Soy Aquaculture Alliance Project 2024

Project Report to Soy Aquaculture Alliance (SAA)

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Implementing integrated approaches to select for feed efficient rainbow trout families to enhance the soy protein utilization in salmonid aquaculture

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1. Introduction

Aquaculture has been one of the fastest growing animal-producing industries with great potential to provide healthy protein and unsaturated fat for the growing population. With the rising concerns of global food crisis, aquaculture might be a solution to feed the future (FAO, 2020). But one of the major impediments to the sustainable development of aquaculture is the availability of high quality, economical, and environmentally friendly protein ingredients for aquafeed production (Hardy, 2010). Plant protein sources (i.e. soybean) have been recognized as candidates to partially or completely replace fishmeal or other animal protein ingredients in the fish diet (Lim et al., 2008). The biggest drawback is that some carnivorous fish species cannot handle high levels of soybean meal in the diet due to poor digestibility and presence of antinutritional factors (Glencross, 2020). To solve this problem, over two decades of selective breeding research has been conducted by our team to develop a rainbow trout (*Oncorhynchus mykiss*) line that thrives on properly formulated, plant protein-based diets (Venold et al., 2012; Overturf et al., 2013; Lee et al., 2020). But, to the best of our knowledge, no commercial breeding programs have selected fish based on improved feed utilization efficiency. This could be mainly due to the difficulties in accurately measuring individual feed intake of fish reared in groups.

An increase in feed efficiency would lower feed costs, which makes up to 60% of variable costs of aquaculture production. Also, improvement of feed efficiency can directly mitigate the negative impacts of aquaculture on the environment (Cho and Bureau, 1997). Economic and environmental issues are two important pillars of sustainability with high impact on rainbow trout aquaculture, which is one of the most produced freshwater species in the US (FAO, 2020; Glencross et al., 2023). Our selected rainbow trout families are unique models to identify genetic and physiological parameters associated with sustainable plant protein utilization in fish. They grow much faster when fed plant-protein based diets compared to unselected trout fed fishmeal-based diets (Venold et al., 2012; Overturf et al., 2013; Lee et al., 2020). In this study, we have planned to validate our results with alternative approaches including using stable isotopes in feed, measuring metabolic rates and correlating gut microbiome with feed efficiency of individual trout families. By improving feed efficiency, the results of this study can help fish farmers to meet the increasing global demands for fish protein, limit food insecurity, abrogate the negative environmental impacts associated with producing fish, and increase producer profitability. The overall long-term aim of this project is to increase the use of soybean-based

protein in the aquafeed industry. This project will provide a unique opportunity to develop efficient and economical methods to fully explore the roles of genetics and nutrition for enhancing sustainable aquaculture in all species and thus providing a source of healthy protein to a growing world population.

2. Materials and Methods

This study was reviewed and approved by the University of Idaho Institutional Animal Care and Use Committee (IACUC). The 15 full-sib families used in this study were produced through crossing in 1:1 ratio by crossing a single female's eggs with milt from a single male. These 15 families were a part of 200 nucleus families produced in spawning cycle (Overturf et al, 2013). Fertilized eggs were transferred to heath trays (MariSource, Legend Brands, Inc., WA, US), then viable eggs were transferred to 140 L fiberglass tanks at 14 □. Rainbow trout from 15 families were reared at the Hagerman Fish Culture Experimental Station (HFCES) of the University of Idaho. All the fish groups were acclimated to the HFCES environment. Families were distinguished as CX-118, CX-125, CX-134, CX-135, CX-137, CX-138, CX-141, CX, 143, CX, 144, CX-145, CX-146, CX-147, CX-148, CX-149, and CX-152. This study was divided into two major parts. The first part (Experiment 1) aimed to develop an indirect benchmark to select the families of rainbow trout to enhance the efficiency of plant protein-based diets and the second part (Experiment 2) aimed at selecting the trout families for improved feed efficiency using stable isotope in feed.

2.1.Experiment 1:

A total of 1300 fish were PIT (passive integrated transponder) tagged on the dorsal muscle (Biomark, ID, US; Figure 1, A) and the tagged fish were acclimated for one week to check for mortalities caused by handling. After the acclimation period, PIT tags of each fish were recorded, and weight and length were measured using a PIT tag reader (HPR Plus Handheld PIT Tag Reader, Biomark, ID, US) attached to a measurement board (Big Fin Scientific, TX, USA) and a scale (Figure 1, B). A total of 1200 fish (80 fish/family) with an average initial weight of 32.4 g and an average initial length of 139.8 cm were distributed in four tanks (1 m^3, Figure 1, C).

Figure 1. Equipment used for tagging (A) and weight/length measurements (B) of rainbow trout before distribution into experimental tanks (C).

Fish were reared in this environment (flow through spring water at 15^{o}C) for four months and went through two periods of feed deprivation (FD) and two periods of refeeding (RF). The experiment started with a FD period followed by RF, FD, and RF periods, each for one month. During the RF periods fish were fed at satiation with an all-plant extruded diet (Table 1). The extruded diet was made and the Bozeman Fish Technology Center (Bezeman, MT, US). After each period, all fish in each tank were anesthetized (40 mg/l MS-222, buffered to pH 7.0), PIT tangs were scanned, and weight and lengths were measured. One separate tank containing 30 fish was considered as a normal group (N group) and continuously fed with the same diet during the four months.

Table 1. The experimental diet used for Experiment 1 with all plant-based protein.

Ingredient name	**g/100 g**	**Composition**	**(% as-is)**
Soybean Meal, Solvent extracted	25	Moisture	4.39
Wheat flour	13.3	Protein	43.57
Wheat gluten meal	2.24	Lipid	13.68
Fish Oil, Whitting trimmings oil	17	Ash	5.82
Soybean Protein Concentrate, Profine	23	Energy (Kcal/g)	5251
Vitamin Premix, ARS702	1		
Mineral premix, ARS 1520	0.1		
Stay-C	0.2		
Lecithin	2		
Taurine	0.5		
Astazanthin, pink	0.08		
Corn Protein Concentrate, E75	10.23		
Dicalcium Phosphate	2.85		
Lysin-HCL	1.67		
DL-Methionine	0.6		
Threonine	0.23		

Data was analyzed using the R software (The R Foundation, Vienna, Austria) according to Grima et al. (2010). At first, a linear regression model was used based on weight gain (WG=final weight (g) - initial weight (g)) and the residuals of this model were created, and values of each individual were averaged for FD and RF periods. The residuals were then standardized and scaled at a mean of 0 and a standard deviation of 10. Fish performance was classed as FD-, FD+, RF- and RF+ for fish exhibiting loss (FD) and gain (RF) of weight relatively lower (-) and higher

(+) than the population mean and separated into four triplicate groups FD-/RF-, FD+/RF+, FD-/RF+ and FD+/RF-. In order to have more distinctive groups of fish, an arbitrary low value of 2 away from the mean were applied to discard all the individuals concentrated on the border of each group. Each group was distributed into three tanks (triplicates) and the number of fish was adjusted to the lowest fish/tank (48 fish/tank). The daily feed intake was measured among these groups to calculate the feed conversion ratio (FCR) for three months. After the three months, fish will be fed the plant-based diet for another 4 months for the second feeding challenge study.

2.2.Experiment 2:

Another group of 1200 rainbow trout with an average initial weight of 32.0 g and an average initial length of 140.5 cm belonging to 15 families were individually PIT tagged (explained in section 2.1.) and distributed into two groups of 40 fish/tank. Therefore, each family was being represented by two groups of tanks (145-L) supplied with flow-through water at 14 □. The two groups of fish were fed at satiation with Diet 1 and Diet 2. Diet 1 was supplemented with 1.2% of lyophilized powder of spirulina whole cells (Cambridge Isotope Laboratories, Inc., MA, US) and Diet 2 was supplemented with lyophilized powder of spirulina whole cells labeled with U-^{15}N (98%+). The formulation and proximate composition of the basal diet is shown in Table 2. Diets were extruded at the Bozeman Fish Technology Center (Bezeman, MT, US) and delivered to HFCES.

Table 2. The basal diet used for Experiment 2 with all plant-based protein.

Ingredient name	g/100 g	Composition	(% as-is)
Soybean Protein Concentrate	23.00	Moisture	5.69
Corn protein concentrate	10.23	Protein	42.23
Soybean Meal, Solvent extracted	25.00	Lipid	19.24
Wheat gluten meal	2.24	Ash	5.57
Wheat four	11.85	Energy (Kcal/g)	5435
Lecithin	2.00		
Fish oil (Menhaden)	17.00		
Stay-C	0.20		
Vitamin premix, ARS702	1.00		
Trace min premix ARS 1440	0.10		
Taurine	0.50		
Choline CI 50%	0.25		
Monocalcium Phosphate	2.85		
Lysin-HCL	1.67		
DL-Methionine	0.60		
Threonine	0.23		
Astaxanthin	0.08		

After 18 days of feeding, all fish were scanned, and weight and length were measured. Also, 0.4 mm diameter Integra™ Miltex™ Standard Biopsy Punches (Fisher Scientific Inc., Pittsburgh, PA, US) were used to take muscle samples from each individual and transferred into Fisherbrand™ round bottom disposable borosilicate glass tubes with plain end. All fish were then treated with vetbond tissue adhesive lotion (Valley vet, Kansas, US) to prevent infection and allow faster recovery of fish. The glass tubes were transferred to an oven and samples were dried at 105□ for three hours. 1200 dried tissue samples were weighed individually (1-2 mg), carefully encapsulated in tin capsules (8×5 mm), and transferred to 96-well plates. All samples along with the weight data were sent to the University of California Davis, Stable Isotope Facility (SIF, CA, USA). SIF uses an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (IRMS) to provide ^{15}N analysis in solid tissues. Fish tissues were analyzed for ^{15}N isotopes using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Samples were combusted at 1000°C in a reactor packed with chromium oxide and silvered copper oxide. Following combustion, oxides were removed in a reduction reactor (reduced copper at 650°C). The helium carrier then flows through a water trap (magnesium perchlorate and phosphorous pentoxide). N_2 and CO_2 were separated on a Carbosieve GC column (65°C, 65 mL/min) before entering the IRMS. A sample's provisional isotope ratio is measured relative to a reference gas peak analyzed with each sample. These provisional values were finalized by correcting the values for the entire batch based on the known values of the included laboratory reference materials. The long-term standard deviation is 0.3 ‰ for ^{15}N. The final delta values were expressed relative to international standards VPDB (Vienna Pee Dee Belemnite) and air for carbon and nitrogen, respectively (Sharp, 2017). Similar to the previous study, fish performance was classed as W-, W+, N- and N+ for fish exhibiting lower (-) and higher (+) weight gain (W) and ^{15}N accumulation (N) relative to the population mean and separated into four groups W-/N-, W+/N+, W-/N+ and W+/N-.

2.3.Statistical analysis

Data were analyzed for normality and homogeneity of variance using the Kolmogorov-smirnov test and shapiro-wilk test in SPSS (IBM SPSS Statistics 29.0.0.0). One-way ANOVA was used to compare means and when significant differences were observed, Duncan's multiple-range test was used as a post-hoc test. Grouping of fish was performed by generating a linear regression model in R software (The R Foundation, Vienna, Austria) according to Grima et al. (2010).

3. Results

3.1.Experiment 1

During the experiment it was observed that rainbow trout could tolerate the one-month periods of FD and survival rate was >95%. The average weight of the experimental population during the FD/RF periods in contrast to the N group (normal group, continuously fed) is shown in Table 3 and Figure 2.

Table3. The average weight of the experimental population during the FD/RF periods in contrast to the N group (normal group).

	Initial	First month (FD)	Second month (RF)	Third month (FD)	Fourth month (RF)
N group	32.6	108.1	205.7	330.9	442.4
FD/RF	32.6	27.7	62.5	56.2	119.5

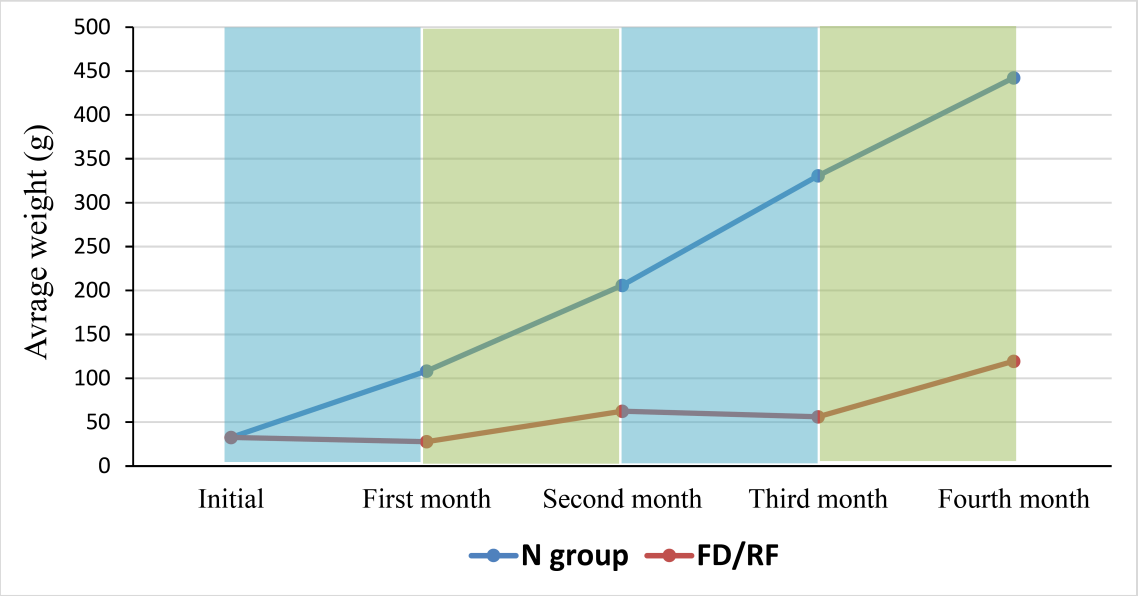

Figure 2. The average weight of the experimental population during the FD/RF periods in contrast to the N group

Figure 3 shows the individual average weight gain (WG, %) of fish in each family throughout the experiment. The WG% of fish during the FD period is obviously negative, showing the amount of weight loss during this period. Differences are more obvious for the RF period as compared to the FD period. Also. Figure 4 shows the statistical differences of WG (%) among families during the FD and RF periods. According to the Duncan's multiple-rage test, family CX-138 had significantly lower weight loss during FD and family CX-125 had the highest weight loss during this period ($P<0.05$). For the RF period, family CX-148 had the highest WG among all the families. Families CX-125 and CX-146 were in second place with highest WG compared to other groups and family CX-143 showed the lowest WG during RF ($P<0.05$).

Figure 3. Average individual WG (%) of fish during re-feeding (RF) and feed deprivation (FD) periods. WG (%) = (final weight – initial weight)/initial weight × 100

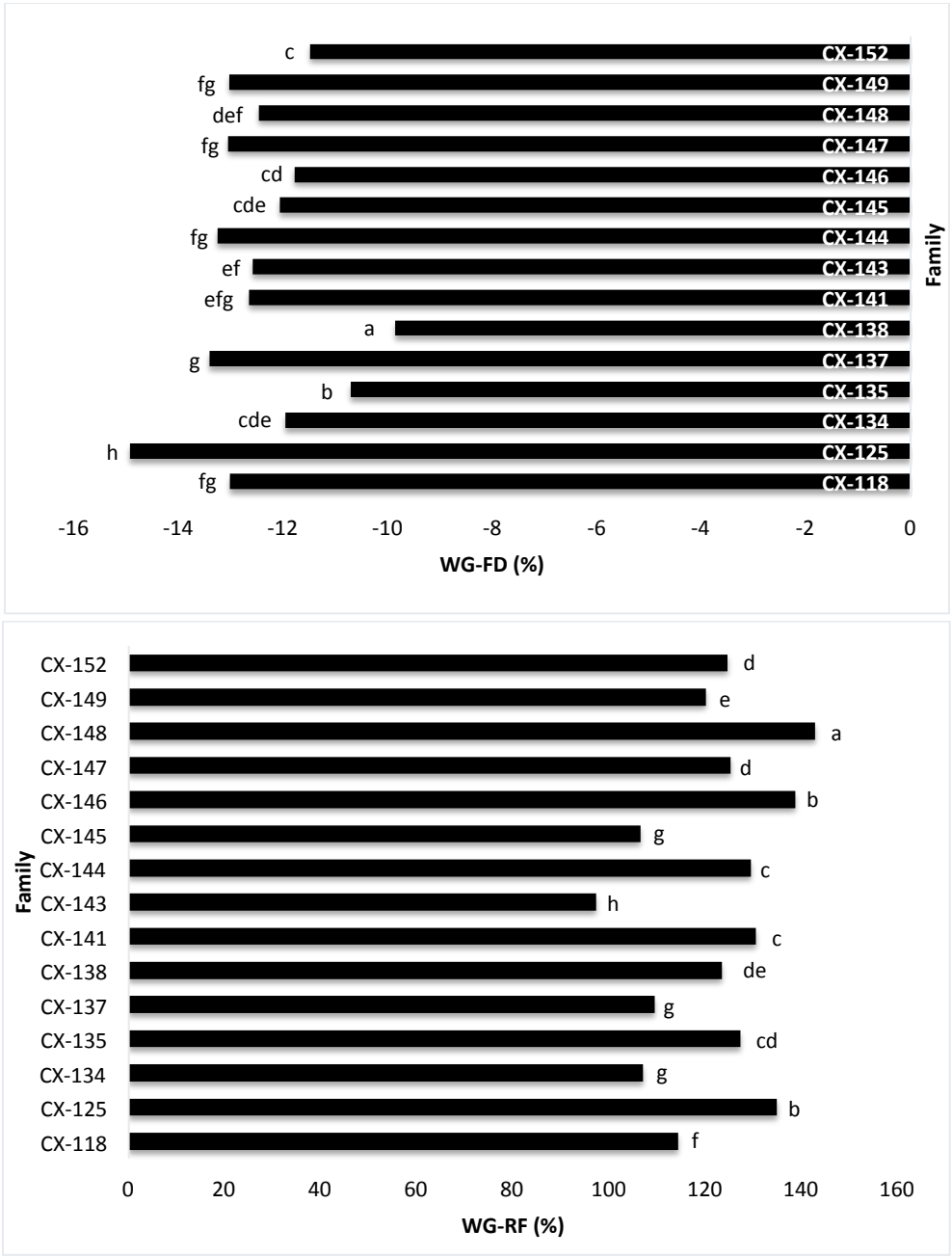

Figure 4. Average WG (%) of different families of fish during re-feeding (RF) and feed deprivation (FD) periods. Bars with different superscripts are significantly different (P<0.05).

Grouping was performed to separate fish based on weight loss during FD and weight gain during RF into four groups of FD-/RF-, FD+/RF+, FD-/RF+ and FD+/RF- (Figure 5). The filtration process retained 667 individuals with FD-/RF- = 154, FD-/RF+ = 146, FD+/RF- = 171, and FD+/RF+ = 196.

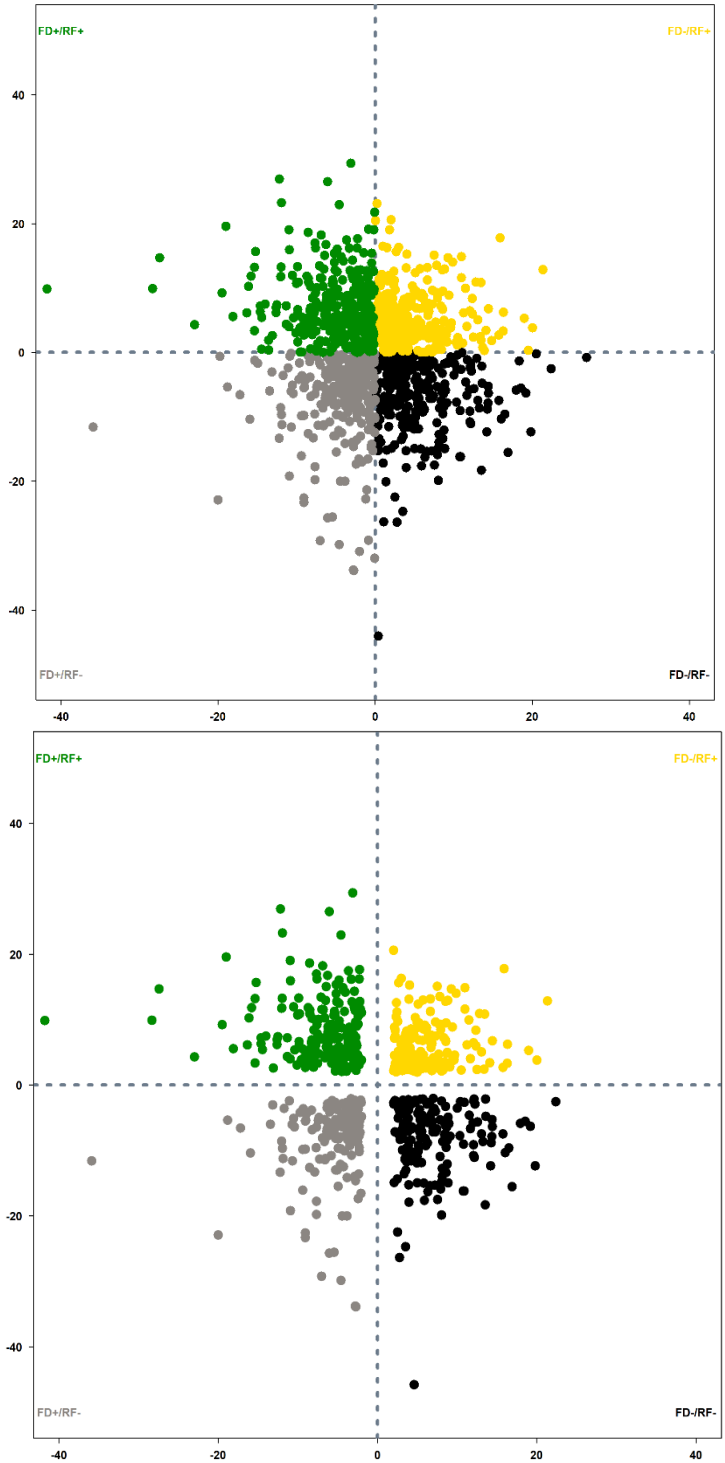

Figure 5. Fish performance was classed as FD-, FD+, RF- and RF+ for fish exhibiting loss (FD) and gain (RF) of weight relatively lower (-) and higher (+) than the population mean and separated into four triplicate groups FD-/RF-, FD+/RF+, FD-/RF+ and FD+/RF- (up). In order to have more distinctive groups of fish, an arbitrary low value of 2 away from the mean were applied to discard all the individuals concentrated on the border of each group (bottom).

After grouping the fish, the percentage of each family falling under each group was calculated and is shown in Table 4 and Figure 6. According to this, family CX-138 and CX-135 seem to have the highest number of fish belonging to the best (FD-/RF+, yellow) group. This is followed by CX-134, CX-148, and CX-149 families. Family CX-125 did not have any representative in the best group. On the other hand, the highest number of worst group (FD+/RF-, gray) was belonging to the CX-118, CX-137, CX-125, and CX-147 families. Family CX-138 had the lowest number of fish belonging to the worst group.

Table 4. The percentage of each family falling under FD-/RF-, FD+/RF+, FD-/RF+ and FD+/RF- groups.

	Groups (%)			
Family	**FD-/RF-**	**FD+/RF-**	**FD-/RF+**	**FD+/RF+**
CX-118	30.95	42.86	9.52	16.67
CX-125	4.26	38.30	0.00	57.45
CX-134	42.86	16.33	30.61	10.20
CX-135	21.05	15.79	42.11	21.05
CX-137	31.25	39.58	12.50	16.67
CX-138	34.09	6.82	52.27	6.82
CX-141	10.87	21.74	17.39	50.00
CX-143	46.51	25.58	11.63	16.28
CX-144	6.82	22.73	15.91	54.55
CX-145	41.03	30.77	12.82	15.38
CX-146	17.14	28.57	17.14	37.14
CX-147	10.91	36.36	21.82	30.91
CX-148	4.35	13.04	30.43	52.17
CX-149	20.83	22.92	29.17	27.08
CX-152	27.91	20.93	25.58	25.58

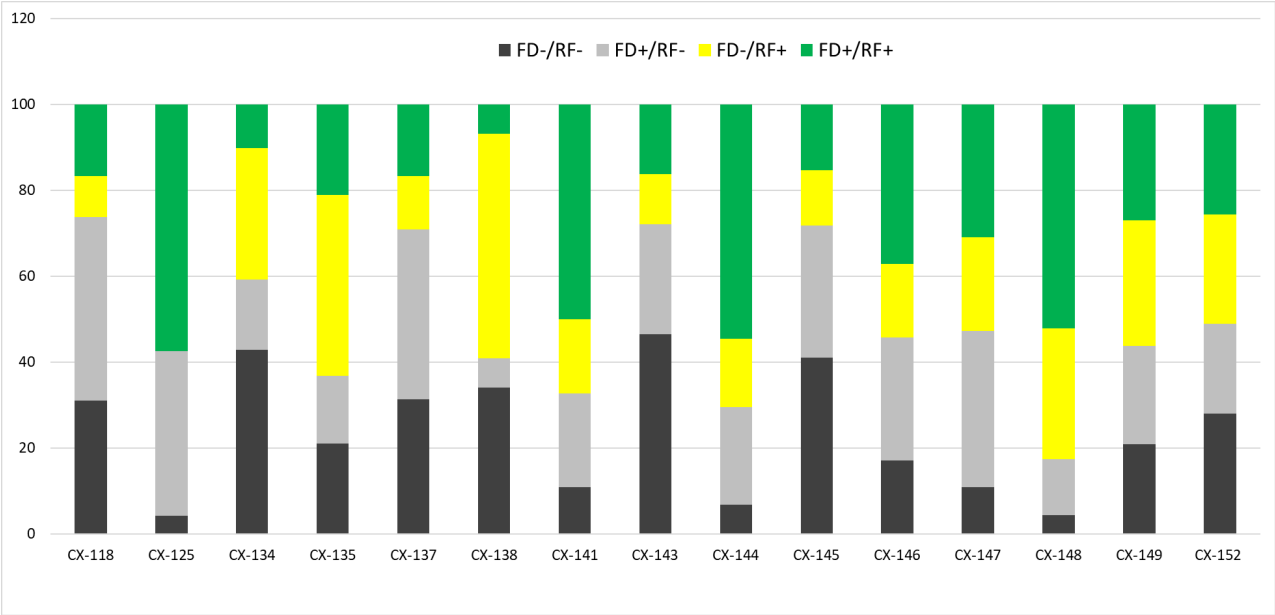

Figure 6. The percentage of each family falling under FD-/RF-, FD+/RF+, FD-/RF+ and FD+/RF- groups

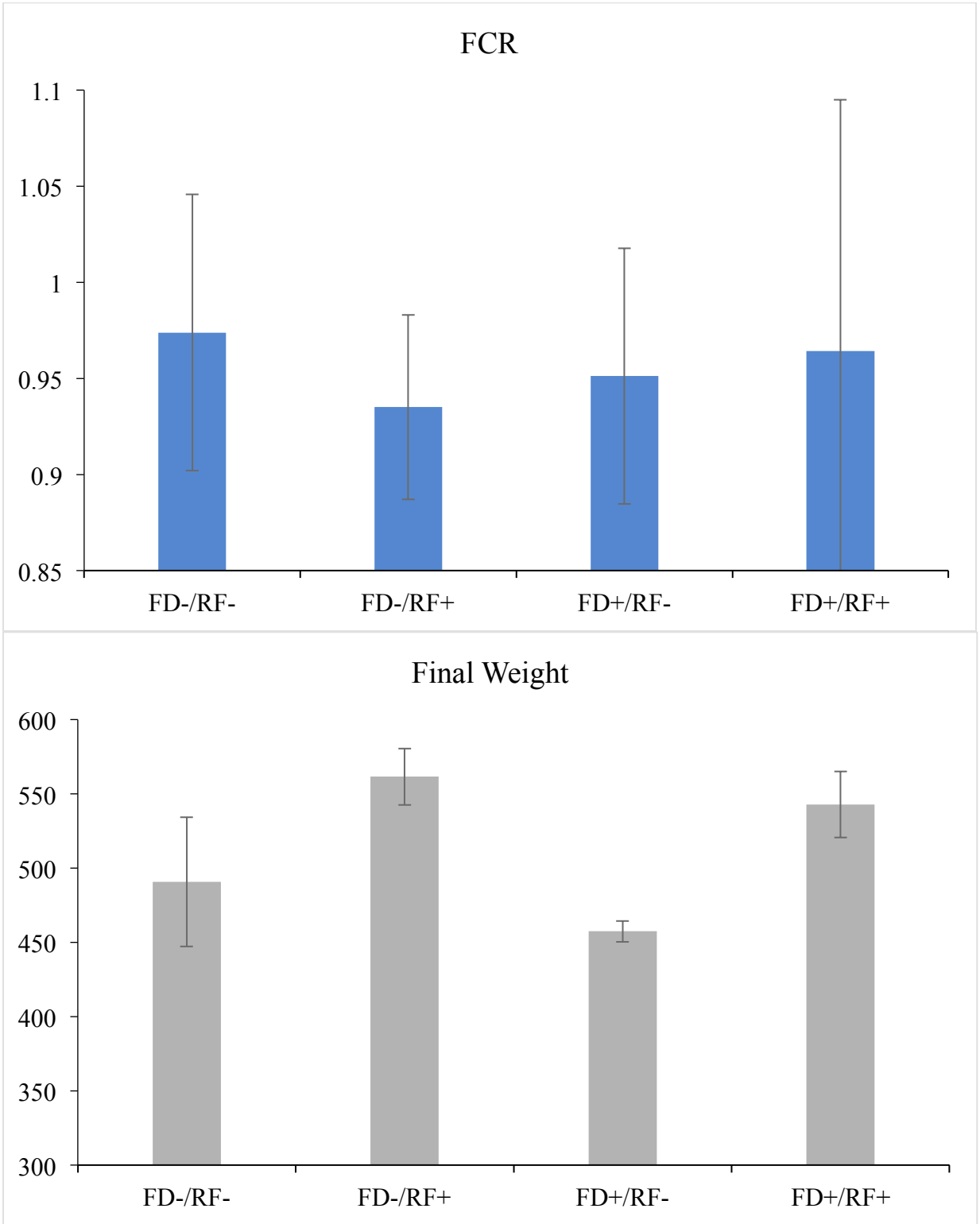

Figure 7. FCR and final weight of four selected groups fed the soy-based diet for 3 months. Data was normalized according to Templeton (2017) then analyzed using One-Way ANOVA at $P<0.05$ significance level.

3.2.Experiment 2

WG (%) of rainbow trout from 15 different families fed for 18 days is shown in Figure 7 and 8.

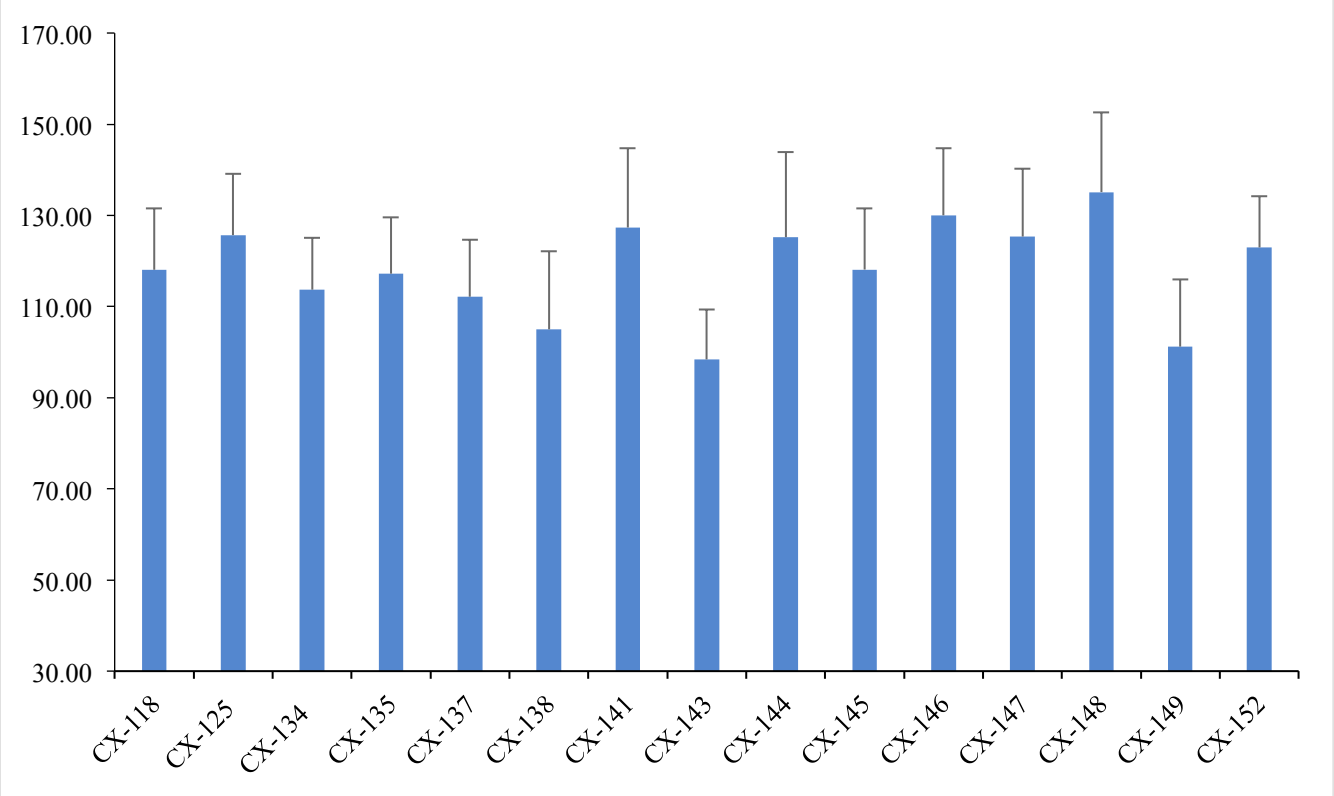

Figure 8. Weight gain (%) of 15 families fed plant-based diets for 18 days. Bars with different superscripts are significantly different ($P<0.05$). Data was not normally distributed and analyzed with Kruskal-Wallis non-parametric test at $P<0.05$ significance level.

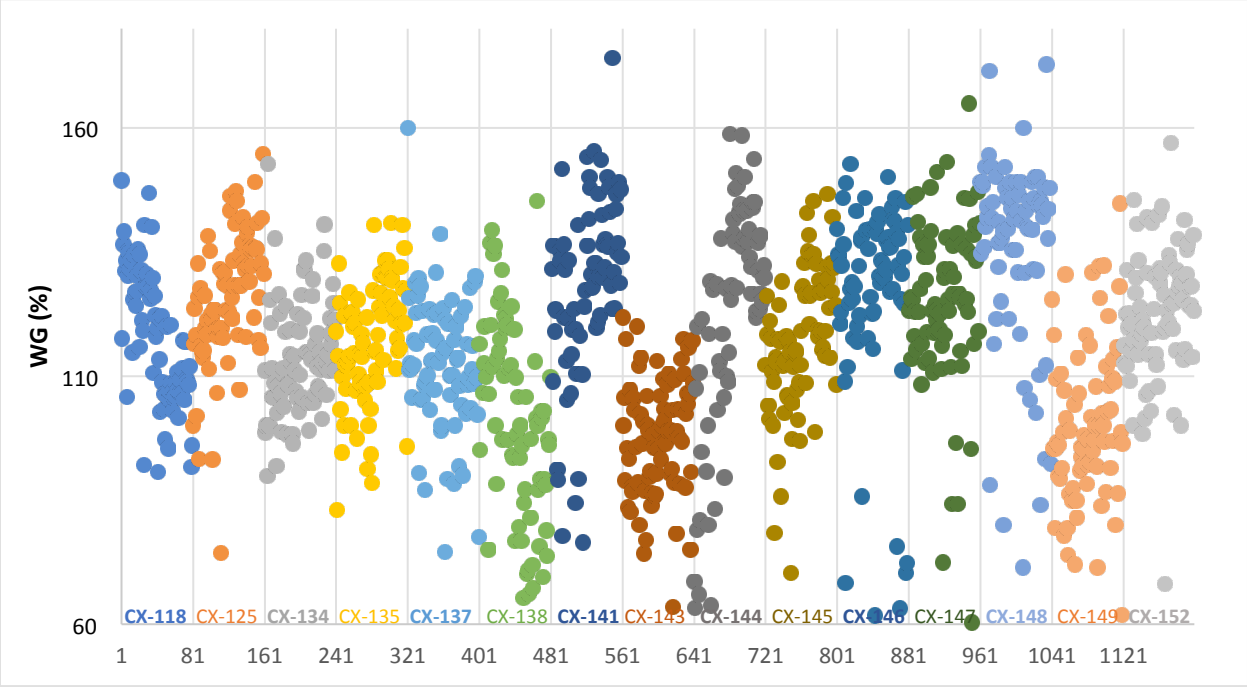

Figure 9. WG (%) of individual rainbow trout from 15 different families fed for 18 days.

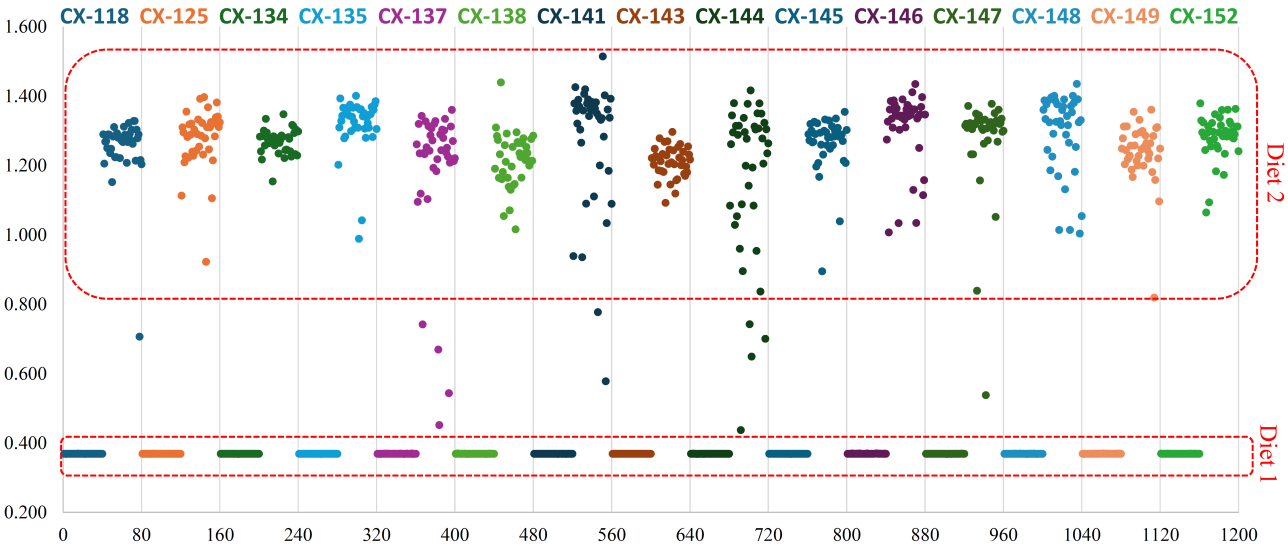

Figure 10. ^{15}N (at-%) in the muscle of rainbow trout from 15 families fed labeled and non-labeled plant-based diets for 18 days.

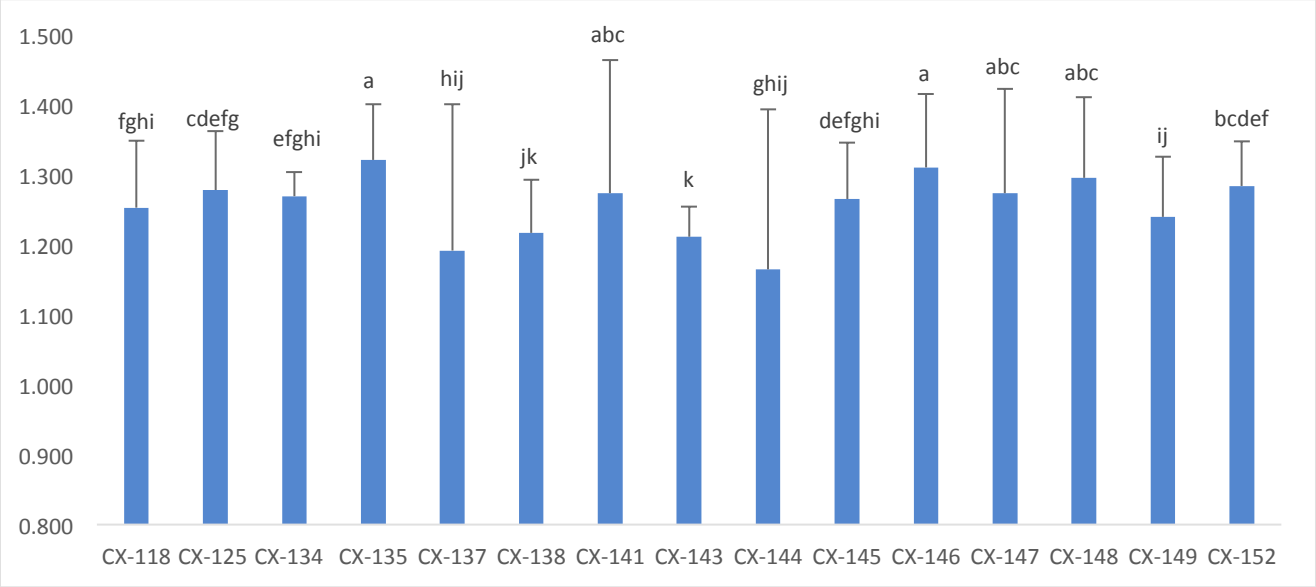

Figure 11. ^{15}N (at-%) in the muscle of rainbow trout fed plant-based diets for 18 days. Data was not normally distributed and analyzed with Kruskal-Wallis non-parametric test at $P<0.05$ significance level.

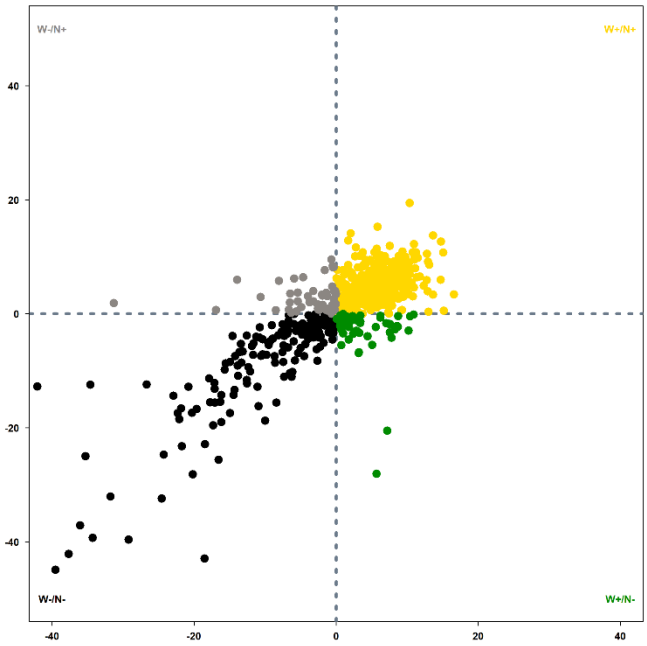

Figure 12. Grouping of individual rainbow trout based on 15N (at-%) in the muscle and growth of 15 families fed plant-based diets for 18 days.

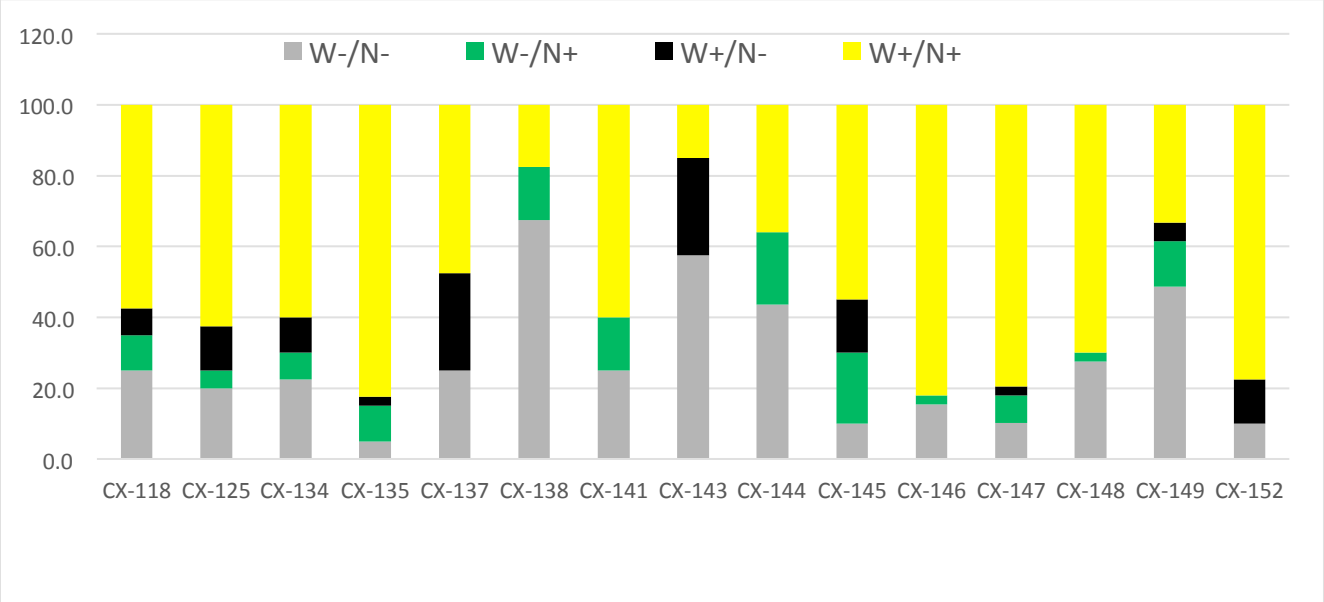

Figure 13. The percentage of each family falling under W-/N-, W+/N+, W-/N+ and W+/N- groups

According to the results of the second experiment, rainbow trout belonging to the CX-148 family had the highest WG but with no significant differences with the CX-125 group ($P>0.05$). Three families belonging to CX-138, CX-143, and CX-149 showed the lowest WG among all families.

4. Discussion

The results of the present study, so far, have demonstrated that there are substantial genetic-based variations among different families of rainbow trout. These results are not surprising since genetic variability related to weight increase/loss has been observed in several studies (Donaldson and Olson, 1957; Grima et al., 2008). The results of our first experiment suggest that fish belonging to the families CX-148, CX-146, and CX-125 had the highest growth during feeding period. Also, fish belonging to the families CX-138, CX-135, and CX-152 showed the lowest weight loss during fasting. This might indicate that rainbow trout belonging to these families are highly capable of digesting and utilizing fishmeal-free diets, can tolerate long periods of feed deprivation, and can recover growth after fasting periods. During the FCR period, FCR was slightly improved in the best group (FD-/RF+) but results were not significant. These are indicators of robustness and healthy fish that would contribute significantly to the production of rainbow trout. Future studies will investigate feed conversion ration of this fish to have a better understanding of their performance. The second experiment has also shown that fish belonging to the CX-118, CX-125, CX-135, CX-141, CX-146, CX-147, CX-148, and CX152 families had better growth compared to other groups. The next part of the experiment will evaluate the deposition of labeled feed in the tissue of rainbow trout.

References

Cho, C.Y. and Bureau, D.P., 1997. Reduction of waste output from salmonid aquaculture through feeds and feeding. The Progressive Fish□Culturist, 59(2), pp.155-160.

Donaldson, L.R. and Olson, P.R., 1957. Development of rainbow trout brood stock by selective breeding. Transactions of the American Fisheries Society, 85(1), pp.93-101.

FAO, 2020. Food and Agriculture Organization of the United Nations. The state of world fisheries and aquaculture 2020: Sustainability in action. Food and Agriculture Organization of the United Nations, 2020.

Glencross, B.D., 2020. A feed is still only as good as its ingredients: An update on the nutritional research strategies for the optimal evaluation of ingredients for aquaculture feeds. Aquaculture Nutrition, 26(6), pp.1871-1883.

Glencross, B., Fracalossi, D.M., Hua, K., Izquierdo, M., Mai, K., Øverland, M., Robb, D., Roubach, R., Schrama, J., Small, B. and Tacon, A., 2023. Harvesting the benefits of nutritional research to address global challenges in the 21st century. Journal of the World Aquaculture Society, 54(2), pp.343-363.

Grima, L., Vandeputte, M., Ruelle, F., Vergnet, A., Mambrini, M., & Chatain, B. (2010). In search for indirect criteria to improve residual feed intake in sea bass (Dicentrarchus labrax): Part I: Phenotypic relationship between residual feed intake and body weight variations during feed deprivation and re-feeding periods. Aquaculture, 300(1-4), 50-58.

Grima, L., Quillet, E., Boujard, T., Robert-Granié, C., Chatain, B. and Mambrini, M., 2008. Genetic variability in residual feed intake in rainbow trout clones and testing of indirect selection criteria (Open Access publication). Genetics Selection Evolution, 40, pp.1-18.

Hardy, R.W., 2010. Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. Aquac. Res. 41, 770-776.

Lee, S., Small, B.C., Patro, B., Overturf, K. and Hardy, R.W., 2020. The dietary lysine requirement for optimum protein retention differs with rainbow trout (Oncorhynchus mykiss Walbaum) strain. Aquaculture, 514, p.734483.

Lim C, Webster CD, Lee C-S. Alternative protein sources in aquaculture diets. Haworth Press; 2008.

Overturf, K., Barrows, F. T., & Hardy, R. W. (2013). Effect and interaction of rainbow trout strain (Oncorhynchus mykiss) and diet type on growth and nutrient retention. Aquaculture Research, 44(4), 604-611.

Sharp, Z.D., 2017. Principles of Stable Isotope Geochemistry, 2nd Edition, The University of New Mexico.

Venold, F.F., Penn, M.H., Krogdahl, Å. and Overturf, K., 2012. Severity of soybean meal induced distal intestinal inflammation, enterocyte proliferation rate, and fatty acid binding protein (Fabp2) level differ between strains of rainbow trout (Oncorhynchus mykiss). Aquaculture, 364, pp.281-292.