11^{ac}	0.11^{abc}	0.08^{bc}
Weight	0.32	0.80	1.85	4.35	9.18	22.54	40.44
CV Weight	0.21^{b}	0.26^{abc}	0.36^{a}	0.33^{ac}	0.32^{abc}	0.31^{abc}	0.25^{bc}
K	0.95	1.08	1.18	1.07	1.10	1.16	1.14
HSI	N/A	N/A	1.00^{a}	1.23^{b}	1.43^{c}	1.53^{c}	1.14^{b}
VSI	N/A	N/A	9.23^{a}	9.60^{a}	10.87^{b}	10.79^{b}	8.16^{c}
Fin Rating	0.41^{a}	0.85^{b}	1.67^{c}	1.95^{de}	1.93^{de}	1.80^{d}	1.99^{de}

EWOS had the lowest suggested feeding rates of the four feed companies, starting at only 2.41% and dropping to 0.88% of the fish body weight per day by the end of the experiment (Table 1.2). Despite these low feeding rates, survival remained greater than 98% for each of the seven feed sizes, and did not differ among feed sizes (Table 1.9). Feed conversion increased with an increase in feed size, as is expected with larger fish and larger feed sizes. Weight gain was variable among the feed sizes and was dependent upon both the length of time fish were on a given size and the amount of feed provided of that size. Specific growth rate and feed intake both generally decreased with an increase in feed size, and were dependent upon feeding rate. This is the case for all four feed companies. Coefficients of variation in length and weight were smaller at the beginning of the experiment when fish were on size 0 and size 1 feed, increased when fish were on size 2, 1.2 mm, 1.5 mm, and 2.0 mm, and dropped towards the end of the experiment when fish were on 3.0 mm. Condition factor (K) was lowest when fish were on size 0 feed. Though variable, K was fairly similar across the remainder of the feed sizes. HSI and VSI were highest in fish on sizes 1.5 and 2.0 mm, suggesting that these feeds contained ingredients that allowed an increase in stored energy despite the low feeding rate. However, both metrics were reduced by the end of the experiment, suggesting that energy reserves were being used to compensate for the low feeding rates. Fin wear increased quickly at the beginning of the experiment, with fin ratings greater than 1.5 (up to 50% of the fin missing) observed in fish fed on the five largest feed sizes (Table 1.9). Fin wear likely occurred due to competitive interactions for the limited food availability as a result of the low feeding rates.

Myxobolus cerebralis-negative subcatchables would be stocked shortly after being on size 2 feed. Although K was highest for fish fed size 2 feed, HSI and VSI were low, suggesting that these fish would have very little stored energy and should be stocked in locations or at a time of year when food availability is high. Fin wear was evident in these fish, but given that they would not be immediately caught by anglers, it is likely the fins would regenerate prior to being caught. *Myxobolus cerebralis*-positive subcatchables would be stocked after being on 3.0 mm feed. Fish K was still relatively high in these fish, but HSI and VSI values suggest that these fish should be stocked when food availability is high. Fin wear was evident in these fish, and would be more noticeable if caught shortly after being stocked. Fish fed EWOS did not reach catchable size by the end of the experiment.

Table 1.10. Comparison of survival (%), feed conversion (g feed/g fish), weight gain (%), specific growth rate (SGR; % BW/d), feed intake (% BW/d), length (mm), CV length, weight (g), CV weight, Fulton's condition factor (K), hepatosomatic index (HSI), viscerosomatic index (VSI), and fin rating among the seven feed sizes used from Skretting. Different letters within the same row for a given metric represent significant differences among the feed sizes.

Metric	Size 0	Size 1	Size 2	1.0 mm	2.0 mm	3.0 mm	4.0 mm
Survival	99.78	99.77	100	100	100	98.12	98.86
Feed Conversion	0.47	0.48	0.53	0.56	0.75	0.90	1.00
Weight Gain	328.33	60.08	108.81	171.90	364.62	88.23	107.61
SGR	5.39	5.17	4.52	4.26	2.74	1.35	0.92
Feed Intake	2.77	2.45	2.43	2.49	2.25	1.24	0.94
Length	45.60	52.87	67.45	93.98	151.62	184.12	241.93
CV Length	0.05^{a}	0.06^{a}	0.08^{ab}	0.07^{a}	0.08^{a}	0.14^{b}	0.10^{ab}
Weight	1.06	1.74	3.72	9.45	43.92	76.70	174.56
CV Weight	0.17^{a}	0.20^{a}	0.24^{ab}	0.21^{ab}	0.23^{ab}	0.32^{b}	0.23^{ab}
K	1.11	1.15	1.19	1.13	1.23	1.17	1.20
HSI	N/A	1.75^{a}	1.61^{a}	2.47^{b}	1.66^{a}	1.77^{a}	2.11^{c}
VSI	N/A	12.75^{bcd}	11.89^{bde}	13.73^{c}	11.89^{de}	11.33^{ae}	10.37^{a}
Fin Rating	0.25^{de}	0.15^{e}	0.15^{e}	0.47^{dc}	0.43^{dc}	1.02^{b}	1.55^{a}

Suggested feeding rates for Skretting fell between those of EWOS and Rangen, and were similar to those of Bio Oregon (Table 1.3). Survival was greater than 98% for each of the seven feed sizes, and did not differ among feed sizes (Table 1.10). Feed conversion increased with an increase in feed size, approaching 1.0 for fish on size 4.0 mm. Coefficients of variation in length and weight were smaller at the beginning of the experiment when fish were on size 0 and size 1 feed, increased when fish were on size 2, 1.0 mm, and 2.0 mm, were highest on size 3.0 mm, but dropped towards the end of the experiment when fish were on 4.0 mm. Though variable, K was fairly similar across the feed sizes, with the exception of size 0 when K was lowest. HSI and VSI were highest in fish on size 1.0 mm, suggesting that this size of feed had ingredients that allowed an increase in stored energy. Both metrics were variable but relatively high compared to other feed companies (Table 1.9 and Table 1.12) at all feed sizes. Fin wear remained fairly minimal until fish were on the last two feed sizes. Increased fin wear in fish fed 3.0 and 4.0 mm was likely due to the size of the fish relative to the size of the holding troughs and increased abrasion on the sides and bottom of the tank.

Myxobolus cerebralis-negative subcatchables would be stocked shortly after switching to a feed size of 1.0 mm. HSI and VSI were highest in fish on 1.0 mm feed suggesting that these fish would have a lot of stored energy and could be stocked in locations or at a time of year when food availability is low since the fish could depend upon these reserves until food availability increased. *Myxobolus cerebralis*-positive subcatchables would be stocked after being on 2.0 mm feed. Fish K was highest in these fish, and HSI and VSI values were still relatively high suggesting that these fish could also be stocked when food availability was low. Fin wear was not very evident in these fish. Catchable size fish stocked after being on size 4.0 mm had high stored energy in the liver, but less visceral fat than in previous sizes as indicated by the HSI and VSI values. However, stored energy is less important in these fish relative to smaller sizes since they are generally stocked in put-and-take fisheries and often caught and removed shortly after being stocked. Fin wear was evident in these fish, and would be noticeable when caught.

Table 1.11. Comparison of survival (%), feed conversion (g feed/g fish), weight gain (%), specific growth rate (SGR; % BW/d), feed intake (% BW/d), length (mm), CV length, weight (g), CV weight, Fulton's condition factor (K), hepatosomatic index (HSI), viscerosomatic index (VSI), and fin rating among the ten feed sizes used from Bio Oregon. Different letters within the same row for a given metric represent significant differences among the feed sizes.

Metric	**Mash**	**Size 0**	**Size 1**	**Size 2**	**1.2 mm**	**1.5 mm**	**2.0 mm**	**2.5 mm**	**3.0 mm**	**4.0 mm**
Survival	99.88	99.78	99.77	100	99.50	100	99.19	99.72	99.40	98.77
Feed Conversion	0.33	0.41	0.45	0.45	0.51	0.72	0.60	0.70	0.63	0.73
Weight Gain	128.96	232.78	113.46	84.50	53.17	57.94	133.36	127.74	78.94	145.49
SGR	6.90	6.01	5.42	5.22	4.56	3.26	3.31	2.35	1.91	1.28
Feed Intake	2.31	2.63	2.51	2.40	2.33	2.36	2.04	1.69	1.23	0.97
Length	27.75	43.72	55.22	66.67	74.32	89.13	118.80	154.47	185.32	249.20
CV Length	0.04	0.05^{a}	0.06^{a}	0.06^{a}	0.08^{a}	0.07^{a}	0.08^{a}	0.09^{a}	0.09^{a}	0.10^{a}
Weight	0.21	0.93	2.07	3.65	5.25	8.52	21.14	46.92	84.20	199.56
CV Weight	0.12	0.14^{a}	0.17^{a}	0.20^{a}	0.25^{a}	0.21^{a}	0.23^{a}	0.24^{a}	0.21^{a}	0.23^{a}
K	0.98	1.10	1.22	1.21	1.25	1.19	1.24	1.23	1.27	1.25
HSI	N/A	N/A	1.66^{d}	1.41^{d}	3.56^{a}	4.14^{c}	4.16^{c}	2.25^{b}	1.59^{d}	1.70^{d}
VSI	N/A	N/A	13.38^{cd}	13.01^{c}	15.95^{f}	14.51^{de}	15.49^{ef}	12.48^{bc}	11.18^{ab}	10.40^{a}
Fin Rating	0.00	0.08^{d}	0.03^{d}	0.02^{d}	0.08^{d}	0.00^{d}	0.18^{cd}	0.33^{bc}	0.43^{b}	1.72^{a}

Suggested feeding rates for Bio Oregon fell between those of EWOS and Rangen, and were similar to those of Skretting (Table 1.3). Survival was greater than 98.5% for each of the ten feed sizes, and did not differ among feed sizes (Table 1.11). Bio Oregon was the only feed company to have a size of feed smaller than size 0, and feed conversion on mash was lower than any other size or type of feed used. Feed conversion increased with an increase in feed size. However, at the end of the experiment, feed conversion was still lower (0.73) than that of similar sized fish fed Skretting (Table 1.10) or Rangen (Table 1.12). Coefficients of variation in length and weight were variable throughout the experiment, but did not differ among the feed sizes, suggesting that fish size was fairly consistent and uniform no matter which size of feed the fish were being fed. Condition was lowest when fish were on mash and size 0 feeds, but fairly similar across the remainder of the feed sizes. HSI and VSI were highest in fish on sizes 1.2 mm, 1.5 mm, and 2.0 mm suggesting that these feed sizes had ingredients that allowed an increase in stored energy. The HSI and VSI values obtained on these three feeds were also twice as high as values obtained on any other size or type of feed, suggesting that fish on these feed sizes were likely quite a bit healthier than similar sized fish fed on the other feed companies. HSI and VSI values dropped to a more normal range exhibited by the other feed companies by the end of the experiment. Fin wear remained low to non-existent throughout the majority of the experiment. Increased fin wear in fish fed 4.0 mm was likely due to the size of the fish relative to the size of the holding troughs and increased abrasion on the sides and bottom of the tank.

Myxobolus cerebralis-negative subcatchables would be stocked after being fed 1.2 mm feed. HSI and VSI values were high in these fish suggesting that these fish would have a lot of stored energy and could be stocked in locations or at a time of year when food availability is low. *Myxobolus cerebralis*-positive subcatchables would be stocked after being on 2.5 mm feed. HSI and VSI values were still relatively high in these fish suggesting that these fish could also be stocked when food availability was low. Fin wear was not evident in these fish. Catchable size fish stocked after being on size 4.0 mm had lower energy reserves, but reserves are not as necessary in these fish when stocked into put-and-take fisheries. Fin wear was evident in these fish, and would be noticeable when caught shortly after being stocked.

Rangen had the highest suggested feeding rates of the four feed companies (Table 1.5). Survival was greater than 97.5% for each of the nine feed sizes, and did not differ among feed sizes (Table 1.12). Survival was lowest at a feed size of 3/32”, lower than the survival exhibited by fish on any other feed size within the other feed companies. Feed conversion increased with an increase in feed size, approaching 1.0 towards the end of the experiment. Coefficients of variation in length and weight did not differ among any of the feed sizes, suggesting that fish size was fairly consistent and uniform no matter which size of feed the fish were being fed. Condition was lowest when fish were on size 0 feed, but fairly similar across the remainder of the feed sizes. HSI and VSI were highest in fish fed on the smaller feed sizes, and lower by the end of the experiment. HSI and VSI values were some of the lowest seen in any of the feed sizes from any feed company. Overall, HSI and VSI values suggest that none of the sizes of fish typically stocked by the state of Colorado should be stocked when food availability is low because fish fed Rangen have low stored energy reserves relative to fish on other feeds, and could starve shortly after being stocked. Fin wear remained low throughout the majority of the experiment. Increased fin wear in fish fed 5/32” and 3/16” was likely due to the size of the fish relative to the size of the holding troughs and increased abrasion on the sides and bottom of the tank. Fin wear would be noticeable in catchable-size fish caught shortly after being stocked.

Table 1.12. Comparison of survival (%), feed conversion (g feed/g fish), weight gain (%), specific growth rate (SGR; % BW/d), feed intake (% BW/d), length (mm), CV length, weight (g), CV weight, Fulton’s condition factor (K), hepatosomatic index (HSI), viscerosomatic index (VSI), and fin rating among the nine feed sizes used from Rangen. Different letters within the same row for a given metric represent significant differences among the feed sizes.

Metric	Size 0	Size 1	Size 2	Size 3	Size 4	3/32"	1/8"	5/32"	3/16"
Survival	99.56	100	99.76	99.26	99.74	97.54	100	100	99.36
Feed Conversion	0.48	0.54	0.54	0.67	0.70	0.84	0.84	0.95	0.97
Weight Gain	59.87	171.24	132.52	149.61	81.49	152.55	69.60	120.45	58.37
SGR	7.82	7.13	6.02	3.92	3.14	2.20	1.89	1.47	1.20
Feed Intake	3.74	4.00	3.35	2.73	2.23	1.91	1.60	1.44	1.17
Length	34.55	45.95	60.55	82.57	102.02	137.52	164.42	210.98	248.59
CV Length	0.06^{a}	0.08^{a}	0.06^{a}	0.08^{a}	0.09^{a}	0.10^{a}	0.08^{a}	0.08^{a}	0.07^{a}
Weight	0.42	1.15	2.80	7.05	12.25	30.87	54.13	121.18	206.23
CV Weight	0.19^{a}	0.20^{a}	0.15^{a}	0.22^{a}	0.27^{a}	0.27^{a}	0.22^{a}	0.20^{a}	0.20^{a}
K	1.00	1.16	1.26	1.22	1.12	1.15	1.19	1.26	1.31
HSI	N/A	N/A	1.46^{bc}	1.53^{b}	1.31^{ab}	1.43^{bd}	1.26^{acd}	1.11^{a}	1.27^{acd}
VSI	N/A	N/A	13.24^{d}	12.94^{d}	12.25^{cd}	11.48^{bc}	11.01^{b}	10.46^{ab}	9.67^{a}
Fin Rating	0.00^{f}	0.08^{f}	0.03^{f}	0.37^{de}	0.55^{e}	0.77^{a}	0.30^{d}	1.58^{b}	1.95^{c}

Fish fed on Bio Oregon grew faster throughout the majority of the experiment than fish fed on EWOS, Skretting, and Rangen (Figure 1.1). However, on average, fish were fed a smaller percentage of their body weight per day on Bio Oregon than on Rangen. In addition, the fish fed Bio Oregon reached the goal weight of 200 grams per fish three weeks sooner than fish fed Rangen. Skretting and Rangen performed similarly through the first half of the experiment. After mid-July, fish fed Rangen grew faster and reached larger body sizes than fish fed Skretting. This is likely because fish fed Skretting were fed at a lower percentage of their body weight per day than fish fed Rangen throughout the second half of the experiment. Fish fed Skretting did not reach a goal weight of 200 g per fish by the end of the experiment, averaging 165 g per fish at the last sample on October 25, 2016. Fish fed EWOS did not perform well compared to the other feeds. This is likely due to the recommended feeding rates provided by the company being 0.22-3.0% lower than the other companies. Fish fed EWOS averaged only 38 g per fish on October 25, 2016 (Figure 1.1).

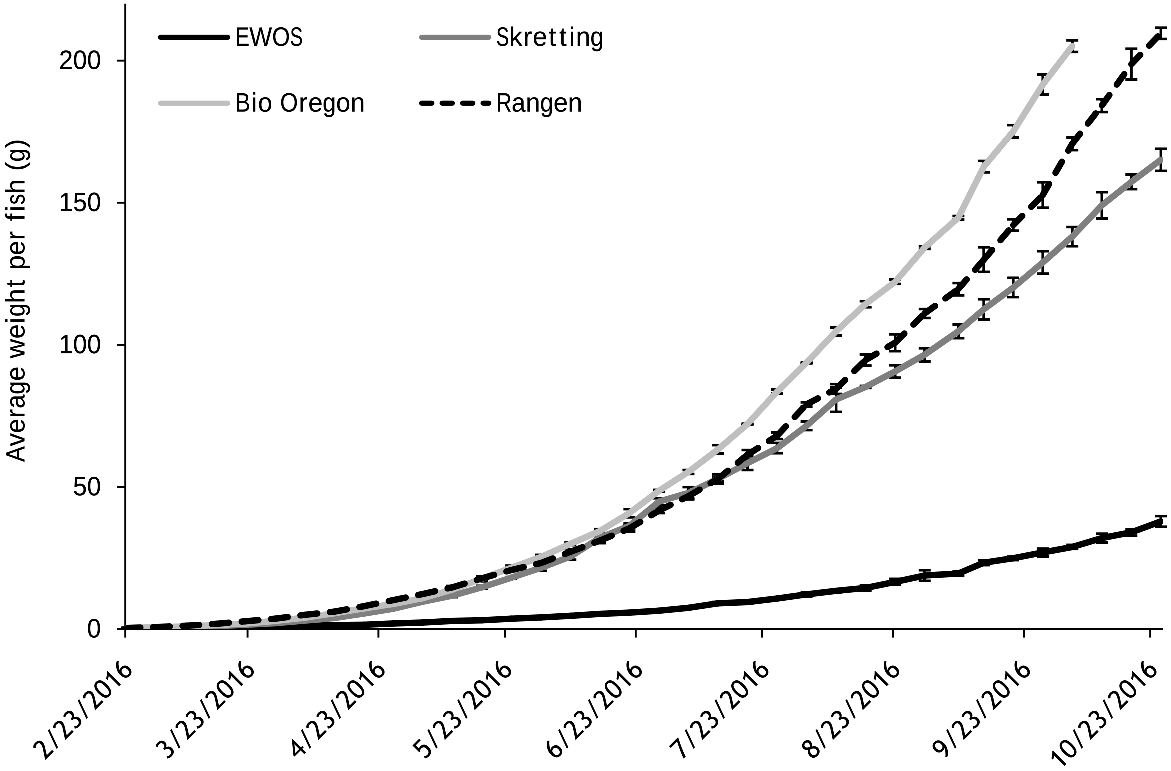

Figure 1.1. Average weekly weights of fish fed EWOS, Skretting, Bio Oregon, and Rangen. Error bars represent differences among replicates (3) within a feed company.

Over the course of the experiment, growth and health metrics varied among the four feed companies (Table 1.13). Overall feed conversion (averaged from the start to end of the experiment) was lowest in fish fed EWOS and Bio Oregon. However, the fish EWOS were still relatively small at the conclusion of the experiment when feed conversion rates are often lower. Feed conversion would likely have been higher at larger sizes for this feed company. As such, feed conversion rates for fish fed Bio Oregon were the lowest for fish reaching catchable size. Skretting and Rangen had the highest feed conversion rates, with Rangen having significantly higher feed conversion rates than Skretting. There was not a significant difference in CV length among the feed companies suggesting that fish were consistently variable in length on all four feeds. However, weight was significantly more variable in fish fed EWOS than in fish fed Skretting, Bio Oregon, or Rangen. Fish fed Bio Oregon exhibited higher HSI and VSI values than the other three feed companies. Although HSI was higher for fish fed Skretting, VSI values did not differ between Skretting and Rangen. EWOS had the lowest HSI and VSI values of the feed companies. Overall fin condition was lowest (better) in fish fed Bio Oregon, and highest in fish fed EWOS. Survival did not differ among the four feed companies (Table 1.13). Across all growth and health metrics, Bio Oregon produced the best fish overall, followed by Skretting, Rangen, and lastly, EWOS.

Table 1.13. Comparison of overall survival (%), feed conversion (g feed/g fish), CV length, CV weight, hepatosomatic index (HSI), viscerosomatic index (VSI), and fin rating (± SE) among the four feed companies. Different letters within the same row for a given metric represent significant differences among the feed companies.

Metric	**EWOS**	**Skretting**	**Bio Oregon**	**Rangen**
Survival	95.98^a (± 1.03)	97.11^a (± 1.18)	96.89^a (± 0.59)	95.56^a (± 0.59)
Feed Conversion	0.66^a (± 0.01)	0.90^c (± 0.01)	0.71^b (± 0.01)	0.94^d (± 0.01)
CV Length	0.10^a (± 0.01)	0.08^a (± 0.01)	0.08^a (± 0.01)	0.08^a (± 0.01)
CV Weight	0.29^b (± 0.02)	0.23^a (± 0.01)	0.21^a (± 0.01)	0.21^a (± 0.01)
HSI	1.22^c (± 0.02)	1.97^b (± 0.04)	2.32^a (± 0.09)	1.32^c (± 0.02)
VSI	9.14^c (± 0.14)	11.45^b (± 0.15)	12.51^a (± 0.18)	11.01^b (± 0.14)
Fin Rating	1.66^c (± 0.02)	0.97^b (± 0.03)	0.77^a (± 0.03)	1.04^b (± 0.03)

Skretting, Bio Oregon, and Rangen produced catchable-size fish by the end of the experiment. On average, it took 0.37 lbs of feed to produce a catchable fish on Skretting, 0.33 lbs of feed to produce a catchable fish on Bio Oregon, and 0.46 lbs of feed to produce a catchable fish on Rangen. Colorado produced and stocked 2,691,614 catchable rainbow trout in 2015. In order to produce this many catchable rainbow trout, 556 tons of feed would be needed of Skretting, compared to 490 tons of Bio Oregon, and 673 tons of Rangen. With natural protein sources, such as fish meal, becoming scarcer, it is important to reduce the amount of feed used to sustainably rear fish in aquaculture. Based on these results, Bio Oregon is the most sustainable for producing Colorado's catchable size rainbow trout.

Fish Preference Results

Testers preferred the appearance of fish fed Bio Oregon over those fed Skretting and Rangen, and fish fed Skretting were preferred over those fed Rangen (Table 1.14). With regard to fish color, fish fed Bio Oregon were preferred over those fed Rangen. Fish fed Skretting did not differ from those fed either Bio Oregon or Rangen. Preference for fin quality did not differ among the feed companies. The total length, body depth, and body shape of fish fed Bio Oregon were preferred over those fed Skretting and Rangen, which did not differ. Testers similarly preferred the head shape of fish fed Bio Oregon over those fed Rangen, although the head shape of fish fed Skretting did not differ from those fed either Bio Oregon or Rangen. When it came to overall satisfaction, testers preferred the appearance of fish fed Bio Oregon over those fed Rangen, but preference did not differ between Skretting and Bio Oregon or Rangen (Table 1.14).

Table 1.14. Opinion-based ratings for appearance of fish reared on three of the four feed companies included in the hatchery feed experiment. Different letters within the same column for a given metric represent significant differences among the feed companies.

Feed Company	**Average Rating**	**Fish Color**	**Fin Quality**	**Total Length**	**Body Depth**	**Head Shape**	**Body Shape**	**Overall Satisfaction**
Skretting	7.02^c	7.23^{ab}	6.97^a	6.91^b	6.68^b	6.88^{ab}	7.25^b	7.24^{ab}
Bio Oregon	7.8^a	7.59^a	7.27^a	8.01^a	8.15^a	7.41^a	8.13^a	8.04^a
Rangen	6.43^b	6.53^b	6.14^a	6.24^b	6.81^b	6.24^b	6.47^b	6.57^b

Due to a small sample size (two chefs) there were few significant differences in the raw fish among the four groups included in the taste test, three groups reared at the BFRH and the Whole Foods Market

(WFM) fish. Fillet color was the only category in which differences were seen, with the WFM fish being darker than the fish reared at the BFRH, with the exception of fish fed Bio Oregon. There was no difference in fillet color among the fish reared at the BFRH (Table 1.15).

Table 1.15. Chef ratings for the four groups of fish included in the rainbow trout taste test, three from feed companies reared at the BFRH and the Whole Foods Market (WFM) fish. Different letters within the same column for a given metric represent significant differences among the feed companies/Whole Foods Market fish.

Feed/ Supplier	Color	Aroma	Texture	Moisture	Tenderness	Workability	Acceptability
Skretting	2^{b}	2^{a}	7.5^{a}	6.5^{a}	8^{a}	5^{a}	6^{a}
Bio Oregon	3^{ab}	1.5^{a}	4^{a}	3.5^{a}	7^{a}	3^{a}	4^{a}
Rangen	2^{b}	2.5^{a}	5.5^{a}	5^{a}	7.5^{a}	5.5^{a}	6^{a}
WFM	6.5^{a}	2^{a}	8^{a}	7.5^{a}	6.5^{a}	8.5^{a}	9.5^{a}

Table 1.16. Opinion-based ratings for the taste of fish reared on three of the four feed companies included in the hatchery feed experiment and the Whole Foods Market (WFM) fish provided as controls. Different letters within the same column for a given metric represent significant differences among the feed companies/WFM fish.

Feed/ Supplier	Average Rating	Fillet Color	Fishiness	Fish Texture	Palatability	Overall Flavor	Overall Satisfaction
Skretting	5.85^{bc}	5.51^{a}	5.33^{a}	5.79^{a}	6.15^{ab}	6.12^{ab}	6.13^{ab}
Bio Oregon	6.3^{a}	6.27^{a}	5.46^{a}	6.04^{a}	6.52^{a}	6.78^{a}	6.78^{a}
Rangen	5.64^{c}	5.56^{a}	5.21^{a}	5.69^{a}	5.68^{b}	5.85^{b}	5.85^{b}
WFM	6.18^{ab}	6.21^{a}	5.46^{a}	6.12^{a}	6.28^{ab}	6.57^{ab}	6.52^{ab}

On average, fish fed Bio Oregon had the best taste, although they did not differ from the fish provided by WFM. The taste of the fish fed Bio Oregon was rated higher than fish fed either Skretting or Rangen, with participants being least satisfied with fish fed Rangen. The four groups did not differ with regards to fillet color, fishiness, or fish texture. However, fish fed Bio Oregon were more palatable and had a better overall flavor than fish fed Rangen, and people were more satisfied overall with the fish fed Bio Oregon than those fed Rangen (Table 1.16). Overall, participants preferred the pan-seared preparation (8.2 out of 10) over the smoked preparation (5.1 out of 10). Both recipes were made available for use by Colorado's anglers by putting them in the Colorado Parks and Wildlife blog (https://coloradooutdoorsmag.com/2016/11/21/taste-tested-recipes-for-your-next-trout-cookout/).

Feed Cost Comparisons

Colorado stocks millions of rainbow trout annually. In 2015, Colorado hatcheries stocked 12,447,260 *M. cerebralis*-negative subcatchable rainbow trout, averaging 2.53 in total length (TL) and 0.01 lbs, 58,604 *M. cerebralis*-positive subcatchable rainbow trout, averaging 6.16 in TL and 0.16 lbs, 1,900,652 *M. cerebralis*-negative catchable rainbow trout, averaging 10.23 in TL and 0.43 lbs, and 790,962 *M. cerebralis*-positive catchable rainbow trout, averaging 10.02 in TL and 0.41 lbs. Using the total amount of feed fed per individual, as well as the cost per pound of feed for each of the feed sizes used in the experiment (Table 1.17), the cost per fish was calculated for each size and number of rainbow trout stocked by Colorado (using the 2015 data presented above) for Skretting, Bio Oregon, and Rangen, the three feed companies that produced a catchable-size rainbow trout by the end of the experiment.

The average cost to produce a fish in the hatchery feed experiment was lowest for fish reared on Rangen, increased slightly for fish reared on Skretting, and was greatly increased for fish reared on Bio Oregon (Table 1.18). The cost per pound of Bio Oregon is \$1.00-\$1.50 greater than for Skretting or Rangen (Table 1.17). One potential reason could be the protein sources included in these feeds. Fish meal is the only protein source listed for Bio Oregon, a much more expensive protein source than some listed for Skretting and Rangen, including blood meal, feather meal, poultry by-product meal, and soybean meal, in addition to fish meal. Because the cost of the size 0 feed from Rangen is higher than Skretting (Table 1.17), it is fairly comparable to produce over 12,000,000 rainbow trout on both of these feeds (Table 1.18). However, it would be less expensive to produce the other sizes of fish on Rangen than on Skretting.

Table 1.17. Cost breakdown, by feed size, for each of the four feed companies used in the hatchery feed experiment.

EWOS		Skretting		Bio Oregon		Rangen	
Size	**Cost/lb**	**Size**	**Cost/lb**	**Size**	**Cost/lb**	**Size**	**Cost/lb**
Size 0	$1.98	Size 0	$0.98	Mash	$2.43	Size 0	$2.55
Size 1	$1.98	Size 1	$0.98	Size 0	$2.43	Size 1	$0.99
Size 2	$1.98	Size 2	$0.98	Size 1	$2.43	Size 2	$0.99
1.2 mm	$1.54	1.0 mm	$0.65	Size 2	$2.43	Size 3	$0.55
1.5 mm	$1.18	2.0 mm	$0.60	1.2 mm	$1.88	Size 4	$0.55
2.0 mm	$1.05	3.0 mm	$0.65	1.5 mm	$1.58	3/32”	$0.501
3.0 mm	$0.99	4.0 mm	$0.60	2.0 mm	$1.55	1/8”	$0.431
				2.5 mm	$1.51	5/32”	$0.431
				3.0 mm	$1.47	3/16”	$0.431
				4.0 mm	$1.36		
Average	**$1.529**	***Average***	**$0.777**	***Average***	**$1.907**	***Average***	**$0.825**

Table 1.18. Cost per fish estimates based on feed cost per size (Table 1.17) and amount of feed used per fish for Skretting, Bio Oregon, and Rangen. The cost to produce the number of fish of each size reared and stocked by the state of Colorado in 2015 are also shown, as is the total cost of feed to meet the numbers of fish produced.

Feed Company	Cost per fish	Neg Sub	Pos Sub	Neg Catch	Pos Catch	Total
Skretting	$0.23	$80,871	$5,514	$435,432	$181,206	$703,024
Bio Oregon	$0.47	$180,930	$11,104	$894,971	$372,445	$1,459,451
Rangen	$0.20	$79,064	$4,797	$386,558	$160,867	$631,286

Discussion

Fish fed Bio Oregon grew faster, had a better feed conversion rate, had more stored energy reserves for use if stocked when food availability is low, and generally had less fin wear than the other feed companies. Less feed was used to produce a catchable fish on Bio Oregon, making it more sustainable than either Skretting or Rangen. Participants also preferred the appearance and taste of fish fed Bio Oregon. However, it costs at least twice as much to raise a fish on Bio Oregon than on the other feed companies. Although fish grew slower on Skretting than on Rangen, fish tended to exhibit lower feed conversions and have more stored energy reserves than fish reared on Rangen. In addition, less feed was used with Skretting than with Rangen, and fish fed Skretting were preferred for appearance over those fed Rangen. It is slightly more expensive to produce a fish on Skretting than on Rangen, but the increased cost may be worth the benefits gained in fish health and angler satisfaction with the final product.

Currently, cost calculations are based on feed costs alone, and do not incorporate other costs incurred by the hatchery system such as transportation, equipment use and maintenance, and employee pay. In addition, other factors identified by this experiment, such as fish fed Bio Oregon reaching a goal weight of 200 g three weeks sooner than fish fed Rangen, have not been included in the cost calculations. It is expected that there would be a significant savings in equipment maintenance, water use, and personnel costs if fish could be stocked out sooner on one feed over another. Stocking fish sooner also opens up space for slower-growing strains or species, and could allow more, larger fish of these slower-growing species to be produced annually. Finally, growth differences could allow more biologist stocking requests to be met on time with the target size fish.

The difference in time to reach the target weight at the end of the experiment between Bio Oregon and Rangen, despite the fact that Bio Oregon was fed at a lower rate than Rangen suggests that there is a large difference in quality between these two feeds. The way that this experiment was designed, feed rate had more of an influence on growth than did feed quality, in general. To determine if there was a difference in feed quality resulting in differences in growth rate between the feed companies, this experiment was conducted again in 2017. In general, the design of the experiment is largely the same as Experiment 1. However, feed rates were standardized across the feed companies, ranging from 3.3% to 1.0% (see Experiment 2 below).

Experiment 2 – Standardized Feeding Rates

This experiment expands on Experiment 1, the motivation and feed descriptions for which can be found above. The first experiment examined the growth rate and health of fish fed on the basic feeds from four commercial feed manufacturers, EWOS, Skretting, Bio Oregon, and Rangen. The manufacturer's recommended feeding rate (percent body weight per day [%BW/d]) for their feeds was followed in the first experiment, with rates ranging from 5.4 to 0.72 %. Fish not only grew at different rates, but growth rate was not commensurate with feeding rate, with fish fed Bio Oregon reaching the goal weight of 200 g per fish three weeks sooner than fish fed Rangen, despite Rangen having a significantly higher feeding rate, especially for smaller fish. These results suggested that in addition to feeding rate, feed quality also likely affected growth rate.

Feeds Evaluated

To examine the effects of feed quality on growth rate, feeds from the same four commercial feed manufacturers were evaluated in this experiment. However for this experiment, the feeding rate was standardized such that all fish switched to a lower feeding rate when they reached the same size, despite which feed company was being used. Skretting and Bio Oregon have similar recommended feeding rates, ranging from 3.3 to 1.0% BW/d, and fell between the wider range of feeding rates recommended by Rangen (5.4 to 1.4% BW/d) and the narrower range of feeding rates recommended by EWOS (2.41 to 0.72% BW/d). As such, the feeding rate for all feed companies was standardized to those recommended by Skretting (Tables 2.1, 2.2, 2.3, and 2.4).

Table 2.1. EWOS standardized feeding rate (percent body weight per day [% BW/d]) by feed size, fish size, and at a temperature of 52-54°F.

Feed Size	Weight (g)	Feeding Rate
#0	Hatch-0.30	3.3
#1	0.30-0.76	3.3
#2	0.76-0.8	3.3
#2	0.8-1.5	3.1
1.2 mm	1.5-3.0	3.0
1.2 mm	3.0-4.5	2.9
1.5 mm	4.5-8.0	2.9
1.5 mm	8.0-9.1	2.4
2.0 mm	9.1-22.7	2.4
3.0 mm	22.7-40	2.4
3.0 mm	40-45.4	1.4
4.0 mm	45.4-80	1.4
4.0 mm	80-91	1.0
5.0 mm	91-908	1.0

Table 2.2. Skretting standardized feeding rate (percent body weight per day [% BW/d]) by feed size, fish size, and at a temperature of 54°F.

Feed Size	Weight (g)	Feeding Rate
#0	Hatch-0.8	3.3
#1	0.8-1.5	3.1
#2	1.5-3.0	3.0
1.0 mm	3-8	2.9
2.0 mm	8-40	2.4
3.0 mm	40-80	1.4
4.0 mm	80-300	1.0

Table 2.3. Bio Oregon standardized feeding rate (percent body weight per day [% BW/d]) by feed size, fish size, and at a temperature of 54°F.

Feed Size	Weight (g)	Feeding Rate
#0	Hatch-0.8	3.3
#1	0.8-1.5	3.1
#2	1.5-3.0	3.0
1.2 mm	3.0-5.0	2.9
1.5 mm	5.0-8.0	2.9
2 mm	8.0-18	2.4
2.5 mm	18-40	2.4
3 mm	40-75	1.4
4 mm	75-80	1.4
4 mm	>80	1.0

Table 2.4. Rangen standardized feeding rate (percent body weight per day [% BW/d]) by feed size, fish size, and at a temperature of 53°F.

Feed Size	Weight (g)	Feeding Rate
#0	Hatch-0.4	3.3
#1	0.4-0.8	3.3
#2	0.8-1.5	3.1
#2	1.5-2.3	3.0
#3	2.3-3.0	3.0
#3	3.0-6.0	2.9
#4	6.0-8.0	2.9
#4	8.0-11.0	2.4
3/32”	11.0-30.0	2.4
1/8”	30.0-40.0	2.4
1/8”	40.0-50.0	1.4
5/32”	50.0-80.0	1.4
5/32”	80.0-114.0	1.0
3/16”	114.0-151.0	1.0

Hatchery Feed Experiment Methods

Rainbow Trout (Hofer [GR]) used for this experiment were spawned at the BFRH in December 2016. A single male-female pair was used to create all of the eggs needed for this experiment as relationships among feed intake, growth, and feed efficiency are easier to determine using full-sib families (Silverstein 2006). Eggs were distributed to egg cups contained within four, 76-L (20-gallon) experimental tanks. Eggs were sized using a von Bayer trough (Piper et al. 1982), and initially counted by hand to determine the volume of eggs (mL) needed for each egg cup. This known volume was used to distribute eggs to each of the four egg cups. Egg mortality was monitored and recorded throughout the egg rearing process. After hatching, dead eggs and cripples were removed from the egg cups and recorded. Upon 50% swim-up, which occurred on January 31, 2017, fish were released into their tanks to begin feeding. Each tank initially contained between 1,175 and 1,190 swim-up fry.

Fish may take to feed better on different diets depending on attraction and palatability of the feed. Therefore, fish were fed the starter diet for the feed company to which each tank had been assigned. Feed companies were assigned to starter tank using a random number generator. Prior to feeding, a subset of 20 fish was removed from the tank and individually measured (total length; TL) and weighed to provide a baseline for estimation of feed conversion and growth in the first week post-swim-up. The average weight per fish and the number of fish per tank were used to set the daily feed amounts based on the standardized rate (%BW/d; Tables 2.1, 2.2, 2.3, and 2.4). Fish were fed eight times daily. Twenty fish were similarly measured and weighed to adjust feed amounts after the first week. Mortality was monitored and recorded to determine the percentage of fish that did not take to feed in each tank. At the end of the second week, another 20 fish were measured and weighed to estimate feed conversion and growth in the second week post-swim-up. Feeding fish for two weeks post-swim-up helped ensure that all fish included in this experiment were actively feeding prior to the start of the experiment. Data from the first two weeks was used to compare initial growth rates and feed conversion rates among the feed companies.

The hatchery feed experiment was started at two weeks post-swim-up, at which time 180 fish each were counted out of the starter tank and distributed into three replicate, 38-L (10-gallon) glass tanks for each feed company in FR1 (see Table 2.5 for tank assignments). More fish were used in this experiment compared to the first experiment because extra fish were needed to conduct the feed and size-at-stocking experiments (see Experiment 3 below). All remaining fish in the starter tank were counted and euthanized. Counts and mortality records were used to determine the starting number of fish per tank at swim-up, and to back-calculate the mortality rate of fish that did not take to feed. An initial sample weight was taken for each tank by placing all 180 fish for a given replicate tank in a tared water bucket on a scale, obtaining individual weights by dividing the total weight by the known number of fish, and calculating the number of fish per pound. This known weight was used to calculate total amount of feed per day (g) for each tank using the standardized feeding rates. In addition, a subset of 20 fish were individually measured and weighed to calculate a Fulton's condition factor (K; Ney 1999) at the onset of the experiment.

Table 2.5. Assignment of feed company to tank, assigned using a random number generator.

Feed Company	Tank
Rangen	1
Bio Oregon	2
EWOS	3
Skretting	4
Rangen	5
Bio Oregon	6
Skretting	7
EWOS	8
Skretting	9
Bio Oregon	10
Rangen	11
EWOS	12

Feeding occurred six times daily while fish remained in FR1, with one sixth of the day's total ration delivered to the tank at each feeding. It was assumed that all feed provided to the fish was consumed for the purpose of calculating feed conversion ratios. Given the GR's voracious appetite and ability to consume a large portion of the food presented to them, this assumption was likely met during this experiment. Throughout the entirety of the experiment, tanks and raceways were fed in a clockwise or counterclockwise direction, alternating rotations between the two directions, and the tank with which feeding began advanced by one tank daily. For example, on day one, tank 1 was fed first, and feeding occurred in a clockwise direction. On day two, tank 2 was fed first, and feeding occurred in a counterclockwise direction. This prevented an anticipated feeding response resulting from feeding in the same order every day that could have increased pre-feeding energy use and affected consumption efficiency.

Two to three batch weights of 20 fish each were obtained from each tank on a weekly basis and amount of feed fed per day was adjusted based on these weights. Daily feed amounts were adjusted for mortalities by subtracting the average weight of an individual fish from the previous weekly batch weight and recalculating the total feed per tank. Once a given tank reached the maximum average individual weight of the range for a given feed size, the tank was switched to the next size of feed and/or to a different feeding rate. A subset of 20 fish were individually measured and weighed on the day that feed rate was changed, which allowed for comparisons both across feeding rates within a feed company, and across feed companies at a given fish size. Fifteen of the 20 fish were returned to the tank after being processed. The remaining five fish were euthanized and dissected to obtain liver and viscera weights. Fin condition was also accessed on all 20 fish. Fin condition can be assessed to determine differences in fish appearance when using different feeds and feed delivery methods utilizing the Health/Condition Profile system (HCP; Goede and Barton 1990), which uses a rating scale between 0 and 3 and is based on the degree of hemorrhaging. Wagner et al. (1996) modified the HCP fin index to base scores on fin length, with 0 = perfect fin, 1 = slight erosion, and 2 = severe erosion. Fins were visually assessed for fin length using the scale developed by Wagner et al. (1996), but allowing for 0.5 scores between whole numbers. Each fin or fin pair (i.e., dorsal, caudal, anal, pelvic and pectoral) was assessed separately, and an average score for the fish was obtained since visual assessments of fish during the first experiment showed that all fins did not exhibit the same amount of wear at the time of assessment, making it difficult to assign an average fin score.

To maintain suggested density indices of pounds per cubic foot less than or equal to half of the fish length in inches (Piper et al. 1982), fish started in the 38 L (10 gallon) glass tanks in FR1. Upon reaching an average of 3 grams per fish, fish were moved to 76 L (20 gallon) aluminum tanks within FR1, and the number of fish was counted and confirmed. Once fish reached an average of 15 grams per fish, they were moved from the tanks in FR1 to the BFRH fiberglass hatchery troughs. Again, the number of fish was counted and confirmed upon moving fish to the hatchery. Twelve hatchery troughs were used to rear the fish inside the hatchery to maintain replication. Fish in the hatchery were fed four times daily. Fish were held in one half of the trough until they reached an average of 65 grams per fish, at which point the divider was removed and the fish were allowed to use the entire trough for the remainder of the growth experiment. The experiment was concluded once fish reached an average of ≥ 210 grams of fish. At the end of the experiment, all fish remaining in a hatchery trough were euthanized, measured, and weighed, and 20 fish were dissected to obtain liver and viscera weights. Fifteen fish from each tank were kept alive and moved to round tanks where they continued to be fed on the same size and ration of feed until all fish from all feed companies had reached the final goal weight. Round tanks (four) contained 45 fish at the end of the experiment, 15 from each of three replicate troughs per feed company.

There are a number of standard metrics used to evaluate growth performance in feed comparison experiments, including weight gain (%), feed conversion ratio (FCR; grams of feed per gram of fish [g feed/g fish]), specific growth rate (SGR; % BW/d), feed intake (% BW/d), hepatosomatic index (HSI), and viscerosomatic index (VSI; Trushenski et al. 2011; Gause and Trushenski 2013), calculated using the following formulas:

$$Weight\ gain = 100 \times \frac{average\ \ final\ \ weight - average\ \ initial\ \ weight}{average\ \ initial\ \ weight}$$

$$Feed\ conversion\ ratio = 100 \times \frac{average\ \ initial\ \ feed\ \ consumption\ (dry\ \ matter)}{average\ \ individual\ \ weight\ gain}$$

$$Specific\ growth\ rate = 100 \times \frac{log_e(average\ \ final\ \ weight) - log_e(average\ \ initial\ \ weight)}{days\ \ of\ \ feeding}$$

$$Feed\ intake = 100 \times \frac{total\ \ dry\ \ matter\ \ intake\ /(initial\ \ individual\ \ \ weight \times final\ \ individual\ \ \ weight)^{0.5}}{days\ \ of\ \ feeding}$$

$$HSI = 100 \times \frac{liver\ \ weight}{BW}$$

$$VSI = 100 \times \frac{total\ \ viscera\ \ weight}{BW}$$

Average individual values were calculated by dividing tank values by the number of fish in the tank at the time the data were collected. Parameters associated with feed consumption were based on average individual values calculated on a daily basis (i.e., average consumption values were calculated daily and summed over the course of the trial; Gause and Trushenski 2013). Weight gain, FCR, SGR, and feed intake were also calculated between each feeding rate change. HSI and VSI were computed for fish that were ≥ 2 grams; HSI and VSI were not calculated for feeding rate changes at which the average weight per fish was < 2 grams due to difficulty of dissection. The HSI and VSI indicate the amount of energy reserves stored in the liver and as fat in the viscera, excess energy that could be used during periods of low food availability after being stocked. The higher the HSI and VSI, the higher the amount of stored energy that can be utilized at a later date.

In addition to the growth metrics listed above, the coefficient of variation in length and weight was used to determine if certain feeds produce a wider range of variation in size than others (Wagner et al. 1996). The coefficient of variation (CV) is calculated as $CV = \frac{s}{\bar{y}}$, where s is the standard deviation in length or weight, and $\bar{y}$ is the mean. The CV was calculated when feeding rate changed for each feed company and used to determine when size variation began to occur during the experiment, if at all. Mortality, an important metric for assessing feed quality, especially at smaller sizes while fish are taking to feed (Kientz et al. 2012), was calculated between each feeding rate change, as well as for the entire growth period from hatch to the end of the experiment.

For each feeding rate change at which fish were dissected to obtain estimates of HSI and VSI, the intestine was preserved in 10% neutral buffered formalin and kept for later histological analysis performed by the FishVet Group (Portland, Maine) and the Aquatic Animal Medicine Research Laboratory (Grenada, West Indies). Sections were taken from the distal portion of the large intestine and examined for density of supranuclear vacuoles, goblet cell density, infiltration of eosinophilic granulocytes, and infiltration of mononuclear cells, all of which were scored on a semi-quantitative scale of one to five, and mucosal length, lamina propria width, and submucosal width, which were performed via digital measurements (μm; Table 2.6). Supranuclear vacuoles are clear glycogen deposits within the epithelium of the large intestine secondary to dietary pinocytosis and endocytosis, and are usually reduced during inflammation. Goblet cells are mucous secreting cells that increase in number secondary to chronic inflammation. Eosinophilic granulocytes and mononuclear cells (e.g., lymphocytes, plasma cells, and macrophages) infiltrate the submucosa and lamina propria secondary to antigenic stimulation. Although it is not uncommon to find small numbers of infiltrates within the intestine, increased numbers occur with inflammation. Mucosal length is the length of the mucosal villi beginning at the muscularis mucosa and ending at the distal tip of the epithelium. Mucosal villi decrease in length secondary to severe chronic inflammation. The width of the lamina propria can increase secondary to edema fluid inflammatory cell infiltrates, and other space-occupying lesions. Finally, the width of the submucosa changes similarly to that of the lamina propria with increased size due to edema fluid, inflammatory cell infiltrates, and other space-occupying lesions. Intestines were collected from fish reared on all four feed companies at five sizes: 1) extra small (70.7 mm TL, 3.8 g; following a feeding rate of 3.0% BW/d), 2) small (91.7 mm TL, 8.5 g; following a feeding rate of 2.9% BW/d), 3) medium (151.8 mm TL, 42.3 g; following a feeding rate of 2.4% BW/d), 4) large (192.2 mm TL, 85.4 g; following a feeding rate of 1.4% BW/d), and 5) extra large (252.6 mm TL, 195.3 g; following a feeding rate of 1.0% BW/d). All histological analyses were performed blind to feed company to prevent bias.

Table 2.6. Parameters of interest for the histological assessment of the intestine, and the semi-quantitative scoring system used to assess each parameter and to compare levels of inflammation across feed companies in the 2017 hatchery feed experiment.

Parameter	**Scoring system**				
	1	**2**	**3**	**4**	**5**
Density of supranuclear vacuoles	Occupy almost entire apical area of enterocytes	Medium-sized vacuoles occupying less than half of the enterocytes	Small-sized near the apical membrane in many enterocytes	Scattered and small vacuoles in few enterocytes	No vacuoles observed
Goblet cell density	Scattered goblet cells observed	Increased number but sparsely distributed	Diffuse and widely spread	Densely grouped in some mucosal folds	Highly abundant and tightly packed
Infiltration of eosinophilic granulocytes	Scattered granulocytes observed in submucosa and lamina propria	Increased number but sparsely distributed	Diffuse and widely spread	Densely grouped in some mucosal folds	Highly abundant and tightly packed
Infiltration of mononuclear cells	Scattered lymphocytes and plasma cells in submucosa and lamina propria	Increased number but sparsely distributed	Diffuse and widely spread	Densely grouped in some mucosal folds	Highly abundant and tightly packed
Mucosal length	Performed via digital measurements. Average of three areas.				
Lamina propria width	Performed via digital measurements. Average of three areas.				
Submucosal width	Performed via digital measurements. Average of three areas.				

An analysis of variance (ANOVA) implemented in SAS PROC GLM (SAS Institute 2016) was used to determine if there were differences in survival, length, and weight among the feed companies following the first two weeks of feeding. Similarly, an ANOVA was used to determine if there were differences in overall FCR, CV length, CV weight, HSI, VSI, fin condition, and survival among the feed companies at the end of the experiment. Unlike the first experiment, survival, FCR, fin condition, HSI, VSI, CV length, CV weight, and K were comparable across feed rate changes both within and across the feeds, and were compared using a two-factor ANOVA, with feed company and feeding rate change as the factors. Summary statistics are provided for SGR, weight gain, feed intake, length, and weight for each feeding rate within each feed company. Parameters of interest from the histological analysis of the intestines were also compared using a two-factor ANOVA, with feed company and fish size as the factors. Note that all results for which the feeding rate is shown, fish were collected when changing to a lower feeding rate. For example, results for 3.3% BW/d are from fish that had been fed at 3.3% BW/d prior to collection, and were being switched to 3.1% BW/d at the time of collection.

Hatchery Feed Experiment Results

There were no significant differences in overall survival in the first two weeks among the feed companies (Table 2.7), indicating that fish took to feed equally on all four feed companies. Feed conversion ratios (grams of feed needed to produce one gram of mass) varied among the feed companies, with Bio Oregon having the lowest FCR and Rangen having the highest FCR. The lower the FCR, the more efficiently fish were able to convert feed to mass. Feed conversion ratios for Skretting and Bio Oregon were fairly similar to the first experiment, likely a result of the similar feeding rates used in the two experiments, whereas the FCR for EWOS was slightly lower, likely due to the higher feeding rate in the second experiment. The FCR for Rangen was higher than in the first experiment, likely a result of the much lower feeding rate used in the second experiment. Weight gain, SGR, and feed intake also varied among the feed companies, and only fish fed on Bio Oregon had a feed intake that approached the actual feeding rate compared to the other feed companies, which may suggest a difference in palatability among starter feeds. Length and weight did not differ among feed companies at the start of the experiment. However, both differed by the end of weeks one and two, with fish fed Rangen significantly smaller than fish fed EWOS, Skretting, or Bio Oregon at the end of the two week pre-feeding period. No fin wear was observed in the first two weeks, with fish from all feed companies having a fin rating of 0 (Table 2.1.7).

Table 2.7. Comparisons of overall survival (%), weekly survival (%), feed conversion ratios (FCR; g feed/g fish), weight gain (%), specific growth rate (SGR; % BW/d), feed intake (% BW/d), weekly length (mm; CV length in parentheses), weekly weight (g; CV weight in parentheses), and average fin rating among the four feed companies (EWOS, Skretting, Bio Oregon, and Rangen) within the first two weeks post-swim-up. Different letters within the same row for a given metric represent significant differences among the feed companies.

Metric	**EWOS**	**Skretting**	**Bio Oregon**	**Rangen**
Overall Survival	99.74^{a}	99.75^{a}	99.83^{a}	99.58^{a}
Week 1	99.83	99.75	99.83	99.66
Week 2	99.91	100.00	100.00	99.91
FCR	0.39	0.41	0.31	0.62
Weight Gain	165.85	155.00	214.87	96.39
SGR	5.75	5.51	9.56	3.97
Feed Intake	2.34	2.34	3.09	2.52
Length (CV)				
Start	24.25^{a} (0.04)	24.20^{a} (0.03)	24.15^{a} (0.03)	24.00^{a} (0.04)
Week1	28.80^{a} (0.03)	27.65^{bc} (0.05)	28.25^{ab} (0.03)	26.95^{c} (0.04)
Week2	31.85^{ab} (0.04)	31.30^{b} (0.05)	32.80^{a} (0.03)	27.80^{c} (0.04)
Weight (CV)				
Start	0.10^{a} (0.09)	0.10^{a} (0.07)	0.10^{a} (0.11)	0.10^{a} (0.13)
Week1	0.19^{a} (0.12)	0.18^{ab} (0.19)	0.18^{ab} (0.12)	0.16^{b} (0.09)
Week2	0.27^{b} (0.14)	0.26^{b} (0.17)	0.31^{a} (0.10)	0.19^{c} (0.15)
Average Fin Rating	0.00	0.00	0.00	0.00

Metrics associated with feed varied both among the feed companies and across the feeding rates within a feed company (Table 2.8). Survival was greater than 97.5% for all feeding rates and feed companies, and in general, varied little among the feed companies with the exception of lower survival rates exhibited by fish fed Bio Oregon compared to Rangen at the 1.4 and 1.0% feeding rates. Similarly, within EWOS, Skretting, and Bio Oregon, there were no differences in survival across the feeding rates, but fish fed Bio Oregon exhibited significantly lower survival at feeding rates of 1.4 and 1.0% (Table 2.8). Feed conversion ratios increased with an increase in fish size and decrease in feeding rate, as had been observed in the first experiment. Feed conversion ratios were similar among the feed companies at the higher feeding rates, but diverged as feeding rate decreased. Overall, EWOS and Bio Oregon had similarly lower feed conversion ratios compared to Skretting and Rangen, the feed conversion ratios of which were similar. Feed conversion ratios approached 1.0 for Bio Oregon at the lower feeding rates. In general, feed conversion ratios were similar within a feed company between the two experiments, suggesting that feed quality had a larger effect on feed conversion ratios than did feeding rate. Weight gain, SGR, and feed intake varied similarly among the feeds across the feeding rates, as would be expected with fish that were of similar average size due to the standardized feeding rates (Table 2.8).

Table 2.8. Comparisons of survival (%), feed conversion ratios (FCR; g feed/g fish), weight gain (%), specific growth rate (SGR; % BW/d), and feed intake (% BW/d) among the four feed companies (EWOS, Skretting, Bio Oregon, and Rangen) and at standardized feeding rates (%BW/d) in the 2017 hatchery feed experiment. Different letters on the left side of survival and FCR values indicate significant differences among feeds within a feeding rate (columns), and different letters on the right side of these values indicate significant differences among feeding rates within a feed company (rows).

Parameter	Feeding Rates						
	3.3%	**3.1%**	**3.0%**	**2.9%**	**2.4%**	**1.4%**	**1.0%**
EWOS							
Survival	^{a} 99.81 ^{z}	^{a} 100 ^{z}	^{a} 99.81 ^{z}	^{a} 100 ^{z}	^{a} 99.16 ^{z}	^{ab} 99.13 ^{z}	^{b} 99.54 ^{z}
FCR	^{a} 0.44 ^{z}	^{a} 0.47 ^{z}	^{a} 0.49 ^{z}	^{b} 0.68 ^{y}	^{a} 0.69 ^{y}	^{ab} 0.79 ^{y}	^{a} 0.77 ^{y}
Weight gain	225.54	99.82	98.42	158.68	408.75	72.44	155.78
SGR	5.62	4.94	4.89	3.39	2.90	1.56	1.18
Feed intake	2.65	2.41	2.46	2.41	2.22	1.25	0.95
Skretting							
Survival	^{a} 99.81 ^{z}	^{a} 100 ^{z}	^{a} 100 ^{z}	^{a} 99.81 ^{z}	^{a} 99.78 ^{z}	^{ab} 99.11 ^{z}	^{b} 100 ^{z}
FCR	^{a} 0.49 ^{z}	^{a} 0.45 ^{z}	^{ab} 0.53 ^{z}	^{b} 0.65 ^{y}	^{a} 0.71 ^{y}	^{bc} 0.86 ^{x}	^{b} 0.90 ^{x}
Weight gain	252.97	88.06	108.717	159.02	399.26	94.73	152.14
SGR	5.40	5.31	4.49	4.07	2.87	1.43	0.99
Feed intake	2.83	2.43	2.47	2.77	2.25	1.25	0.93
Bio Oregon							
Survival	^{a} 100 ^{z}	^{a} 100 ^{z}	^{a} 100 ^{z}	^{a} 100 ^{z}	^{a} 99.79 ^{z}	^{a} 98.06 ^{y}	^{a} 97.74 ^{y}
FCR	^{a} 0.44 ^{z}	^{a} 0.48 ^{z}	^{a} 0.50 ^{z}	^{a} 0.53 ^{z}	^{a} 0.67 ^{y}	^{a} 0.69 ^{y}	^{a} 0.72 ^{y}
Weight gain	240.68	105.72	96.27	151.56	347.89	94.36	159.08
SGR	5.82	5.15	4.81	4.39	3.06	1.77	1.28
Feed intake	2.7	2.51	2.45	2.41	2.26	1.24	0.95
Rangen							
Survival	^{a} 100 ^{z}	^{a} 100 ^{z}	^{a} 99.81 ^{z}	^{a} 100 ^{z}	^{a} 100 ^{z}	^{b} 100 ^{z}	^{b} 99.73 ^{z}
FCR	^{a} 0.50 ^{z}	^{a} 0.50 ^{z}	^{b} 0.62 ^{y}	^{b} 0.76 ^{x}	^{b} 0.99 ^{w}	^{c} 0.96 ^{w}	^{b} 0.93 ^{w}
Weight gain	324.98	99.83	99.16	164.42	398.33	89.00	160.11
SGR	4.78	4.93	4.03	3.20	2.03	1.30	0.97
Feed intake	2.60	2.51	2.52	2.51	2.22	1.26	0.94

Individual growth, health, and appearance metrics also varied both among the feed companies and across the feeding rates within a feed company (Table 2.9). Coefficients of variation (CV) in both length and weight were generally lowest at a feeding rate of 3.3%, increasing with a decrease in feeding rate. Although there were no differences in CV length or CV weight among the feeding rates for fish fed EWOS or Skretting, fish fed Bio Oregon and Rangen exhibited greater variability in both length and weight at lower feeding rates. CV weight for fish fed Rangen was especially high at feeding rates of 3.0 and 2.9%, and exceeded those exhibited by fish fed Rangen in the first experiment. Fulton's condition factor (K) was lowest at the higher feeding rates and increased as fish grew and feeding rates decreased, especially at feeding rates of 2.4, 1.4, and 1.0%. Although K differed among the feeds, the differences are likely not biologically relevant.

Hepatosomatic index (HSI) and viscerosomatic index (VSI) differed greatly among feeds and feeding rates (Table 2.9). For EWOS and Bio Oregon, HSI and VSI were highest at feeding rates between 3.0% and 2.4%, and Bio Oregon exhibited a significantly higher HSI and VSI at a feeding rate of 2.9%. This feeding rate corresponds to feed sizes between 1.2 and 2.0 mm, in which higher HSI and VSI values were observed in the first experiment. Skretting showed an increasing trend in HSI to a feeding rate of

1.4%, decreasing at a feeding rate of 1.0%. EWOS, Skretting, and Bio Oregon had lower HSI and VSI values at a feeding rate of 1.0%, suggesting that larger feeds fed at lower feeding rates contained less storable energy than smaller, higher energy feeds fed at higher feeding rates. Rangen exhibited a trend of increasing HSI values through a feeding rate of 1.0%, although the VSI values obtained from fish reared at a feeding rate of 1.0% were similarly low relative to the other feed companies. Fin wear increased with an increase in fish size and decrease in feeding rate, and similar to the first experiment, fin wear was lowest in Bio Oregon fish, highest in Rangen fish, and fell between these two for fish fed EWOS and Skretting (Table 2.9).

Table 2.9. Comparisons of length (mm), coefficient of variation in length (CV length), weight (g), CV weight, Fulton's condition factor (K), hepatosomatic index (HSI), viscerosomatic index (VSI), and fin rating among the four feed companies (EWOS, Skretting, Bio Oregon, and Rangen) and at standardized feeding rates (% BW/d) in the 2017 hatchery feed experiment. Different letters on the left side of CV length, CV weight, K, HSI, VSI and fin rating values indicate significant differences among feeds within a feeding rate (columns), and different letters on the right side of these values indicate significant differences among feeding rates within a feed company (rows).

Parameter	**Feeding Rates**						
	3.3%	**3.1%**	**3.0%**	**2.9%**	**2.4%**	**1.4%**	**1.0%**
EWOS							
Length	44.67	55.03	69.68	95.62	157.32	189.93	256.37
CV Length	$^{a}0.05^{z}$	$^{a}0.07^{z}$	$^{a}0.09^{z}$	$^{a}0.05^{z}$	$^{a}0.05^{z}$	$^{a}0.07^{z}$	$^{a}0.07^{z}$
Weight	0.94	1.83	3.51	9.17	48.08	86.05	207.91
CV Weight	$^{a}0.16^{z}$	$^{a}0.22^{z}$	$^{ab}0.28^{z}$	$^{a}0.14^{z}$	$^{a}0.17^{z}$	$^{a}0.21^{z}$	$^{a}0.21^{z}$
K	$^{a}1.05^{zy}$	$^{a}1.08^{z}$	$^{ab}1.01^{y}$	$^{b}1.04^{zy}$	$^{ab}1.22^{x}$	$^{a}1.24^{x}$	$^{a}1.22^{x}$
HSI	N/A	N/A	$^{a}1.91^{z}$	$^{b}2.00^{z}$	$^{b}1.97^{z}$	$^{ab}1.65^{zy}$	$^{a}1.58^{y}$
VSI	N/A	N/A	$^{a}14.38^{z}$	$^{b}14.02^{zy}$	$^{a}14.59^{z}$	$^{a}12.60^{y}$	$^{a}11.24^{x}$
Fin rating	$^{a}0.01^{z}$	$^{a}0.11^{z}$	$^{a}0.31^{y}$	$^{b}0.64^{x}$	$^{c}0.79^{x}$	$^{a}0.80^{x}$	$^{b}1.16^{w}$
Skretting							
Length	44.9	53.45	69.63	94.08	153.52	194.17	260.30
CV Length	$^{a}0.05^{z}$	$^{a}0.04^{z}$	$^{a}0.06^{z}$	$^{a}0.05^{z}$	$^{ab}0.06^{z}$	$^{a}0.06^{z}$	$^{a}0.07^{z}$
Weight	0.94	1.63	3.39	9.15	43.58	85.31	207.97
CV Weight	$^{a}0.18^{z}$	$^{a}0.15^{z}$	$^{ab}0.20^{z}$	$^{ab}0.17^{z}$	$^{a}0.21^{z}$	$^{a}0.17^{z}$	$^{a}0.20^{z}$
K	$^{a}1.01^{zy}$	$^{a}1.06^{yx}$	$^{b}0.98^{z}$	$^{b}1.08^{x}$	$^{b}1.19^{w}$	$^{b}1.16^{w}$	$^{c}1.17^{w}$
HSI	N/A	N/A	$^{b}1.49^{z}$	$^{c}1.55^{z}$	$^{ab}1.59^{z}$	$^{b}1.71^{z}$	$^{a}1.46^{z}$
VSI	N/A	N/A	$^{b}12.24^{zy}$	$^{c}11.44^{y}$	$^{b}13.09^{z}$	$^{a}11.68^{zy}$	$^{b}9.99^{x}$
Fin rating	$^{b}0.26^{z}$	$^{ab}0.23^{z}$	$^{a}0.27^{zy}$	$^{b}0.49^{yx}$	$^{b}0.59^{x}$	$^{b}1.12^{w}$	$^{c}1.27^{w}$
Bio Oregon							
Length	46.11	55.67	71.32	92.57	147.72	189	259.51
CV Length	$^{a}0.03^{z}$	$^{a}0.06^{z}$	$^{a}0.05^{z}$	$^{a}0.05^{z}$	$^{ab}0.08^{z}$	$^{a}0.07^{z}$	$^{a}0.07^{z}$
Weight	1.00	1.87	3.91	9.46	41.60	85.66	212.97
CV Weight	$^{a}0.13^{z}$	$^{a}0.18^{zy}$	$^{a}0.16^{zy}$	$^{ab}0.17^{zy}$	$^{a}0.26^{y}$	$^{a}0.19^{zy}$	$^{a}0.21^{zy}$
K	$^{a}1.01^{z}$	$^{a}1.07^{z}$	$^{a}1.07^{z}$	$^{a}1.18^{y}$	$^{a}1.26^{x}$	$^{a}1.26^{x}$	$^{ab}1.20^{y}$
HSI	N/A	N/A	$^{ab}1.63^{z}$	$^{a}3.77^{y}$	$^{ab}1.67^{z}$	$^{ab}1.51^{z}$	$^{a}1.48^{z}$
VSI	N/A	N/A	$^{b}12.35^{y}$	$^{a}16.22^{z}$	$^{b}12.47^{y}$	$^{a}11.21^{yx}$	$^{a}11.05^{x}$
Fin rating	$^{a}0.00^{z}$	$^{a}0.10^{zy}$	$^{a}0.23^{yx}$	$^{a}0.26^{yx}$	$^{a}0.30^{x}$	$^{a}0.89^{w}$	$^{a}0.96^{w}$
Rangen							
Length	43.30	53.70	68.33	94.00	152.38	193.8	262.53
CV Length	$^{a}0.05^{z}$	$^{a}0.07^{zy}$	$^{a}0.09^{zy}$	$^{a}0.09^{zy}$	$^{b}0.10^{y}$	$^{a}0.09^{zy}$	$^{a}0.07^{zy}$
Weight	0.86	1.72	3.35	9.04	40.88	85.20	217.60
CV Weight	$^{a}0.16^{z}$	$^{a}0.23^{zy}$	$^{b}0.32^{y}$	$^{b}0.30^{y}$	$^{a}0.29^{zy}$	$^{a}0.27^{zy}$	$^{a}0.24^{zy}$
K	$^{a}1.04^{zy}$	$^{a}1.09^{yx}$	$^{ab}1.01^{z}$	$^{b}1.05^{zy}$	$^{c}1.11^{x}$	$^{b}1.14^{xw}$	$^{bc}1.18^{w}$
HSI	N/A	N/A	$^{c}1.08^{z}$	$^{c}1.41^{z}$	$^{a}1.31^{z}$	$^{a}1.26^{z}$	$^{b}1.87^{y}$
VSI	N/A	N/A	$^{ab}13.48^{z}$	$^{b}13.73^{z}$	$^{b}12.71^{zy}$	$^{a}11.62^{y}$	$^{ab}10.36^{x}$
Fin rating	$^{b}0.33^{z}$	$^{b}0.37^{z}$	$^{a}0.32^{z}$	$^{b}0.68^{y}$	$^{bc}0.76^{y}$	$^{c}1.49^{x}$	$^{d}1.60^{x}$

Table 2.10. Semi-quantitative scores for density of supranuclear vacuoles, goblet cell density, infiltration of eosinophilic granulocytes, and infiltration of mononuclear cells, and measurements of mucosal length, lamina propria width, and submucosal width (µm) by feed company and fish size. Different letters on the left side of mucosal length values indicate significant differences among feeds within a fish size (columns), and different letters on the right side of these values indicate significant differences among fish sizes within a feed company (rows). Different letters in the overall column represent significant average differences among feed companies, and in the overall rows represent significant average differences among fish sizes.

Parameter	Extra Small	Small	Medium	Large	Extra Large	Overall
			EWOS			
Vacuoles	2.7	2.8	3.3	3.5	3.5	3.2 ^{ab}
Goblet Cell Density	2.5	2.7	2.5	2.3	2.3	2.5 ^{a}
Granulocytes	1.3	2.5	3.0	3.2	2.8	2.6 ^{a}
Mononuclear Cells	1.0	1.0	1.2	1.2	1.0	1.1 ^{a}
Mucosal Length	^{a} 300.1 ^{z}	^{a} 296.7 ^{z}	^{a} 424.3 ^{z}	^{a} 365.3 ^{z}	^{b} 410.7 ^{z}	356.9 ^{b}
Lamina Propria Width	6.1	6.7	7.5	9.8	14.0	8.8 ^{a}
Submucosal Width	6.5	6.4	6.4	8.4	9.0	7.3 ^{a}
			Skretting			
Vacuoles	2.7	2.5	3.3	4.0	2.7	3.0 ^{ab}
Goblet Cells	2.7	2.3	2.3	2.7	2.2	2.4 ^{a}
Granulocytes	1.5	2.3	3.0	3.0	2.5	2.5 ^{a}
Mononuclear Cells	1.0	1.0	1.3	1.0	1.0	1.1 ^{a}
Mucosal Length	^{a} 291.7 ^{z}	^{a} 280.9 ^{z}	^{a} 443.5 ^{yz}	^{a} 362.7 ^{z}	^{ab} 576.1 ^{y}	398.5 ^{ab}
Lamina Propria Width	6.1	7.1	9.0	10.3	13.4	9.2 ^{a}
Submucosal Width	6.5	6.2	7.7	8.9	8.8	7.6 ^{a}
			Bio Oregon			
Vacuoles	2.0	2.0	3.3	3.5	2.5	2.7 ^{b}
Goblet Cells	2.2	2.2	2.5	2.7	1.7	2.2 ^{a}
Granulocytes	1.3	2.5	3.0	2.8	2.5	2.4 ^{a}
Mononuclear Cells	1.0	1.0	1.2	1.2	1.0	1.1 ^{a}
Mucosal Length	^{a} 287.8 ^{z}	^{a} 364.1 ^{z}	^{a} 382.3 ^{z}	^{a} 374.6 ^{z}	^{a} 642.1 ^{y}	411.8 ^{a}
Lamina Propria Width	5.8	6.9	7.7	8.7	12.1	8.2 ^{a}
Submucosal Width	6.1	6.1	6.8	9.2	9.1	7.4 ^{a}
			Rangen			
Vacuoles	4.0	2.0	4.0	3.8	3.5	3.5 ^{a}
Goblet Cells	3.0	2.0	2.3	2.7	2.0	2.4 ^{a}
Granulocytes	2.2	2.5	3.0	3.0	2.2	2.6 ^{a}
Mononuclear Cells	1.0	1.0	1.0	1.2	1.0	1.0 ^{a}
Mucosal Length	^{a} 255.0 ^{z}	^{a} 317.4 ^{yz}	^{a} 325.0 ^{yz}	^{a} 342.3 ^{yz}	^{b} 455.0 ^{y}	338.9 ^{b}
Lamina Propria Width	6.2	7.3	7.9	10.3	10.2	8.4 ^{a}
Submucosal Width	5.9	6.7	7.8	8.9	12.1	8.3 ^{a}
			Overall			
Vacuoles	2.8 ^{ab}	2.3 ^{a}	3.5 ^{b}	3.7 ^{b}	3.0 ^{ab}	
Goblet Cells	2.6 ^{a}	2.3 ^{a}	2.4 ^{a}	2.6 ^{a}	2.0 ^{a}	
Granulocytes	1.6 ^{a}	2.5 ^{b}	3.0 ^{c}	3.0 ^{c}	2.5 ^{b}	
Mononuclear Cells	1.0 ^{a}	1.0 ^{a}	1.1 ^{a}	1.2 ^{a}	1.0 ^{a}	
Mucosal Length	351.7 ^{a}	415.1 ^{ab}	337.1 ^{c}	356.2 ^{bc}	421.7 ^{d}	
Lamina Propria Width	6.1 ^{a}	7.0 ^{ab}	8.0 ^{b}	9.8 ^{c}	12.5 ^{d}	
Submucosal Width	6.3 ^{a}	6.3 ^{a}	7.2 ^{a}	8.8 ^{b}	9.7 ^{b}	

There was a significant feed and size effect for the average density of supranuclear vacuoles in the intestines of fish reared on feeds from the four feed companies (Table 2.10). A size effect is common in these types of analyses, with increasing semi-quantitative scores from smaller to larger fish sizes due to longer antigen exposure. In this experiment, the semi-quantitative score for the density of supranuclear vacuoles, as well as the infiltration of eosinophilic granulocytes, dropped in the extra large fish, which is not typical. It is suspected that this is a result of sample location along the intestine in the extra large fish rather than a physiological effect of feed. With regard to the feed effect, Bio Oregon had a significantly lower semi-quantitative score for density of supranuclear vacuoles than did Rangen, meaning that the density of the supranuclear vacuoles in fish fed Bio Oregon were higher than for fish fed Rangen. A higher density of supranuclear vacuoles is considered an indication that more of the nutrients in the feed are being absorbed, which could be one reason why fish reared on Bio Oregon and Rangen show a significant difference in growth rate (Figure 2.1). Overall, there was not a feed effect for goblet cell density, infiltration of eosinophilic granulocytes, or infiltration of mononuclear cells, suggesting that none of the feeds cause an increase in intestinal inflammation relative to the others. The lack of differences in the infiltration of mononuclear cells suggests that fish are not reacting to proteins in the feed and that, antigenically, the feeds are all well tolerated. This is supported by the lack of feed differences in the lamina propria and submucosal widths, which suggest that none of the feeds induced increased inflammation. Although there was a feed, size, and interactive effect for mucosal length, the lack of evidence of inflammation from the other parameters of interest suggests that mucosal length differences among the feeds was more likely a result of growth performance differences than feed effects on the intestines.

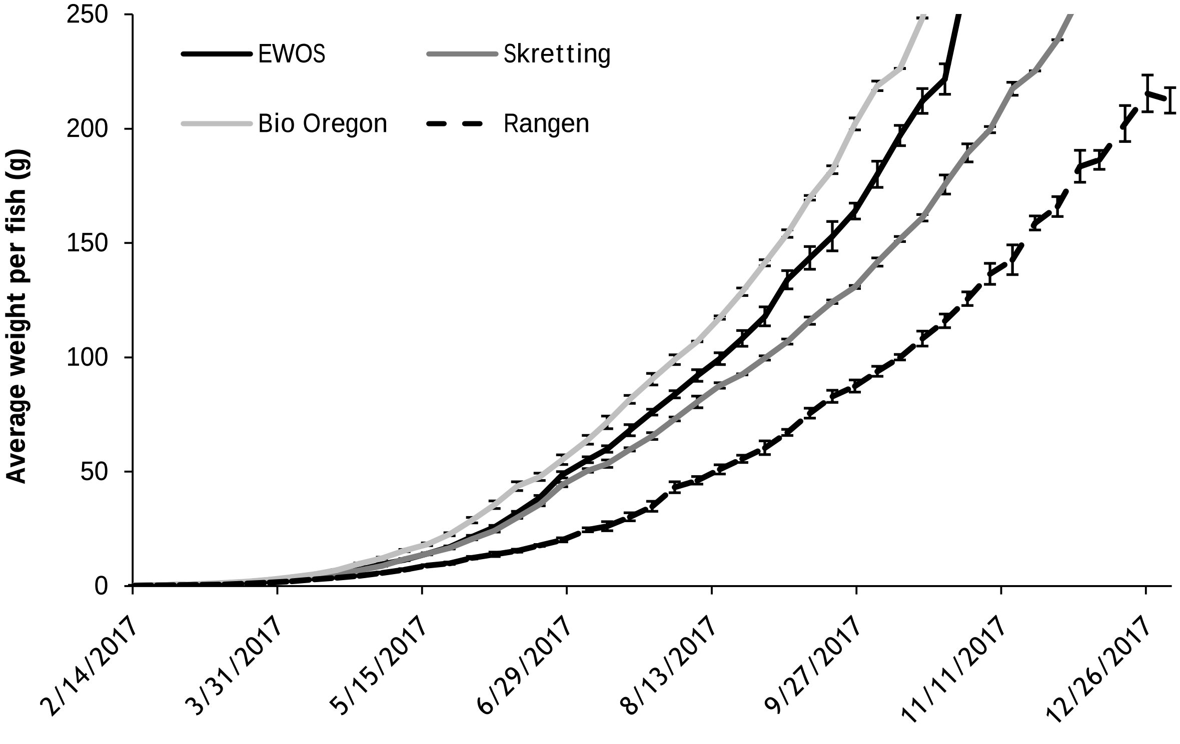

Figure 2.1. Average weekly weights of fish fed EWOS, Skretting, Bio Oregon, and Rangen. Error bars represent differences among replicates (3) within a feed company.

Fish fed Bio Oregon grew faster throughout the majority of the experiment than fish fed EWOS, Skretting, and Rangen (Figure 2.1). Fish fed EWOS reached the goal weight of ≥ 201 g only two weeks after fish fed Bio Oregon and exhibited a similar growth curve throughout the experiment, suggesting that feed quality was similar between the two companies. These results also suggest that the feeding rate recommended by EWOS and used in the first experiment was too low for the GR reared at the BFRH, and that the poor growth performance of these fish in the first experiment was entirely a result of the feeding rate. Fish fed Skretting grew slower than those fed EWOS and Bio Oregon, reaching the goal weight six weeks later than Bio Oregon and four weeks later than EWOS. Finally, fish fed Rangen exhibited much lower growth rates than those fed on the other three feeds, and did not reach the goal weight until 13 weeks after fish fed Bio Oregon (Figure 2.1), suggesting that this feed was of lower quality than the other feeds used in this experiment. After reaching the goal weight, fifteen fish from each replicate from EWOS, Skretting, and Bio Oregon were moved to round tanks and maintained at a feeding rate of 1.0% until the goal weight was reached by Rangen. By the time fish reared on Rangen reached the goal weight of ≥ 210 g on January 2, 2018, fish fed EWOS weighed 479 (± 23)g, fish fed Skretting weighed 347 (± 12.6) g, and fish fed Bio Oregon weighed 577 (± 23.8) g.

Table 2.11. Comparison of overall survival (%), feed conversion ratios (FCR; g feed/g fish), coefficient of variation in length (CV length), CV weight, hepatosomatic index (HSI), viscerosomatic index (VSI), and fin rating (± SE) among the four feed companies (EWOS, Skretting, Bio Oregon, and Rangen). Different letters within the same row for a given metric represent significant differences among the feed companies.

Metric	EWOS	Skretting	Bio Oregon	Rangen
Survival	97.78^{ab} (± 0.81)	98.70^{ab} (± 0.74)	96.30^{b} (± 0.81)	99.63^{a} (± 0.37)
Feed Conversion	0.75^{b} (± 0.01)	0.85^{c} (± 0.01)	0.70^{a} (± 0.01)	0.93^{d} (± 0.01)
CV Length	0.06^{a} (± 0.004)	0.06^{a} (± 0.002)	0.06^{a} (± 0.003)	0.08^{b} (± 0.005)
CV Weight	0.20^{a} (± 0.012)	0.18^{a} (± 0.007)	0.18^{a} (± 0.010)	0.25^{b} (± 0.015)
HSI	1.73^{ab} (± 0.04)	1.53^{c} (± 0.02)	1.81^{a} (± 0.08)	1.56^{bc} (± 0.04)
VSI	12.57^{a} (± 0.18)	11.06^{c} (± 0.14)	12.06^{ab} (± 0.19)	11.63^{bc} (± 0.16)
Fin Rating	0.81^{b} (± 0.02)	0.88^{b} (± 0.02)	0.64^{a} (± 0.02)	1.11^{c} (± 0.02)

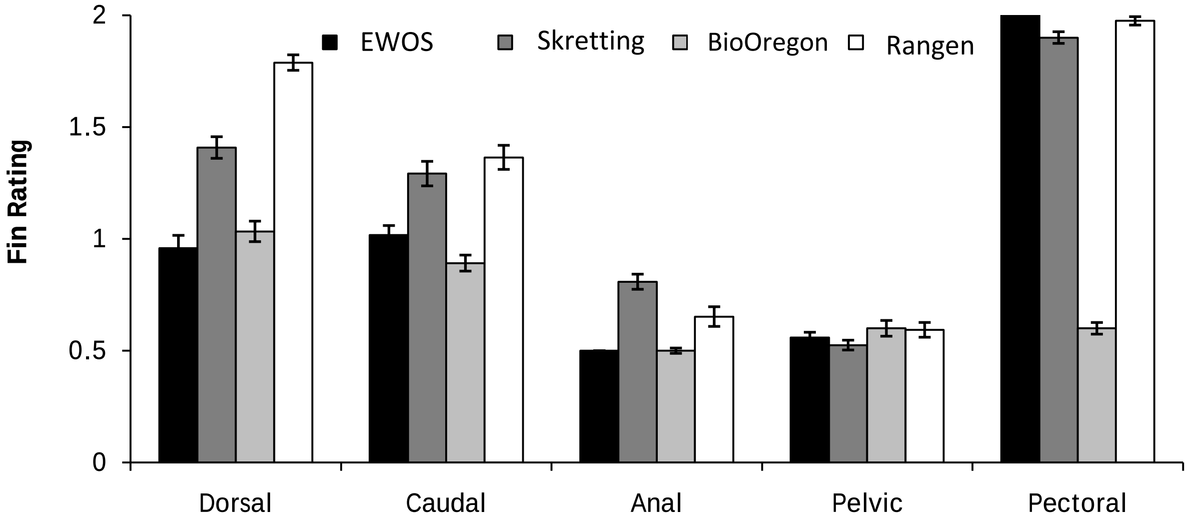

Figure 2.2. Average fin rating by fin type (dorsal, caudal, anal, pelvic, and pectoral) for fish fed EWOS, Skretting, Bio Oregon, and Rangen that reached the goal weight of ≥ 210 g.

Over the course of the experiment, growth and health metrics varied among the four feed companies (Table 2.11). Overall feed conversion ratio (averaged from the start to end of the experiment) was lowest in fish fed Bio Oregon. EWOS had a lower feed conversion ratio than did the other two feed companies, though it was higher than in the first experiment, likely a result of the larger sizes attained by these fish in the second experiment. Skretting and Rangen had the highest feed conversion ratios, with fish fed Rangen having a significantly higher feed conversion ratio than fish fed Skretting. Length and weight were significantly more variable in fish fed Rangen than fish fed EWOS, Skretting, or Bio Oregon. Fish fed Bio Oregon exhibited higher HSI values than those fed Skretting and Rangen, which had the lowest overall HSI values. VSI values differed from HSI values in that fish fed EWOS had the highest overall VSI value, and was significantly higher than for fish fed Skretting and Rangen, though did not differ from fish fed Bio Oregon. Overall fin condition was lowest (better) in fish fed Bio Oregon, and highest in fish fed Rangen (Table 2.11). Fin condition differed by fin type, with the anal and pelvic fins showing significantly less wear than the dorsal, caudal, and pectoral fins. In general, fish fed EWOS and Bio Oregon showed significantly less fin wear across all fin types than did fish fed Skretting and Rangen, with the exception of the pectoral fins, of which fish fed EWOS, Skretting, and Rangen all showed severe erosion, and fish fed Bio Oregon showed very slight erosion (Figure 2.2). Survival was one of the only metrics in which Rangen had an advantage over Bio Oregon, with a significantly higher survival in fish reared on Rangen (Table 2.11).

All feed companies produced catchable-size fish by the end of the experiment. On average, it took 0.38 lbs of feed to produce a catchable fish on EWOS, 0.44 lbs of feed to produce a catchable fish on Skretting, 0.35 lbs of feed to produce a catchable fish on Bio Oregon, and 0.49 lbs of feed to produce a catchable fish on Rangen. Colorado produced and stocked 2,691,614 catchable Rainbow Trout in 2015. In order to produce this many catchable Rainbow Trout, 570 tons of feed would be needed of EWOS, compared to 657 tons of Skretting, 532 tons of Bio Oregon, and 716 tons of Rangen. With natural protein sources, such as fish meal, becoming scarcer, it is important to reduce the amount of feed used to sustainably rear fish in aquaculture. Based on these results, Bio Oregon is the most sustainable for producing Colorado's catchable size Rainbow Trout.

Feed Cost Comparisons

Table 2.12. Cost breakdown, by feed size, for each of the four feed companies (EWOS, Skretting, Bio Oregon, and Rangen) used in the 2017 hatchery feed experiment.

EWOS		**Skretting**		**Bio Oregon**		**Rangen**	
Size	**Cost/lb**	**Size**	**Cost/lb**	**Size**	**Cost/lb**	**Size**	**Cost/lb**
Size 0	$1.98	Size 0	$0.98	Mash	$2.05	Size 0	$2.55
Size 1	$1.98	Size 1	$0.98	Size 0	$2.05	Size 1	$0.99
Size 2	$1.98	Size 2	$0.98	Size 1	$2.05	Size 2	$0.99
1.2 mm	$1.54	1.0 mm	$0.65	Size 2	$2.05	Size 3	$0.55
1.5 mm	$1.18	2.0 mm	$0.60	1.2 mm	$1.50	Size 4	$0.55
2.0 mm	$1.05	3.0 mm	$0.65	1.5 mm	$1.19	3/32"	$0.501
3.0 mm	$0.99	4.0 mm	$0.60	2.0 mm	$1.14	1/8"	$0.431
4.0 mm	$0.90			2.5 mm	$1.10	5/32"	$0.431
5.0 mm	$0.77			3.0 mm	$1.06	3/16"	$0.431
				4.0 mm	$0.94		
Average	**$1.374**	***Average***	**$0.777**	***Average***	**$1.513**	***Average***	**$0.825**

Colorado stocks millions of Rainbow Trout annually. In 2015, Colorado hatcheries stocked 12,447,260 *M. cerebralis*-negative subcatchable Rainbow Trout, averaging 2.53 in TL and 0.01 lbs, 58,604 *M. cerebralis*-positive subcatchable Rainbow Trout, averaging 6.16 in TL and 0.16 lbs, 1,900,652 *M. cerebralis*-negative catchable Rainbow Trout, averaging 10.23 in TL and 0.43 lbs, and 790,962 *M. cerebralis*-positive catchable Rainbow Trout, averaging 10.02 in TL and 0.41 lbs. Using the total amount of feed fed per individual, as well as the cost per pound of feed for each of the feed sizes used in the experiment (Table 2.12), the cost per fish was calculated for each size and number of Rainbow Trout stocked by Colorado (using the 2015 data presented above). The average cost to produce a fish in the hatchery feed experiment was lowest for fish reared on Rangen, increasing four to five cents each for rearing fish on Skretting, EWOS, and Bio Oregon, respectively (Table 2.13). The cost per pound of Bio Oregon is about $0.75 greater than for Skretting or Rangen (Table 2.12). One potential reason could be the protein sources included in these feeds. Fish meal is the only protein source listed for Bio Oregon, a much more expensive protein source than some listed for Skretting and Rangen, including blood meal, feather meal, poultry by-product meal, and soybean meal, in addition to fish meal. Because the cost of the size 0 feed from Rangen is higher than Skretting (Table 2.12), it is fairly comparable to produce over 12,000,000 subcatchable Rainbow Trout on both of these feeds (Table

2.13). However, it would be less expensive to produce the other sizes of fish on Rangen than on Skretting.

Table 2.13. Cost per fish estimates based on feed cost per size (Table 2.12) and amount of feed used per fish. The cost to produce the number of Rainbow Trout of each size (negative subcatchables [Neg Sub], positive subcatchables [Pos Sub], negative catchables [Neg Catch], and positive catchables [Pos Catch]) reared and stocked by the state of Colorado in 2015 are also shown, as is the total cost of feed to produce 15,197,480 Rainbow Trout.

Feed Company	Cost per fish	Neg Sub	Pos Sub	Neg Catch	Pos Catch	Total
EWOS	$0.33	$177,727	$7,772	$618,548	$257,411	$1,061,457
Skretting	$0.27	$104,084	$5,455	$508,019	$211,414	$828,972
Bio Oregon	$0.36	$178,054	$7,921	$675,730	$281,207	$1,142,912
Rangen	$0.22	$90,432	$4,957	$414,455	$172,477	$682,321

Discussion

Similar to the results of the first experiment, Bio Oregon was the best performing feed of the four feeds tested. Fish fed Bio Oregon reached the goal weight of ≥ 210 g two weeks, six weeks, and 13 weeks sooner than fish fed EWOS, Skretting, and Rangen. Additionally, fish fed Bio Oregon generally had more stored energy reserves, were less variable in size, and exhibited less fin wear than those fed Rangen. However, it costs 1.6 times more to produce a catchable size fish on Bio Oregon than on Rangen. The second best alternative to Bio Oregon appears to be EWOS, however, it still costs 1.5 times more to produce a catchable size fish on EWOS than Rangen. In general, there were not many differences among EWOS and Skretting, so Skretting may be a valid alternative for balancing cost and quality of fish produced by the state of Colorado.

Experiment 3 – Effects of Size-at-Stocking and Feed on Post-Stocking Survival of Rainbow Trout

Fish used in the feed and size-at-stocking survival experiments originated from Experiment 2. To determine potential differences in post-stocking survival and health of fish reared on the four feeds, EWOS, Skretting, Bio Oregon, and Rangen, fish were transported to and held in tanks at the Parvin Lake Research Station (Red Feather Lakes, Colorado). Experimental tanks were supplied with unfiltered lake water so that water quality, food availability, etc. was the same in the tanks as it would have been had fish been stocked into the lake itself. However, conducting this experiment in tanks allowed detection of mortality, if it occurred, and prevented detection probability, which would have been much lower if fish were released into the lake, from affecting inferences regarding survival and health metrics at the conclusion of the trial period.

The first size-at-stocking experiment with fingerling Rainbow Trout, averaging 77.9 (± 8.5) mm total length (TL) and 4.6 (± 1.6) g, began on April 19, 2017. All fish used in the experiment were measured, weighed, and Visual Implant Elastomer (VIE) tagged at the BFRH prior to transport up to the Parvin Lake Research Station. VIE tags were used to maintain replication within a tank, as ten fish per each of three replicates for each feed company were included in each experimental tank, a total of 30 fish per tank. Four, 76-L (20-gallon) experimental tanks were used for the experiment, one for each feed company. Fish remained undisturbed in the tanks for 50 days, with the experiment concluding on June 8, 2017. Fish were not fed during this time period, although food particles from the lake made it into the tanks through the water line, and the tanks were not cleaned to simulate being held in the lake environment. Upon completion of the experiment, fish were identified using VIE tag color, measured, and weighed. Additionally, all fish were dissected to obtain liver and viscera weights.

Fulton's condition factor (K) was calculated for all individuals prior to and after the experiment using individual fish length and weight and the equation for K presented in Ney (1999). Hepatosomatic index (HSI) and viscerosomatic index (VSI) were calculated using the ratio of the liver or viscera weight to total weight of the fish (Trushenski et al. 2011; Gause and Trushenski 2013) at the conclusion of the experiment. The HSI and VSI indicate the amount of energy reserves stored in the liver and as fat in the viscera, excess energy that could be used during periods of low food availability after being stocked. The higher the HSI and VSI values, the higher the amount of stored energy that can be utilized at a later date. Measured HSI and VSI values were not available for fish immediately prior to the start of the experiment since the experiment began at a fish size that fell between feeding rate changes at which these values were assessed in the hatchery feed experiment. To obtain HSI and VSI values for fish at the start of the experiment, HSI and VSI of fish from the feeding rate prior to and after the feed and size-at-stocking experiment began were plotted. A regression line was fit to the data and used to calculate the values at the start of the experiment based on the daily change in HSI and VSI and the day on which the experiment started. A repeated measure analysis of variance (RM ANOVA) implemented in SAS Proc GLM (SAS Institute 2016), was used to determine if there were differences in pre-and post-experiment length, weight, condition factor, HSI, and VSI within and among fish reared on the feeds from the four feed companies.

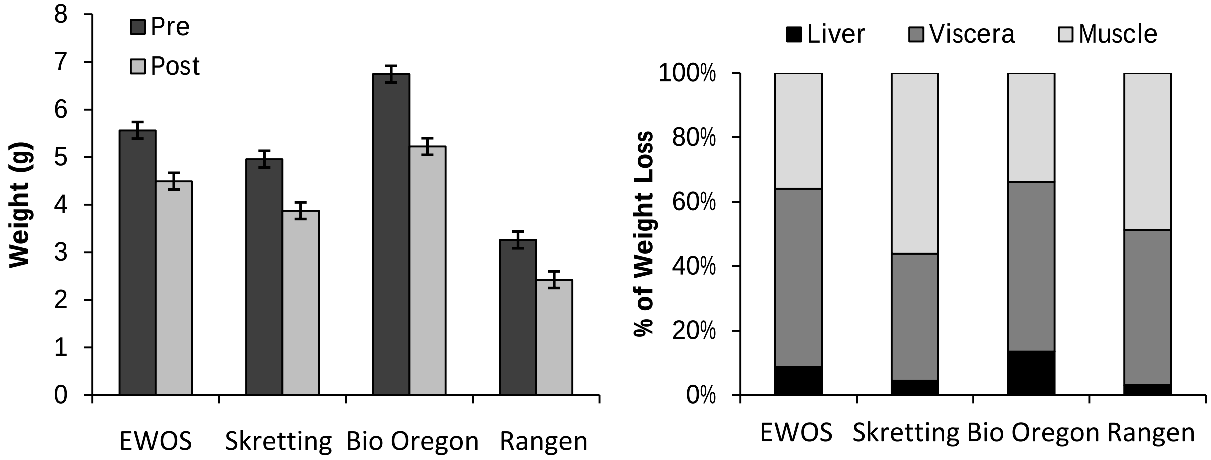

Figure 3.1. *Left panel:* Fingerling Rainbow Trout weight (g; SE bars) at the start (pre) and end (post) of the 50 day feed and size-at-stocking experiment by feed company (EWOS, Skretting, Bio Oregon, and Rangen). *Right panel:* Location from which weight loss in the fingerling Rainbow Trout occurred for each feed company given the change in weight of the liver, viscera, and overall weight of the fish from the beginning to the end of the 50 day feed and size-at-stocking experiment.

No mortality occurred over the course of the experiment. As such, results are focused on the differences in fish condition and health from the beginning and end of the experiment. Overall, fish length did not change over the course of the 50 day experiment ($p = 0.68$) indicating that fish did not grow while in the experimental tanks. There was a significant decrease in weight from the beginning to end of the experiment ($p < 0.01$; Figure 3.1), although the interaction between weight at the beginning and end of the experiment and feed company was not significant ($p = 0.26$). The location in which weight loss occurred varied by feed group, with a larger percentage of the total weight loss occurring from absorption of the liver and viscera fat for fish fed EWOS and Bio Oregon, and occurring as muscle loss for fish fed Skretting and Rangen. As a result of the weight loss exhibited by all fish in the experiment, there was also a significant reduction in K from the beginning to the end of the experiment ($p < 0.01$), with K averaging 1.07 (± 0.03) at the beginning of the experiment, and 0.78 (± 0.01) at the end of the experiment.

Differences in weight loss location were likely a result of the differences in primarily HSI among the feed companies at the beginning of the experiment ($p < 0.04$; Figure 3.2). Fish reared on all four feed companies experienced a significant decrease in HSI over the course of the experiment ($p < 0.01$). Decreases in HSI were larger for fish fed EWOS and Bio Oregon, which is reflected in the location in which weight loss occurred in these fish, the liver, and is likely a result of having more available energy to draw from the liver at the beginning of the experiment compared to fish reared on Skretting and Rangen who relied on muscle absorption to account for smaller HSI at the beginning of the experiment.

Additionally, fish reared on EWOS and Bio Oregon also had significantly larger VSI at the beginning of the experiment compared to fish reared on Skretting and Rangen ($p < 0.01$; Figure 2.3.2). Significant reductions in VSI occurred for fish reared on all four feed companies, and VSI was similar among the feed companies at the end of the experiment, suggesting that fish in all four feed groups had absorbed all available visceral fat and energy in the viscera, and the final VSI reflected the minimum weight of the organs in all fish. Similar to the HSI, the larger reductions in VSI in fish reared on EWOS and Bio Oregon suggest that there were more reserves available in the viscera in these fish compared to fish reared on Skretting and Rangen, resulting in less muscle absorption by the end of the experiment.

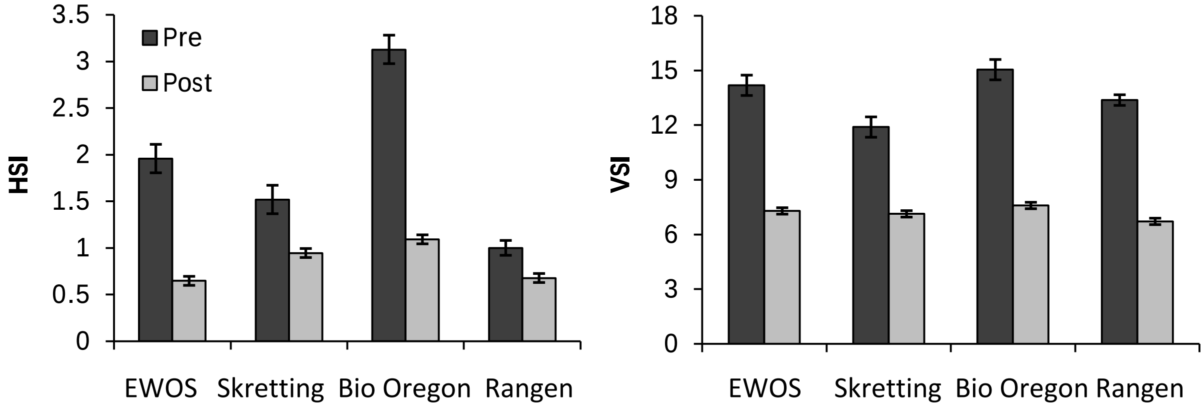

Figure 3.2. Hepatosomatic index (HIS; SE bars; left panel) and viscerosomatic index (VSI; SE bars; right panel) at the start (pre) and end (post) of the 50 day feed and size-at-stocking experiment for fingerling Rainbow Trout reared on the four feeds (EWOS, Skretting, Bio Oregon, and Rangen).

The second size-at-stocking experiment with subcatchable Rainbow Trout, averaging 201.2 (± 21.2) mm TL and 98.3 (± 35.3) g, began on August 23, 2017. All fish used in the experiment were measured, weighed, and VIE tagged at the BFRH prior to transport up to the Parvin Lake Research Station. VIE tags were used to maintain replication within a tank, as ten fish per each of three replicates for each feed company were included in each experimental tank, a total of 30 fish per tank. Four, 946-L (250-gallon) experimental tanks were used for the experiment, one for each feed company. Fish remained undisturbed in the tanks for 50 days, with the experiment concluding on October 12, 2017. Fish were not fed during this time period, although food particles from the lake made it into the tanks through the water line, and the tanks were not cleaned to simulate being held in the lake environment. Unfortunately, as a result of density and temperature issues within the experimental tanks, high mortality occurred. Because mortality was not suspected to be a result of nutrition, since all tanks experienced similarly high mortality rates, and because there were very few fish remaining at the end of experiment from which to compare size, condition, and health metrics, this experiment was dropped from the post-stocking survival and feed comparisons.

The third and final size-at-stocking experiment with catchable Rainbow Trout, averaging 263.2 (± 25.9) mm TL and 218.5 (± 74) g, began on November 8, 2017. All fish used in the experiment were measured, weighed, and VIE tagged at the BFRH prior to transport up to the Parvin Lake Research Station. A subset of five fish from each feed company were dissected to obtain initial estimates of HSI and VSI. This experiment was modified from the first two to reduce densities and to prevent excess mortality due to crowding and poor water quality which occurred in the subcatchable experiment. As such, VIE tags were used to identify fish by feed company. Four, 946-L (250-gallon) experimental tanks were used for the experiment, each containing five fish from each feed company, a total of 20 fish per tank. Fish remained undisturbed in the tanks for 50 days, with the experiment concluding on December 27, 2017. Fish were not fed during this time period, although food particles from the lake made it into the tanks through the water line, and the tanks were not cleaned to simulate being held in the lake environment. Upon completion of the experiment, fish were identified using VIE tag color, measured and weighed. Additionally, all fish were dissected to obtain liver and viscera weights. An RM ANOVA implemented in SAS Proc GLM (SAS Institute 2016), was used to determine if there were differences in pre-and post-experiment length, weight, condition factor, HSI, and VSI within and among fish reared on the feeds from the four feed companies.

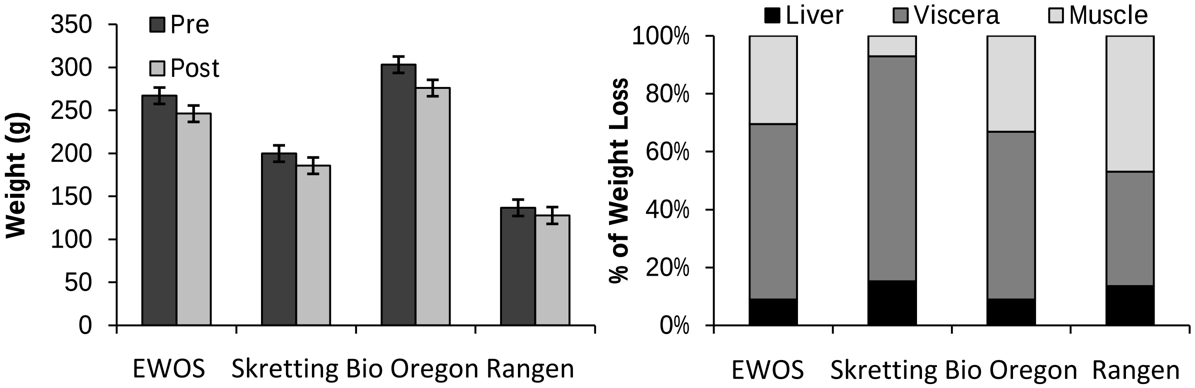

Figure 3.3. *Left panel:* Catchable Rainbow Trout weight (g; SE bars) at the start (pre) and end (post) of the 50 day feed and size-at-stocking experiment by feed company (EWOS, Skretting, Bio Oregon, and Rangen). *Right panel:* Location from which weight loss in the catchable Rainbow Trout occurred for each feed company given the change in weight of the liver, viscera, and overall weight of the fish from the beginning to the end of the 50 day feed and size-at-stocking experiment.

Overall, fish length did not change over the course of the 50 day experiment (p = 0.84) indicating that fish did not grow while in the experimental tanks. There was a significant decrease in weight from the beginning to end of the experiment (p = 0.01; Figure 3.3), although the interaction between weight at the beginning and end of the experiment and feed company was not significant (p = 0.79), suggesting that feed quality did not affect the ability to maintain weight in catchable Rainbow Trout. Patterns of weight loss differed from the fingerling Rainbow Trout. Fish reared on Skretting experienced very little muscle loss in comparison to the fingerling fish reared on the same feed, and muscle loss was much lower in fish reared on Skretting than in the other three feed companies in the catchable Rainbow Trout experiment. Fish reared on Rangen were the only fish to experience up to 50% muscle loss, with fish reared on EWOS and Bio Oregon losing only about 25% muscle mass (Figure 3.3). Similar to the weight data, although there was significant reduction in K in all fish between the beginning and end of the experiment (p < 0.01), decreasing from 1.20 (± 0.04) to 1.10 (± 0.03) over the course of the 50 day experiment, there was not a significant reduction in K within a feed company between the beginning and end of the experiment (p = 0.12).

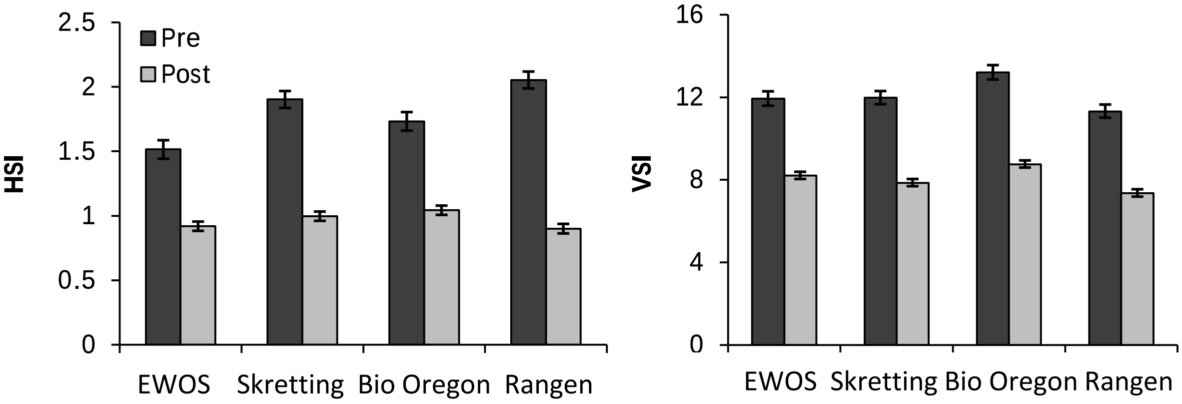

Figure 3.4. Hepatosomatic index (HIS; SE bars; left panel) and viscerosomatic index (VSI; SE bars; right panel) at the start (pre) and end (post) of the 50 day feed and size-at-stocking experiment for catchable Rainbow Trout reared on the four feeds (EWOS, Skretting, Bio Oregon, and Rangen).

HSI and VSI both decreased significantly over the course of the experiment ($p < 0.01$ for both metrics; Figure 3.4), suggesting that the liver and viscera fat were both absorbed to keep fish alive over the course of the experiment, and the weight loss location results suggest that for all fish except those fed on Rangen, the liver and viscera were primarily source of energy consumption over the course of the experiment. HSI decreased significantly within feeds from the beginning to the end of the experiment, but VSI did not ($p = 0.62$), suggesting that the liver was the primary source of energy consumption during the experiment, and that this source was likely drawn from prior to switching to viscera fat for energy.

Discussion

The results of the feed and size-at-stocking experiments show that feed quality is more of factor affecting the health and condition of fish when they are smaller than when they are larger. Fingerling Rainbow Trout experienced much larger declines in weight and condition compared to catchable rainbow trout reared on the same feeds. Additionally, weight loss location differs in food-deprived fingerling and catchable Rainbow Trout, with fingerling Rainbow Trout losing proportionally more muscle mass overall than catchable Rainbow Trout. It is expected that if these fingerling Rainbow Trout were to be released into a body of water in which food availability was low upon release, fish reared on EWOS and Bio Oregon would survive better than those reared on Skretting and Rangen because of the energy reserves the fish were able to access in the liver and viscera, rather than the muscle. Muscle absorption and loss of muscle mass is expected to affect swimming ability and predator avoidance in these fish, decreasing their survival in the wild. Fingerling Rainbow Trout reared on EWOS and Bio Oregon exhibited higher HSI and VSI than did the catchable Rainbow Trout, a pattern that had been observed in Experiments 1 and 2 above, likely a result of the higher energy content in the smaller feed sizes from these feed companies compared to Skretting and Rangen.

Overall, the results of these experiments support the conclusions of the second hatchery feed experiment that EWOS and Bio Oregon should be considered for use on a larger scale within Colorado's hatcheries. Both feeds produce healthier, higher quality Rainbow Trout that would be expected to exhibit higher post-stocking survival rates. Additionally, both feeds produce catchable Rainbow Trout in a shorter amount of time than do Skretting and Rangen, with Bio Oregon producing catchable fish two weeks sooner than EWOS, and both producing catchable fish at least 1 to 3 months sooner than Skretting or Rangen.

Qualifying Statement

The results contained herein were obtained from trout reared in Colorado hatcheries under controlled study conditions. Results may vary under other hatchery conditions or with different strains or species from those used in these experiments. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the State of Colorado.

Literature Cited

Bailey, J., and A. Alanärä. 2006. Effect of feed portion size on growth of rainbow trout, *Oncorhynchus mykiss* (Walbaum), reared at different temperatures. Aquaculture 253:728-730.

Gause, B. R., and J. T. Trushenski. 2013. Sparing fish oil with beef tallow in feeds for rainbow trout: effects of inclusion rates and finishing on production performance and tissue fatty acid composition. North American Journal of Aquaculture 75:495-511.

Goede, R. W., and B. A. Barton. 1990. Organismic indices and an autopsy-based assessment as indicators of health and condition of fish. Pages 93-108 *in* S. M. Adams, editor. Biological indicators of stress in fish. American Fisheries Society Symposium 8. Bethesda, Maryland.

Kientz, J., M. E. Barnes, and D. J. Durben. 2012. Performance of commercial starter diets during first-feeding of landlocked fall Chinook salmon. Proceedings of the South Dakota Academy of Science 91:101-106.

Kim, K. I. 1997. Re-evaluation of protein and amino acid requirements of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 151:3-7.

Ney, J. J. 1999. Practical use of biological statistics. Pages 167-191 *in* C. C. Kohler and W. A. Hubert, editors. Inland fisheries management in North America, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Noble, C., K. Mizusawa, K. Suzuki, and M. Tabata. 2007. The effect of differing self-feeding regimes on the growth, behavior and fin damage of rainbow trout held in groups. Aquaculture 264:214-222.

Piper, R. G., I. B. McElwain, L. E. Orme, J. P. McCaren, L. G. Fowler, and J. R. Leonard. 1982. Fish hatchery management. U. S. Fish and Wildlife Service, Washington, D. C.

SAS Institute. 2016. SAS system software, release 9.4. SAS Institute, Cary, North Carolina.

Silverstein, J. T. 2006. Relationships among feed intake, feed efficiency, and growth in juvenile rainbow trout. North American Journal of Aquaculture 68:168-175.

Trushenski, J. T., C. S. Kasper, and C. C. Kohler. 2006. Challenges and opportunities in finfish nutrition. North American Journal of Aquaculture 68:122-140.

Trushenski, J., J. Rosenquist, and B. Gause. 2011. Growth performance, tissue fatty acid composition, and consumer appeal of rainbow trout reared on feeds containing terrestrially-derived rendered fats. North American Journal of Aquaculture 73:468-478.

Wagner, E. J., M. D. Routledge, and S. S. Intelman. 1996. Assessment of demand feeder spacing on hatchery performance, fin condition, and size variation of rainbow trout *Oncorhynchus mykiss*. Journal of the World Aquaculture Society 27:130-136.

Appendix A
Feed Quality and Deformity Formation in Snake River Cutthroat Trout

Feed quality can affect many processes in salmonid fishes including growth, health indices, appearance, and taste. The effects of basic feeds from four commercial feed manufacturers were evaluated for Rainbow Trout reared in Colorado hatcheries (see Experiments 1-3 above), but had not been evaluated for other species of salmonids. Anecdotal evidence from the CPW Crystal River Hatchery suggested that deformity incidence in Snake River Cutthroat Trout may vary with feed. This was determined after switching feeds from Rangen to Bio Oregon in subsequent years and following standard practice of removing deformed individuals from future brood stocks at the fingerling life stage. Fewer deformed individuals were removed after feeding Bio Oregon than in previous years of feeding Rangen. The following describes an experiment conducted at the BFRH used to test if deformity incidence in Snake River Cutthroat Trout fingerlings differs by feed company.

Seven unique male-female families were spawned at the CPW Crystal River Hatchery in fall 2017 and shipped in individual egg crates to the BFRH for experimentation. Upon receiving the eggs, the three families that contained enough eggs for experimentation (≥ 2000; families 1, 4, and 5) were chosen based on egg counts performed at the CPW Crystal River Hatchery. Space constraints at the BFRH prevented all seven families from being used in the experiment. Eggs from each family were placed in floating egg boxes in the experimental raceways and counted out using egg siphons. Two thousand eggs from each family were split into two adjoining hatchery troughs, each containing one thousand eggs, and held until they hatched. During this time, dead eggs were removed from the trough to prevent fungal infections.

Fish were reared in the same troughs in which they had hatched. Crippled fish and mortalities were removed daily. Feeding began upon swim-up, which occurred on January 4, 2018. The two troughs for each family were each fed different feeds, Bio Oregon or Rangen. Similar to Experiment 1 above, the manufacturer's recommendations for feed size and feeding rate were followed in this experiment (Tables A.1 and A.2). Fish were fed six times daily through the conclusion of the experiment on March 26, 2018. Upon conclusion of the experiment, 50 fish were removed from the trough, measured and weighed, and checked for deformity type and number. Additionally, five fish from this group were dissected to obtain liver and viscera weights for comparisons of hepatosomatic index (HSI) and viscerosomatic index (VSI) between the feed companies.

Table A.1. Bio Oregon suggested feeding rate (% BW/d) by feed size, fish size, and at a temperature of 54°F.

Feed Size	Count per Pound	Length (in)	Weight (g)	Feeding Rate
#0	3000-570	Hatch-1.7	0.15-0.8	3.3
#1	570-300	1.7-2.1	0.8-1.5	3.1
#2	300-150	2.1-2.6	1.5-3.0	3.0

Table A.2. Rangen suggested feeding rate (% BW/d) by feed size, fish size, and at a temperature of 53°F.

Feed Size	Count per Pound	Length (in)	Weight (g)	Feeding Rate
#0	< 1,200	< 1.3	< 0.4	5.4
#1	1,200	1.3	0.4-0.8	5.4
#2	600	1.5	0.8-1.5	4.5
#2	300	2.0	1.5-2.3	3.9
#3	200	2.3	2.3-4.5	3.5

The remaining fish were individually checked for deformities, and type of deformity or deformities were recorded for each fish. Nine deformities were identified in this experiment, 1) spinal deformities, bends or twists of the spine that caused a deviation from the typical fusiform shape, 2) lower jaw deformities, shortened lower jaws or lower jaws that were bent to one side, 3) upper jaw and cranial deformities, deformities causing shortening of the upper jaw or depressions in the skull, 4) caudal deformities, most often a nearly 90 degree dip in the spine posterior of the adipose fin, 5) missing eyes, where the eye did not appear to form correctly causing it to be small or nonexistent, 6) bulging eyes or exopthalmia, where the eye extended out of its socket, 7) caudal fin deformities, typically consisting of deformations where the caudal fin connected to the body along the top of the fish, 8) dorsal fin deformities, where the dorsal fin was not fully formed, containing only a couple of deformed rays, or nonexistent, and 9) opercular deformities, where the operculum was pulled back or eroded along the edge or in the center, exposing the gills. Although all deformities ranged in severity, a severity score was not associated with the deformities, and all deformity types were recorded as present or absent.

Since the effect of feed on growth and health indices had never been evaluated for Snake River Cutthroat Trout, an analysis of variance (ANOVA), implemented in SAS PROC GLM (SAS Institute 2017), was used to determine if there were differences in survival, fish weight, feed conversion ratios, HSI, and VSI among the families and feed companies. Additionally, an ANOVA was used to determine family and feed effects on deformity presence, number of deformities per fish, and deformity type and expression, as well as whether fish weight was affected by deformity presence or number.

There was not an effect of feed on survival, but survival did differ among families. Family 5 exhibited significantly higher survival (97%) than families 1 and 4 (84% and 86%, respectively). Feed conversion ratios differed by feed, with fish fed Bio Oregon having a significantly lower feed conversion ratio (0.70) than fish fed Rangen (1.0). Although the feed conversion ratio was similar for Rainbow Trout and Snake River Cutthroat Trout fed Bio Oregon, Snake River Cutthroat Trout fed Rangen had a higher feed conversion rate than did Rainbow Trout fed Rangen (see Experiment 1 above). Despite the lower feed conversion ratios, fish fed Rangen weighed significantly more at the end of the experiment than fish fed Bio Oregon (4.41 and 4.07 g, respectively), likely a result of the higher feeding rate used for Rangen. Fish reared on Bio Oregon had a significantly higher HSI and VSI (1.28 and 11.1, respectively) than did fish fed Rangen (1.09 and 9.8, respectively). Overall, HSI and VSI values for the Snake River Cutthroat Trout were lower than for Rainbow Trout (see Experiment 1 above), suggesting that the two species differ in the way that the feeds are processed and excess energy stored in the liver and viscera.

Deformity incidence differed among the families, but was not affected by feed. A significantly larger percentage of the fish in family 1 developed a deformity in comparison to families 4 and 5 (21, 13 and 10%, respectively), and a larger percentage of fish expressed deformities in family 4 than family 5. On average, 14% and 15% of fish fed Bio Oregon and Rangen expressed deformities, respectively. Among deformed individuals, the number of deformities differed by both family and feed, with family 1 expressing a larger number of deformities (1.5) than family 4 (1.3) or family 5 (1.1), and fish fed Bio Oregon expressing a higher number of deformities (1.4) than fish fed Rangen (1.2). The presence of a deformity affected fish weight, with fish that did not have a deformity weighing an average of 4.3 g, and fish with a deformity weighing an average of 3.7 g. Additionally, in the fish that expressed at least one deformity, the number of deformities also affected fish weight such that the more deformities a fish expressed, the smaller the fish (Figure A.1).

Overall, opercular deformities were the most common deformity observed in the experiment, followed by upper jaw and cranial deformities, caudal deformities, spinal deformities, and lower jaw deformities (Table A.3). Bulging eyes were also fairly common, and were generally observed in conjunction with cranial deformities, explaining why more bulging eyes were observed in family 1 than the other two families. Missing eye, caudal fin and dorsal fin deformities were the least common deformities, and were generally observed in one family, suggesting these deformities were likely associated with family genetics rather than feed company. Certain deformities were differentially expressed by family or feed company (Table A.3). For example, the number of fish expressing opercular deformities was similar among the feed companies in families 1 and 4, but fish fed Bio Oregon in family 5 expressed fewer opercular deformities than did fish fed Rangen in that same family. Upper jaw and cranial deformities were more common in family 1 compared to families 4 and 5. A higher number of fish fed Bio Oregon exhibited caudal deformities than did fish fed Rangen in all families.

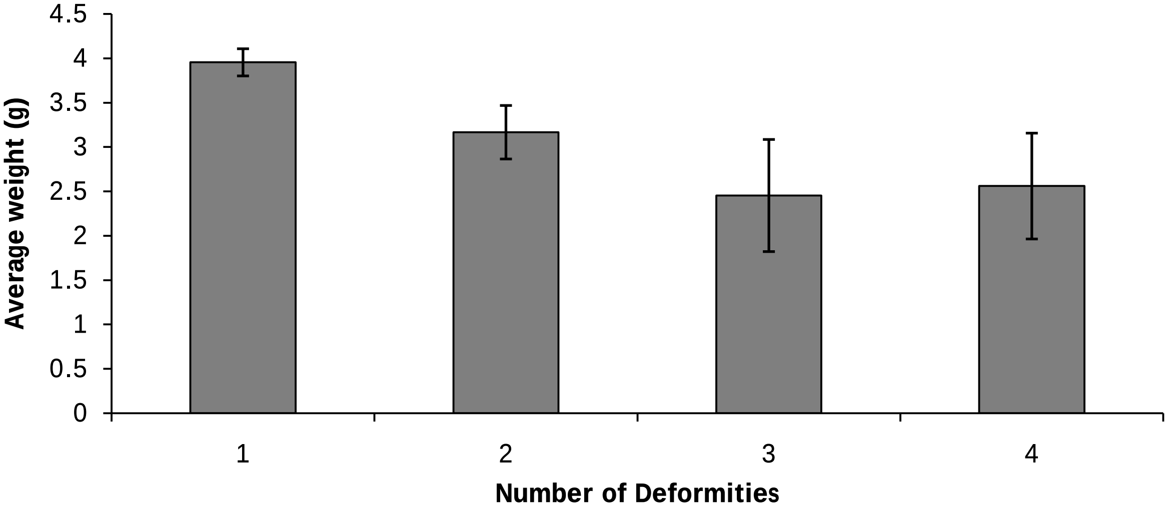

Figure A.1. Average weight (g; SE bars) of Snake River Cutthroat Trout expressing one, two, three, or four deformities.

Table A.3. Number of deformities observed by deformity type for all fish included in the experiment, and by family and feed company.

Deformity Type	All	Family 1		Family 4		Family 5	
		Bio Oregon	Rangen	Bio Oregon	Rangen	Bio Oregon	Rangen
Spinal	92	19	12	14	17	26	4
Opercular	333	53	67	56	53	29	75
Lower Jaw	80	18	15	10	9	14	14
Upper Jaw/Cranial	164	51	59	18	21	9	6
Caudal	106	56	18	15	3	13	1
Missing Eye	4	3	0	0	1	0	0
Bulging Eye	66	26	26	6	6	2	0
Caudal Fin	8	5	2	1	0	0	0
Dorsal Fin	2	0	0	0	0	2	0

Although deformity type and deformity number were affected by feed company, overall deformity expression did not differ by feed. As such, switching feeds from Rangen to Bio Oregon is not likely to result in lower deformity formation in Snake River Cutthroat Trout, especially if hatchery practice is to remove fish exhibiting any kind of deformity during the brood stock selection process. However, given the critical evaluation of even slight deformities made by the observers in this experiment, deformity severity may have more of an effect on deformity identification during hatchery sorting. Family had a large effect on incidence of deformities, with some families containing 11% more deformed fish than other families in this experiment. It is therefore likely that the observed reduction in deformity incidence in the CPW Crystal River Hatchery was a result of spawning fish that were less likely to have offspring that expressed deformities than families spawned in previous years rather than changing the feed company. There could be additional potential benefits, however, of switching feeds from Rangen to Bio Oregon in Snake River Cutthroat Trout brood stocks, including lower (better) feed conversion ratios, which should result in higher growth rates when extending feeding beyond the fingerling life stage, and higher HSI and VSI values, which could result in healthier fish and potentially better egg quality if excess energy storage carries over into egg formation in future brood stock fish.