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Effects of Four Commercial Diets on Rainbow Trout *Oncorhynchus mykiss Growth,* Feeding Efficiency and Mortality at a Production Hatchery with Endemic Bacterial Coldwater Disease

Tabor J Martin[1], Jill M Voorhees[2], Cody E Treft[1], Brian Fletcher[1] and Michael E Barnes[2]*

1 South Dakota Department of Game, Fish and Parks, Cleghorn Springs State Fish Hatchery, 4725 Jackson Blvd Rapid City, SD 57702, USA

2 South Dakota Department of Game, Fish and Parks, McNenny State Fish Hatchery, 19619 Trout Loop Spearfish, SD 57783, USA

***Corresponding author:** Michael E Barnes

mike.barnes@state.sd.us

South Dakota, Department of Game, Fish and Parks, USA.

Tel: +605-642-1262

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Abstract

Flavobacterium psychrophilum is a pathogen causing bacterial Coldwater disease in salmonid hatcheries worldwide. This study evaluated the feeding of four commercial diets to rainbow trout *Oncorhynchus mykiss* at a hatchery with endemic bacterial Coldwater disease in three sequential feed trials, beginning at 28 days after initial feeding and lasting for a total duration of 83 days. Two Bio-Oregon (Longview, Washington, USA) diets, Bio-Vita and BioPro2 and two Skretting (Toele, Utah, USA) diets, Classic Trout and Protec, were used. While the overall mortality rates were the same among all the diets, there were differences in the timing at which the mortality occurred. Significant differences in tank ending weight, weight gain and feed conversion ratio only occurred in the third and final trial, with the Classic Trout diet producing the poorest results. Overall results, with all of the trials combined, indicated tanks receiving the Bio-Vita and BioPro2 diets had significantly higher weight gain than tanks receiving Classic Trout. Additionally, feed conversion ratios were significantly elevated in the Classic Trout treatment group compared to the other three dietary treatments. With few impacts on overall mortality among all diets tested, the faster growth obtained from the Bio-Oregon diets may lead to more rapid development of acquired immunity with possible implications for bacterial Coldwater disease management beyond the duration of this study.

Keywords: *Flavobacterium psychrophilum*; Rainbow trout; Bacterial Coldwater disease; *Oncorhynchus mykiss*; Diet

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Introduction

Bacterial coldwater disease, caused by Flavobacterium psychrophilum, affects hatcheries rearing salmonids around the world. Mortality from this disease typically ranges from 10 to 30%, but losses of up to 70% have been reported [1]. Although bacterial coldwater disease affects many different salmonid species, juvenile rainbow trout Oncorhynchus mykiss are particularly susceptible [2,3].

With no approved commercial vaccines currently available, antibiotics are typically used to treat bacterial coldwater disease infections. However, Flavobacterium psychrophilum quickly develops antibiotic resistance , and there are numerous concerns about the over use of antibiotics [1,4-11]. Other techniques that may help alleviate coldwater disease-induced mortality include improving water quality, decreasing fish handling and promptly removing fish mortalities from the rearing units [2,3,12].

Nutrition affects the fish immune response and particular attention has been focused on the impacts of beta-glucans, nucleotides, and probiotics to prevent and remediate fish disease outbreaks [13-19]. Barnes and Brown suggested that nutrition may influence Coldwater disease, but there is a lack of research evaluating diet and bacterial coldwater disease [3].

Cleghorn Springs State Fish Hatchery, Rapid City, South Dakota, USA experiences substantial mortality in juvenile rainbow

trout due to bacterial Coldwater disease [20]. The predictable occurrence of this disease at the Cleghorn hatchery and the availability of several commercial diets provided the opportunity to evaluate potential dietary influences on bacterial coldwater disease-induced mortality in a production aquaculture setting. Thus, the objective of this study was to examine the mortality and rearing performance of juvenile rainbow trout fed one of four different commercial diets during exposure to *Flavobacterium psychrophilum*.

Materials and Methods

All experimentation occurred at Cleghorn Springs State Fish Hatchery using 11°C spring water (total hardness as $CaCO_3$, 36 mg L^{-1}; alkalinity as $CaCO_3$, 210 mg L^{-1}; pH, 7.6; total dissolved solids, 390 mg L^{-1}). Nitrogen was removed from the incoming water via a degassing column, and liquid oxygen dissolved into the main incoming water line prior to the water being routed to each rearing tank used in this study. Dissolved oxygen levels were maintained at or above 7.0 mg/L in the discharge water from all of the tanks.

Three sequential trials were conducted, with the first trial starting after Shasta strain rainbow trout were feed trained for 28 days using Bio-Oregon (Longview, Washington, USA) starter. During the feed-training period, symptoms typical of bacterial Coldwater disease, such as anorexia, sporadic circular swimming patterns, deformation of vertebrae and skin ulcerations, were observed and the presence of *Flavobacterium psychrophilum* was confirmed [2,3,12]. No therapeutic antibiotic treatments were administered to the trout, and the symptoms and mortality associated with bacterial Coldwater disease continued throughout the duration of the study.

In the first trial 5,000 rainbow trout (mean ± SE, length 36.2 ± 0.42 mm, weight 0.45 g ± 0.014 g, n=30) were each placed into 16, 1.61 m^3 diameter circular fiberglass tanks on March 23, 2016. One of four commercial diets was assigned to each of the 16 tanks (n=4). The diets used were Classic Trout #1 (Skretting, Toele, Utah, USA), Protec #1 (Skretting, Toele, Utah, USA), Bio-Vita Starter #1 (Bio-Oregon, Longview, Washington, USA), and BioPro2 #1 (Bio-Oregon, Longview, Washington, USA). **Tables 1 and 2** describe the proximate composition and ingredients for each diet. The initial trial lasted for 27 days. At the trial conclusion on April 21, 2016, total tank weights were recorded to the nearest 0.01 g and five fish per tank were weighed to the nearest 0.01 g and measured (total length) to the nearest 0.01 mm.

Feed amounts were determined using the hatchery constant (HC) method with a planned feed conversion of 1.1 and a maximum growth rate of 0.07 cm/day. This is the typical rate used at Cleghorn hatchery [21]. Daily feed rations were dispensed hourly from 0800 to 1600 using automatic feeders (Sweeney Enterprises, Inc., Boerne, TX). Feed amount and mortality were recorded daily for each tank. Daily feed amounts were weighed to the nearest 0.1 g.

The second trial began immediately after conclusion of the first trial. After total tank weights were obtained, all of the fish from the four tanks of each treatment were placed into one tank. From this pool 4,000 fish were placed into each of four tanks. This process was repeated for each of the diets (4 tanks) used in trial 1. Feeding of #2 granules commenced the following day and continued for 26 days until the end of the trial on May 17, 2016. The diets used in trial 1 were continued in trial 2; only the particle size increased to #2 granules (e.g. fish fed #1 Classic Trout in trial 1 received #2 Classic Trout in trial 2). Feeding rates, techniques, and data collection were the same as trial 1.

The third trial began immediately after conclusion of trial 2. All four tanks of each treatment fish were again pooled into one tank. From each pool, 3,500 fish were placed into each of four tanks. Feeding of 1.5 mm granules commenced the following day and continued for 30 days until the end of the trial on June 16, 2016. The diets used in trial 3 were the same as in the first two trials, but particle size increased to 1.5 mm pellets. The fish received the same diet as they did in the first trials, but at the larger size. Feeding rates, techniques, and data collection were the same as in the first two trials.

For all trials, weight gain was measured by subtracting initial tank weight from final tank weight. Feed conversion ratio was calculated for each tank by dividing the total amount of feed fed by the total weight gained.

One-way Analysis of Variance was used for data analysis, and if significant effects were observed, Tukey's means of comparison procedure was conducted. Percent mortality data was log transformed prior to analysis to stabilize variances [22].

Table 1 Proximate analysis of diets used in this study.

	Feed Size	Classic Trout	Protec	BioPro2	Bio-Vita
#1	Protein (%)	52	52	52	52
	Fat (%)	16	16	20	20
	Fiber (%)	3	3	1	1
	Ash (%)	12	10	12	12
	Moisture (%)	8	10	8.5	8.5
	NFE[1] (%)	9	9	6.5	6.5
	Digestible Energy	18.2	18.2	19.3	19.3
#2	Protein (%)	52	52	52	52
	Fat (%)	16	16	20	20
	Fiber (%)	3	3	1	1
	Ash (%)	12	10	12	12
	Moisture (%)	8	10	8.5	8.5
	NFE[1] (%)	9	9	6.5	6.5
	Digestible Energy	18.2	18.2	19.3	19.3
1.5 mm	Protein (%)	52	47	50	50
	Fat (%)	16	17	22	20
	Fiber (%)	3	3	1	1
	Ash (%)	1 12	10	13	13
	Moisture (%)	8	8	8.5	8.5
	NFE[1] (%)	9	15	5.5	7.5
	Digestible Energy	18.2	18.2	19.6	19.6

NFE: Nitrogen-Free Extract

Table 2 Ingredient list on manufacturer's ingredient label. Ingredients are listed in order of decreasing diet composition (i.e., the diets contain less of each subsequent ingredient moving down the list).

Feed Size	Classic Trout	Protec	BioPro2	Bio-Vita
#1	Fish meal	Fish meal	Fish meal	Fish meal
	Wheat flour	Wheat flour	Fish oil	Fish oil
	Poultry by-product meal	Fish oil	Wheat flour	Wheat flour
	Fish oil	Wheat gluten	Wheat gluten	Wheat gluten
	Squid meal	Feather meal	Whey powder	Dried fish soluble
	Brewer's yeast	Poultry by-product meal	Porcine gelatin	Dried whey powder
	Choline chloride	Brewer's yeast	Lecithin	Porcine gelatin
	L-ascorbyl-2-polyphosphate	Squid meal	Vitamin/mineral premix	Mono ammonium phosphate
	Vitamin/mineral premix	L-lysine hydrochloride	Brewer's yeast	Brewer's yeast
	Ethoxyquin	L-ascorbyl-2-polyphosphate	Vitamin E	DL methionine
		Choline chloride	Astaxanthin	Vitamin/mineral premix
		Astaxanthin	Flavoring	Astaxanthin
		Vitamin/mineral Premix	Ethoxyquin	Ethoxyquin
		Flavoring		
		Ethoxyquin		
#2	Fish meal	Fish meal	Fish meal	Fish meal
	Wheat flour	Wheat flour	Fish oil	Fish oil
	Poultry by-product meal	Fish oil	Wheat flour	Wheat flour
	Fish oil	Wheat gluten	Wheat gluten	Wheat gluten
	Squid meal	Feather meal	Whey powder	Dried fish solubles
	Brewer's yeast	Poultry by-product meal	Porcine gelatin	Dried whey powder
	Choline chloride	Brewer's yeast	Lecithin	Porcine gelatin
	L-ascorbyl-2-polyphosphate	Squid meal	Vitamin/mineral premix	Mono ammonium phosphate
	Vitamin/mineral premix	L-lysine hydrochloride	Brewer's yeast	Brewer's yeast
	Ethoxyquin	L-ascorbyl-2-polyphosphate	Vitamin E Supplement	DL methionine
		Choline chloride	Astaxanthin	Vitamin/mineral premix
		Astaxanthin	Flavoring	Astaxanthin
		Vitamin/mineral Premix	Ethoxyquin	Ethoxyquin
		Flavoring		
		Ethoxyquin		
1.5 mm	Fish meal	Fish meal	Fish meal	Fish meal
	Wheat whole	Wheat flour	Fish oil	Fish oil
	Soybean meal	Soybean meal	Wheat flour	Wheat flour
	Blood meal	Blood meal	Wheat gluten	Wheat gluten
	Feather meal	Poultry oil	Mono ammonium phosphate	Mono ammonium phosphate
	Poultry by-product meal	Fish oil	Brewer's yeast	Brewer's yeast
	Fish oil	Wheat gluten meal	Vitamin/mineral premix	Vitamin/mineral premix
	Poultry oil	Feather meal	Astaxanthin	Astaxanthin
	Corn gluten meal	Corn gluten meal	Flavoring	Ethoxyquin
	Mono calcium phosphate	Mono calcium phosphate	Ethoxyquin	
	L-ascorbyl-2-polyphosphate	L-ascorbyl-2-polyphosphate		
	D L Methionine	Brewer's yeast		
	Calcium Propionate	Choline chloride		Fish meal
	Vitamin/Mineral premix	Calcium Propionate		Fish oil
	Ethoxyquin	Vitamin/Mineral premix		Wheat flour
		Flavoring		
		Ethoxyquin		

Table 3 Mean (± SE) gain, feed conversion ratio (FCR) and mortality rates for tanks of rainbow trout experiencing an outbreak of Bacterial Coldwater Disease while receiving one of four diets during three sequential feeding trials. Means followed by different letters within a row are significantly different (N=4, P<0.05).

Trial		Classic Trout	Protec	BioPro2	Bio-Vita
1	Initial biomass (kg)	2.23 ± 0.00	2.23 ± 0.00	2.23 ± 0.00	2.23 ± 0.00
	Final biomass (kg)	8.66 ± 0.19	8.89 ± 0.16	8.76 ± 0.21	8.79 ± 0.11
	Gain (kg)	6.43 ± 0.19	6.66 ± 0.16	6.53 ± 0.21	6.56 ± 0.11
	Food fed (kg)	6.76 ± 0.01	6.77 ± 0.00	6.73 ± 0.01	6.74 ± 0.01
	FCR*	1.05 ± 0.03	1.02 ± 0.02	1.03 ± 0.03	1.03 ± 0.02
	Mortality (%)	5.7 ± 0.2 z	5.4 ± 0.1 z	6.5 ± 0.2 y	6.4 ± 0.1 y
2					
		8.51 ± 0.00	8.56 ± 0.00	8.58 ± 0.00	8.53 ± 0.00
		18.60 ± 0.38	18.41 ± 0.21	18.88 ± 0.14	18.02 ± 0.11
		10.10 ± 0.37	9.90 ± 0.21	10.30 ± 0.14	9.50 ± 0.11
		11.24 ± 0.02	11.26 ± 0.01	11.32 ± 0.00	11.24 ± 0.01
		1.12 ± 0.04	1.15 ± 0.03	1.12 ± 0.03	1.19 ± 0.01
		3.9 ± 0.4 zy	4.7 ± 0.1 z	3.3 ± 0.2 y	3.7 ± 0.2 zy
3					
	Initial biomass (kg)	16.86 ± 0.08	16.83 ± 0.71	17.29 ± 0.1	16.61 ± 0.27
	Final biomass (kg)	32.76 ± 0.91 z	35.42 ±1.18 zy	38.54 ± 0.58 y	37.24 ± 0.85 y
	Gain (kg)	15.90 ± 0.89 z	18.59 ± 0.58 zy	21.26 ± 0.58 y	20.64 ± 0.89 y
	Food fed (kg)	19.87 ± 0.17	19.81 ± 0.17	20.59 ± 0.13	19.91 ± 0.29
	FCR	1.26 ± 0.07 z	1.07 ± 0.03 y	0.97 ± 0.03 y	0.97 ± 0.03 y
	Mortality (%)	3.1 ± 0.2 z	3.0 ± 0.1 z	2.3 ± 0.2 y	2.4 ± 0.2 zy
Comprehensive					
Trial		Classic Trout	Protec	BioPro2	Bio-Vita
	Initial biomass (kg)	2.23 ± 0.00	2.23 ± 0.00	2.23 ± 0.00	2.23 ± 0.00
	Final biomass (kg)	32.76 ± 0.91	35.42 ± 1.2	38.54 ± 0.588	37.24 ± 0.85
	Gain (kg)	30.52 ± 0.91 z	33.19 ± 1.2 zy	36.31 ± 0.58 y	35.01 ± 0.85 y
	Food fed (kg)	37.88 ± 0.18	37.84 ± 0.17	38.64 ± 0.14	37.89 ± 0.30
	FCR	1.24 ± 0.03 z	1.10 ± 0.04 y	1.07 ± 0.02 y	1.08 ± 0.02 y
	Mortality (%)	8.8 ± 0.3	8.4 ± 0.1	8.8 ± 0.1	8.8 ± 0.2

*Feed conversion ratio (FCR)=Food fed/Weight gain

Significance was predetermined at P<0.05. To prevent pseudo-replication, mean individual fish length and weight was first calculated for each tank, and then these means were used in analysis of variance.

Results

Symptoms of Bacterial Coldwater Disease were observed in each of the three trials, and the presence of *Flavobacterium psychrophilum* was verified.

Trial 1

Rainbow trout mortality was significantly different among the diets. Mortality in the tanks receiving Protect and Classic Trout was 5.4% and 5.7%, respectively, which was significantly less than the 6.4% and 6.5% observed in Bio-Vita and BioPro2 **(Table 3)**. Weight gain and feed conversion ratio were not significantly different among the diets. There was also no significant difference in individual fish length and weight **(Table 4)**.

Trial 2

The 4.7% mortality in tanks of trout fed Protec was significantly greater than the 3.3% mortality in the tanks fed BioPro2. Total tank weight gain ranged from 9.5 to 10.3 kg, but was not significantly different among the dietary treatments. Feed conversion ratio also was not significantly different. At the end of the second trial, mean rainbow trout length was 68.3 mm and mean weight was 3.9 g, with no significant differences observed among the four diets.

Trial 3

The 2.3% mortality in the tanks of trout fed BioPro2 was significantly lower than the 3.0% and 3.1% mortality observed in tanks fed Classic Trout or Protec. At 1.26, feed conversion ratio was significantly elevated in tanks receiving Classic Trout compared to the feed conversion ratio of 1.07 or less observed with all of the other diets. The Classic Trout diet also produced

Table 4 Mean (± SE) lengths and weights fish sizes from rainbow trout rainbow trout experiencing an outbreak of Bacterial Coldwater Disease while receiving one of four diets during three sequential feeding trials (N=4).

Trial	Feed Size		Classic Trout	Protec	BioPro2	Bio-Vita
1	#1 granules	Final length (mm)	55.2 ± 1.1	54.0 ± 1.6	56.9 ± 1.0	55.9 ± 1.1
		Final weight (g)	2.0 ± 0.2	1.7 ± 0.2	2.1 ± 0.1	1.9 ± 0.1
	#2 granules	Final length (mm)	69.4 ± 2.9	66.0 ± 2.6	70.5 ± 2.1	67.5 ± 2.2
2		Final weight (g)	4.0 ± 0.4	3.5 ± 0.4	4.3 ± 0.3	3.7 ± 0.3
	1.5 mm	Final length (mm)	94.2 ± 3.1	97.6 ± 2.7	98.4 ± 2.2	96.7 ± 2.4
3		Final length (mm)	9.3 ± 0.9	10.4 ± 0.8	11.4 ± 0.8	10.7 ± 0.9

significantly lower tank final weights and weight gain. Although mean individual fish lengths were over 2 mm less in trout fed Classic Trout, and mean weights were also 1.0 g less, neither fish lengths nor weights were significantly different among the diets.

Overall

With the results of all three of the trials combined, mortality rates were very similar and were not significantly different among the dietary treatments. However, tanks receiving the Bio-Vita and BioPro2 diets had significantly higher weight gain than tanks receiving Classic Trout. Feed conversion ratios were significantly elevated in the Classic Trout treatment group compared to the other three dietary treatments.

Discussion

While the overall mortality rates were the same among all the diets, there were differences in the timing at which the mortality occurred. Most of the mortality in the tanks of fish receiving Bio-Oregon diets occurred early and then subsequently decreased. Conversely, mortality in the tanks of fish receiving the Skretting diets was initially low and then increased. Even though each diet met or exceeded rainbow trout for protein and lipid nutritional requirements differences in ingredients, lipid content and digestible energy among the diets may at least partially explain the differences in mortality timing [23,24].

Astaxanthin, a carotenoid precursor to vitamin A , was present in all three sizes of both Bio-Oregon diets and #1 and #2 Protec granules; it was absent from Skretting Classic Trout and 1.5 mm Protec. Astaxanthin may have benefits other than just as a fillet colorant [24]. Christiansen et al. reported that astaxanthin influenced Atlantic salmon (*Salmo salar*) fish growth and survival, while Hertrampf and Piedad-Pascual indicated that salmonids with a higher content of astaxanthin were more resistant to microbial infection [25].

Lecithin is a phospholipid that, when included in fish diets, has been associated with improved growth and increased survival [24,26]. It has also been show to increase stress resistance [27]. The Bio-Vita and Classic Trout diets were the only ones that did not contain lecithin. In contrast, porcine gelatin was only present in the two Bio-Oregon diets. Porcine gelatin has only minimal known nutritional value but is highly digestible, making it suitable for carnivorous fish diets [28].

Lipid content also differed among the diets. The BioVita diets had lipid concentrations of 20 to 22% compared to the Skretting diets which were at 16 to 17%. Although the relationship between dietary lipid and immunological functions in terrestrial animals has been studied extensively, only a few attempts have been made to investigate these interactions in aquatic animals [29]. Kiron et al. noted that essential fatty acid deficiencies reduce antibody production and the *in vitro* killing of bacteria by macrophages in rainbow trout [15]. The higher digestible energy available in both Bio-Oregon diets, in comparison to the Skretting diets, may have provided the additional energy needed by the fish during *F. psychrophilum* infection, and thus may have influenced the observed mortality patterns [29].

Typical bacterial Coldwater disease mortality symptoms such as anorexia, erosion of caudal peduncle, sporadic circular swimming patterns, deformation of vertebrae, and skin ulcerations were observed throughout the duration of the study [2,3,12]. Mortality rates were typically concentrated during a short period of time and then would eventually subside. However symptoms persisted throughout the duration of the study. Historical bacterial Coldwater disease induced mortality of Shasta strain rainbow trout at Cleghorn Springs State Fish Hatchery has ranged from 1% to nearly 70%, making the nearly 5% mortality observed in this study at the lower end of the mortality spectrum. Treft et al. documented bacterial coldwater disease induced mortality ranging from 55%-69% in Shasta strain rainbow trout fry at Cleghorn [30]. The relatively low bacterial Coldwater disease-induced mortality observed in this trial could be due to the size of the fish used. Treft et al. recorded mortality starting with initial feeding, while this study evaluated mortality in larger fish that had been feed trained [30].

The results of this study may be specific to the strain of rainbow trout used, because different rainbow trout strains can exhibit different mortality patterns [31]. Mortality may also have been influenced by the unique water chemistry of Cleghorn hatchery and the fish rearing densities used [2,32-34]. In addition, the strain of Flavobacterium psychrophilum in this trial is unique, and less virulent strains may react differently to different diets [20,30].

The significantly lower weight gain and higher feed conversion ratio in trial 3 and overall of the fish fed the Skretting Classic Trout diet compared to the BioPro2 and Bio-Vita diets may have been due to differences in digestible energy and lipid content among these diets. However, gain and feed conversion ratio were also significantly different in fish fed either the Classic Trout or Protec diet, both of which had similar manufacturer-reported values of digestible energy and fat. But, different amounts of different protein and lipid sources were used in these diets. In the 1.5 mm sizes, formulations indicate higher concentrations of poultry oil

and fish oil in the Protec diet, where-as Skretting Classic Trout contained poultry by-product meal and higher concentrations of feather meal. The Protec diet in #1 and #2 sizes listed fish oil as the third ingredient, where-as Skretting Classic Trout listed fish oil fourth and poultry by-product meal third. In contrast, fish oil was listed as the second ingredient in both BioPro2 and Bio-Vita.

The individual fish length and weight results appear to be at odds with the overall tank weight gain and feed conversion ratio results. This is likely due to the very small size of the samples for statistical analysis. Because the tanks were the experimental unit, regardless of the number of individual fish that were weighed or measured, N could be no larger than four.

The timing of weight gain, feed conversion ratio, and mortality differences observed in this study reinforces the need to run experiments long enough to see significant effects. If this study had ended after the first trial (27 days) or the second trial (53 days), no significant differences would have been observed; significant differences in weight gain and feed conversion ratio did not become apparent until the end of the third trial at 83 days. Longer trial durations may be needed to successfully determine the effects of dietary differences. Weatherup and McCraken [35] stated that trials must persist long enough for significant differences to be observed and de Francesco et al. [36] did not observe differences between fish meal and plant-based diets until after 84 days. The National Research Council suggested feeding trial durations of 56-84 days [24].

Unlike the results reported by Treft et al., the results of this study indicate relatively minor impacts on overall mortality among all of the diets tested [30]. However, the faster growth obtained from the Bio-Oregon diets may lead to more rapid development of acquired immunity with possible implications for bacterial Coldwater disease management beyond the duration of this study [37,38].

Conclusion

In conclusion, Bio-Oregon diets are recommended during the rearing of juvenile rainbow trout to improve growth, increase feeding efficiencies and decrease mortality caused by *Flavobacterium psychrophilum*. Additional research is needed on the possible interaction between specific dietary ingredients, including possible immuno-stimulants such as astaxanthin, and bacterial Coldwater disease infections in rainbow trout.

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