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Effects of feeding a fishmeal-free versus a fishmeal-based diet on post-smolt Atlantic salmon *Salmo salar* performance, water quality, and waste production in recirculation aquaculture systems

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ABSTRACT

The Atlantic salmon farming industry has progressively decreased the proportion of fishmeal used in commercial diets due to rising costs and sustainability concerns. A variety of alternate proteins have been identified to partially replace fishmeal; however, very little research has described the effect of feeding alternate protein, fishmeal-free diets to Atlantic salmon, particularly post-smolts cultured in recirculation aquaculture systems (RAS). Therefore, a 6-month study was conducted to compare the effects of feeding a fishmeal-free diet (FMF) versus a fishmeal-based diet (FM) on post-smolt Atlantic salmon performance, water quality, and waste production rates in six replicated RAS. Experimental diets were fed to Atlantic salmon (281 ± 5 g to begin) in triplicate RAS. Protein ingredients used in the FMF diet included mixed nut meal, poultry meal, wheat flour, and corn protein concentrate; while the FM diet contained menhaden meal, poultry meal, soy protein concentrate, and blood meal proteins. Fish oil derived from whiting fish trimmings was used in the FMF diet to establish a wild fisheries input to farmed fish output ratio of 0:1; while menhaden oil was the primary lipid source for the FM diet. Both diets were formulated with approximately 42% crude protein and 27% crude fat. Each RAS was operated with flushing rates that created an average system hydraulic retention time of 20 days (5% system volume flushed daily) and a mean feed loading rate of 3.2 kg feed/m^3 of daily make-up water volume. Atlantic salmon growth, survival, and feed conversion ratios (FCR) were unaffected ($P > 0.05$) by diet. At the conclusion of the study, Atlantic salmon fed the FMF and FM diets were 1.716 ± 0.076 and 1.720 ± 0.065 kg; cumulative survival was >99% for both; and average FCR was 0.89 ± 0.03 and 0.90 ± 0.02, respectively. The FMF diet resulted in greater total phosphorous (TP), carbonaceous biochemical oxygen (cBOD), and total suspended solids (TSS) mass per kg feed in the effluent ($P < 0.05$). The FMF and FM diets produced 0.009 ± 0.001 v. 0.006 ± 0.001 kg TP/kg feed; 0.079 ± 0.005 v. 0.056 ± 0.005 kg cBOD/kg feed; and 0.297 ± 0.028 v. 0.221 ± 0.032 kg TSS/kg feed, respectively. A significantly higher percentage of TSS was captured by radial flow settlers of RAS receiving the FMF diet compared to capture by settlers associated with the FM diet. Mass balance data, radial settler removal efficiency, and observations of flushed solids suggested that the FMF diet produced fecal matter with better settling characteristics. Lower TSS and true color values ($P < 0.05$), indicative of clearer water, were measured in RAS receiving the FMF diet. Total phosphorous (most of which was dissolved) was 4 times greater in the culture water of RAS that received the FMF diet, e.g., 4.3 ± 0.1 mg/L v. 0.9 ± 0.0 mg/L for the FM Diet. This was the first research attempt to formulate a fishmeal-free diet for Atlantic salmon with this ingredient profile and one of few studies to demonstrate uncompromised Atlantic salmon performance when feeding a diet without fishmeal.

1. Introduction

Over the past several decades, the market price for fishmeal and fish oil has risen steadily due to static supply and increasing

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demand, which has driven the aquaculture industry to consider more economical and sustainable ingredients for use in aquafeeds (Naylor et al., 2000; Gatlin et al., 2007; Tacon and Metian, 2008; Naylor et al., 2009; FAO, 2014). A variety of potential alternate proteins have been identified, including: algal (Kiron et al., 2012) and bacterial (Aas et al., 2006) proteins, poultry by-product (Fowler, 1991); invertebrate and nut meals (Barrows and Frost, 2014), and a variety of plant-based proteins (Gatlin et al., 2007). Significant progress has been made to reduce or replace fishmeal in the diets of many aquaculture species without compromise to health or performance (Adelizi et al., 1998; Furuya et al., 2004; Kaushik et al., 2004; Gatlin et al., 2007; Salze et al., 2010; Rossi, 2011). For example, recent research indicates that fishmeal-free diets with alternate protein blends are capable of producing comparable rainbow trout *Oncorhynchus mykiss* growth compared to traditional fishmeal-based diets (Kaushik et al., 1995; Barrows et al., 2007; Gaylord et al., 2007; Davidson et al., 2013; Barrows and Frost, 2014).

Atlantic salmon *Salmo salar*, however, generally have a low tolerance for fishmeal substitution; particularly, for diets containing high levels of plant-derived proteins, such as soybean meal (Baeverfjord and Krogdahl, 1996; Francis et al., 2001). Several studies have shown that Atlantic salmon fed diets with partial fishmeal replacement by plant proteins exhibited reduced feed intake, decreased growth rates, and/or reduced nutrient digestibility and gut health compared to standard fishmeal-based diets (Mundheim et al., 2004; Kraugerud et al., 2007; Refstie et al., 2010; Pratoomyot et al., 2011; Burr et al., 2013). Moreover, Espe et al. (2006) reported slower growth for Atlantic salmon fed fishmeal-free diets with various plant protein blends compared to a fishmeal-based diet. In contrast, a few studies have reported comparable Atlantic salmon growth and health, and/or nutrient digestibility when evaluating diets with partial fishmeal replacement versus diets with full fishmeal inclusion (Refstie et al., 2001; Refstie and Tiekstra, 2003; Aas et al., 2006; Torstensen et al., 2008; Øverland et al., 2009; Bendikson et al., 2011; Burr et al., 2012). To the authors' knowledge, however, only one study has demonstrated uncompromised Atlantic salmon growth performance when feeding a diet devoid of fishmeal. Burr et al. (2012) found that juvenile (31.5 g) Atlantic salmon fed diets containing blends of soy, corn, wheat, algae, and poultry by-product proteins grew at equal rates, to a mean size of approximately 250 g, compared to salmon fed a fishmeal-based diet.

The efficient use of sustainable feed ingredients has become increasingly important to the salmon farming industry over the past several decades due to steady growth in production and the coinciding demand for feed (Bostock et al., 2010; FAO, 2014). Historically, the salmon farming industry has used large proportions of fishmeal in commercial diets and has therefore been a net consumer of marine resources, producing wild fisheries input to farmed fish output ratios as high as 5:1 (Naylor et al., 2009); however, the industry has recognized that continued use of large quantities of fishmeal is not economically or environmentally sustainable and has progressively reduced the amount of fishmeal used in commercial diets. Bostock et al. (2010) reported that use of fishmeal and fish oil by the salmon industry began to decline from 1995 to 2005, experienced an even sharper drop from 2005 to present, and is expected to follow a similar trend until at least 2020. Alternate proteins including soya concentrates, sunflower meal, and poultry by-products are now commonly used to partially replace fishmeal in commercial salmon diets (Marine Harvest, 2015). Nevertheless, additional research is needed to further reduce or completely eliminate fishmeal in Atlantic salmon diets.

Accompanying the trends for increased Atlantic salmon production and reduced use of fishmeal in diets is the need to evaluate new and possibly more environmentally benign culture methods for Atlantic salmon. A growing number of commercial salmon companies are now producing smolts using recirculation aquaculture systems (RAS) (Bergheim et al., 2009), with a few farms raising post-smolts up to 0.5–1 kg prior to transfer to sea cages (Dalsgaard et al., 2013) and more currently operating or under construction in Norway. In addition, there is recent interest in culturing Atlantic salmon to market-size in land-based, closed containment systems that utilize RAS technology (Thorarensen and Farrell, 2011; Summerfelt and Christianson, 2014; Davidson et al., In Press). At present, there are approximately one dozen facilities worldwide that are raising Atlantic salmon to market-size in RAS (Summerfelt et al., 2015a). Thus, research on a range of culture aspects, including evaluation of sustainable diets fed to Atlantic salmon in RAS, would provide beneficial information for this developing industry sector.

Diets that are fed in RAS, particularly systems operated with long hydraulic retention times and/or high feed loading rates, can have a profound effect on culture tank water quality compared to flow-through and open systems that continuously exchange water, because metabolic wastes tend to accumulate in RAS (Davidson et al., 2009; Martins et al., 2009). In addition to providing optimal fish performance, alternate protein diets should be compatible with the production system, should minimize nutrient excretion and dissolution, and should result in water quality that is conducive to fish health. Ideally, newly developed diets should also generate fecal waste that has favorable mechanical properties for RAS applications, such as fecal stability (Brinker and Friedrich, 2012). Research evaluating waste production characteristics of alternate protein diets fed in RAS applications is limited. Davidson et al. (2013) evaluated the effect of feeding a grain-based diet without fishmeal versus a fishmeal-based diet fed to rainbow trout in low exchange RAS and found that the fishmeal-free diet resulted in greater accumulation of total ammonia nitrogen, total suspended solids, and carbonaceous biochemical oxygen demand in the culture water. Total phosphorus levels in the culture water and the effluent were lower for RAS associated with the grain-based diet, and culture water was clearer (based on true color and ultraviolet transmittance levels). Differences in solids removal efficiency across filtration devices were also measured as a consequence of diet. Despite these water quality differences, rainbow trout growth and survival were equal between dietary treatments (Davidson et al., 2013).

The research study described herein mirrored Davidson et al. (2013), but evaluated a fishmeal-free diet for Atlantic salmon that contained mixed nut (pistachio or almond byproduct) and poultry meal proteins. In addition, fish oil used in the fishmeal-free diet was derived from whiting fish trimming waste, which resulted in a wild fisheries input to farmed fish output of 0:1 (Monterey Bay Aquarium, 2011). The primary objective of this study was to compare the effects of feeding a fish meal-free diet versus a fishmeal-based diet on Atlantic salmon performance, water quality, and waste production in replicated recirculation aquaculture systems.

2. Methods

2.1. Experimental design & diet descriptions

Six replicated RAS (9.5 m^3) were randomly assigned to one of two dietary treatments (Fig. 1) to begin the 6-month study. Atlantic salmon cultured in three systems were fed a fishmeal-free (FMF) diet; while salmon cultured in the remaining three systems were fed a fishmeal-based (FM) diet. The experimental diets were manufactured at the USDA-ARS Fish Technology Center (Bozeman, MT, USA). The primary protein ingredients used in the FMF diet were mixed nut meal, wheat flour, corn protein concentrate, and poultry meal (Table 1). The FM diet was formulated to represent a commercial-type Atlantic salmon diet containing menhaden meal,

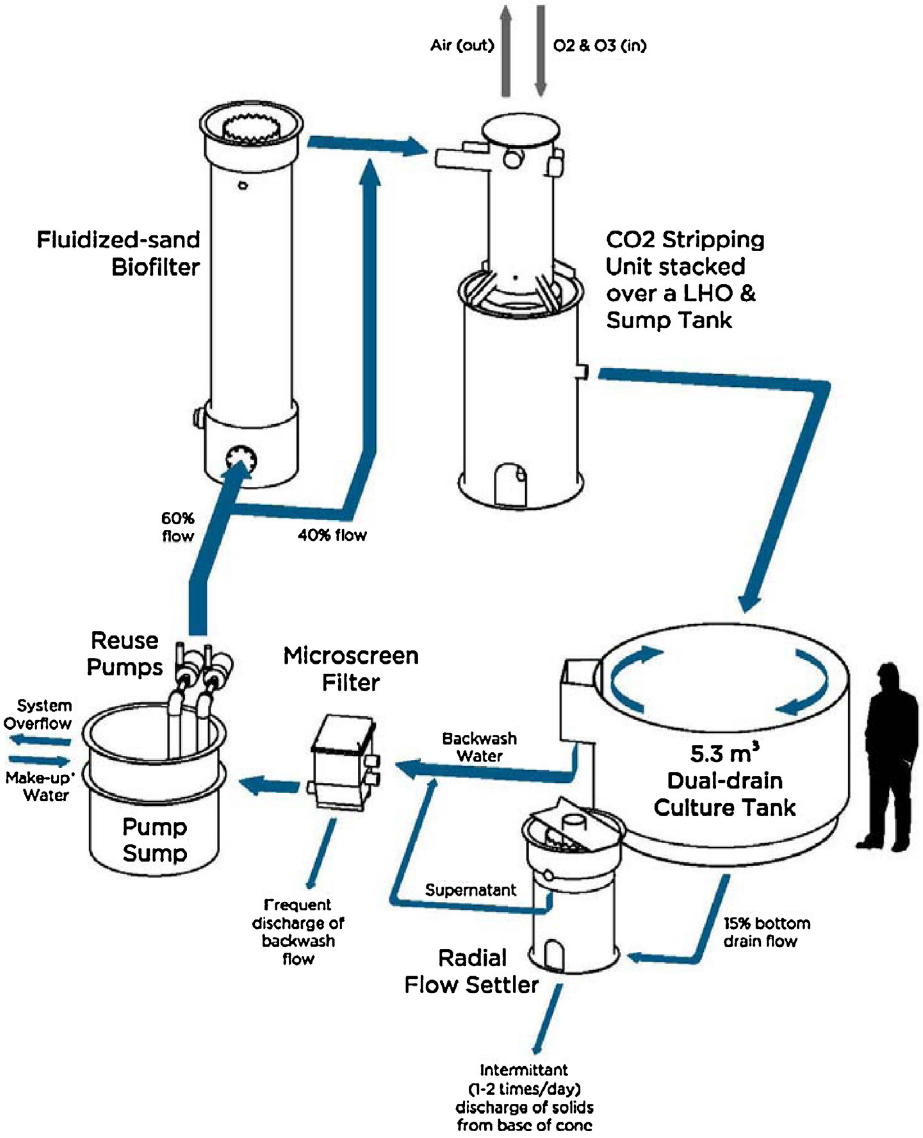

Fig. 1. Process flow drawing of an individual 9.5 m^3 recirculation aquaculture system used in the present study (courtesy Kata Rishel, TCFFI Engineering Services).

poultry meal, soy protein concentrate, and blood meal (Table 1). Fish oil, derived from whiting fish trimming waste, was used in the FMF diet to maintain a wild fisheries-in to farmed fish-out ratio of 0:1 (Monterey Bay Aquarium, 2011); while menhaden oil was the primary lipid used in the FM diet. The diets were formulated with a protein: fat ratio of approximately 42/27 and were provided with the same trace mineral and vitamin premixes. Limited amino acids were supplemented (including taurine in the FMF diet) to match the amino acid profile of rainbow trout muscle as an approximation for salmon muscle (Table 1); e.g., with at least 1.2, 3.3, and 1.7% of methionine, lysine, and threonine, respectively. Astaxanthin (approximately 80 ppm after extrusion) was included in each diet. Proximate composition (moisture, crude protein, crude fat, and ash; Table 2) of dried feed samples was determined according to approved methods (AOAC, 1995) on a Leco thermogravimetric analyzer (TGA701, LECO Corporation, St. Joseph, MI, USA). Protein (N X 6.25) was determined by the Dumas method (AOAC, 1995) on a Leco nitrogen determinator (TruSpec N, LECO Corporation) and lipid was determined by petroleum ether extraction using an Ankom XT10 (Ankom Technologies, Macedon, New York, USA). Macronutrient apparent digestibility coefficients for key diet ingredients were predetermined during a separate study (Barrows et al., 2014; Table 3).

2.2. System description and operation

The experimental RAS used during the study (Fig. 1) were originally described in Davidson et al. (2009). To summarize, each 9.5 m^3 system recirculated 380 L/min (100 gpm) of freshwater through a 5.3 m^3 dual drain culture tank, a radial flow settler, a microscreen drum filter with 60 μm screens, a fluidized sand biofilter, a geothermal heat exchanger or an in-line heater (depending on season), a carbon dioxide stripping column, and a low head oxygenator (LHO) (Fig. 1). In order to establish similar system dilution rates, the complete volume (265 L) of the radial flow settler of each RAS was flushed 1–3 times daily; whereas, flushing frequency was dependent on controlling nitrate-nitrogen levels at <75 mg/L. The reduced water level resulting from flushing was sensed by a float valve in the pump sump which automatically replaced the flushed water volume with spring water to maintain system equilibrium. Makeup water was continuously measured for each RAS with digital flow meters installed upstream of each float valve. Aside from this daily water exchange event, each RAS was operated with 100%

Table 1
Ingredient and nutrient composition of experimental diets.

Ingredient	g/kg (as-fed)	
	FMF	FM
Mixed Nut Meal,[a]	320.0	–
Poultry Meal[b]	295.0	160.0
Wheat flour[c]	99.4	195.1
Menhaden Meal, mechanically extracted[d]	–	195.0
Fish Oil, whitefish trimings oil[e]	182.0	–
Fish Oil, menhaden[f]	–	157.4
Soy Protein Concentrate[g]	–	128.5
Blood Meal, spray dehydrated[h]	–	70.5
Canola Oil	–	56.5
Corn protein concentrate[i]	35.6	–
Dicalcium phosphate	32.5	5.0
Vitamin Premix [j]	10.0	10.0
Lysine-HCL	6.2	6.5
Choline CL	6.0	6.0
Taurine	5.0	–
DL- Methionine	2.8	4.0
Stay-C	3.0	2.0
Threonine	0.5	1.5
Trace Min. Premix[k]	1.0	1.0
Astaxanthin[l]	1.0	1.0

[a] Adaptive Bio-Resources, 540 g/kg protein.
[b] American Dehydrated Foods Inc., 759 g/kg protein.
[c] Manildra Milling, 120 g/kg protein.
[d] Omega Proteins, Menhaden Special Select, 628 g/kg protein.
[e] Bio-Oregon Proteins.
[f] Omega Proteins.
[g] Solae, Pro-Fine VF, 693 g/kg crude protein.
[h] ADF Inc., 839 g/kg protein.
[i] Cargill, Empyreal 75, 761.0 g/kg protein.
[j] ARS 702; contributed, per kg diet; vitamin A 9650 IU; vitamin D 6600 IU; vitamin E 132 IU; vitamin K3 1.1 gm: thiamin mononitrate 9.1 mg; riboflavin 9.6 mg; pyridoxine hydrochloride 13.7 mg; pantothenate DL-calcium 46.5; cyancobalamin 0.03 mg; nicotinic acid 21.8 mg; biotin 0.34 mg; folic acid 2.5; inostitol 600.
[k] ARS 640, Contributed in mg/kg of diet; zinc 40; manganese 13; iodine 5; copper 9.
[l] DSM Nutritional Products.

Table 2
Proximate composition of the fishmeal-free and fish meal-based diets fed during the present study.

(%)	FMF Diet	FM Diet
Dry Matter	94.7	93.7
Moisture	5.22	3.96
Crude Protein	42.2	42.3
Crude Fat	27.0	26.3
Ash	4.35	5.62
Phosphorus Total	1.41	1.30
Calcium	1.09	0.65
Crude Fiber	0.15	0.45

Table 3
Macronutrient apparent digestibility coefficients (%) of key ingredients used in the fishmeal-free and fishmeal-based diets. (Barrows et al., 2014).

Protein	Diet	Dry Matter	Fat	Crude Protein	Energy
Mixed Nut Meal	FMF	64	96	86	75
Poultry Meal	FM, FMF	100	95	98	99
Wheat Flour	FM, FMF	NA	NA	82	56
Menhaden Meal	FM	71	92	83	88
Soy Protein Concentrate	FM	72	75	94	81
Blood Meal	FM	NA	NA	91	86
Corn Protein Concentrate	FMF	67	77	80	NA

flow recycle without additional dilution. This water exchange strategy created an average system hydraulic retention time (HRT) of approximately 20 days or daily water exchange equivalent to 5% of the system volume. The average feed loading rate was 3.2 ± 0.2 kg feed/m^3 of daily makeup water. Tank HRT was approximately 15 min. Sodium bicarbonate (NaHCO$_3$) was added to each RAS as needed to maintain alkalinity at ≥200 mg/L.

2.3. *Atlantic salmon: pre-study rearing*

Atlantic salmon (Salmobreed AS, Bergen, Norway) were received as fertilized, eyed-eggs and hatched on-site in a Heath-tray-style incubation system maintained at an average temperature of 8.1 °C. When juvenile salmon had absorbed the majority of yolk sac, they were temperature acclimated to 12 °C and moved to a 12-tank single-pass system enclosed by an opaque tent. While in this culture system, salmon were reared under 24-h light until they were 3 g or approximately 4 months post-hatch; at this time the lighting regimen was adjusted to 18-h light: 6-h dark to simulate the start of a waning photoperiod. When the salmon were 45 g and approximately 7.5 months of age the lighting regimen was changed to 12-h light: 12-h dark to mimic a winter photoperiod and to stimulate smoltification, or in other words to create S^0 smolts. After approximately 5 weeks of exposure to this photoperiod, 24-h continuous light was reinstituted. Two months later, when the fish weighed 100 g, a portion of the population was moved from the 12-tank flow-through system into four RAS used during the present study. Salmon were allowed to acclimate to the new surroundings for two months and were then randomly distributed into all 6 RAS to provide an equal number of fish per experimental unit (220 fish/tank). To begin the study, salmon stocked among the RAS weighed, on average, 281 ± 5 g, resulting in an initial stocking density of 12 kg/m^3. Salmon were reared entirely in freshwater during early life-stage and post-smolt production.

2.4. *Feeding methods*

Prior to the study, Atlantic salmon were fed a commercially-available diet containing 43% protein and 24% fat (Bio-Oregon, Westbrook, ME, USA). When the study began, salmon were immediately switched to the respective experimental diets. Salmon in each RAS were intentionally fed at the same rate for the first three weeks of the study, while acclimating to the diet change. Thereafter, feeding rates were adjusted separately per RAS based on observations of feeding activity and wasted feed. Fish were fed to apparent satiation using a computer operated feeding system (Freshwater Institute, Shepherdstown, WV, USA), programmed to deliver short feed bursts once an hour via automated feeders (T-drum 2000CE, Arvotec, Huutokoski, Finland). A constant 24-h photoperiod was provided which allowed “around-the-clock” feeding.

2.5. *Water quality sampling and analysis*

Water samples were collected weekly from tank side drains and tested on-site. Specific parameters, methodologies, and frequencies of testing are outlined in Table 4. Water samples were also collected at approximately 2, 4, and 6 months of the study from the following locations of each RAS: tank side drain, tank bottom drain (radial flow settler inlet), drum filter outlet, and radial flow settler outlet. The drum filter inlet flow consisted of 90% side drain flow and 10% radial settler outlet flow. These samples were collected in triplicate (over three separate days) and tested for total nitrogen (TN), total phosphorus (TP), and total suspended solids (TSS) levels; which were used for calculation of waste removal efficiency across radial flow settlers and drum filters. All water quality parameters measured on-site (Table 4) were analyzed according to methods described in APHA (2012) and Hach Company (2003,2015). Dissolved metals were analyzed by Cornell University’s Nutrient Analysis Lab (Ithaca, NY, USA).

Table 4
Water quality parameters evaluated, methodologies, and frequency of testing.

Parameter	Method of Analysis	Frequency of Recording/Testing
Dissolved Oxygen	Hach SC100 Universal Controller & LDO® Probe	Daily
Oxidative Reduction Potential	Hach SC100 Universal Controller & Differential ORP Sensor	Daily
Temperature	Hach SC100 Universal Controller & Differential ORP Sensor	Daily
Alkalinity	Standard Methods 2320 – Sulfuric Acid Titration	Twice weekly
pH	Hach Method 8156–pH electrode	Twice weekly
Carbon Dioxide	Hach Method 8223 – Buret Titration	Once weekly
$CBOD_5$	Standard Methods 5210B – 5 day test (No prefiltration of sample)	Once weekly
Heterotrophic Bacteria Count	Standard Methods 9215D – Membrane Filtration and Agar Plate Counts	Once weekly
Nitrite Nitrogen	Hach Method 8507 – Diazotization	Once weekly
Nitrate Nitrogen	Hach Method 8171 – Cadmium Reduction	Once weekly
Phosphorus	Hach Method 8190 – Acid Persulfate Digestion	Once weekly
Total Ammonia Nitrogen	Hach Method 8038 – Nessler	Once weekly
Total Nitrogen	Hach Methods 10071, 10072 – Persulfate Digestion Method	Once weekly
Total Suspended Solids	Standard Methods 2540D – Dried at 103–105 °C	Once weekly
True Color	Hach Method 8025 – Platinum-Cobalt	Once weekly
Ultraviolet Transmittance	Standard Methods 5910B – Ultraviolet Absorption	Once weekly
Dissolved Metals	Inductively Coupled Plasma Atomic Emission Spectrometry technique	Two events (Months 2 & 4)

*TN, TP, & TSS were collected three additional times (Months 2, 4, 6) from radial flow settler in/out sites, drum filter in/out sites, drum filter backwash, & radial flow settler flush.

2.6. Biosolids collection and waste production calculations

During the same 2, 4, and 6 month events, samples were also collected from two waste discharge flows of each RAS: (1) the drum filter backwash and (2) the concentrated flow flushed from the cone-bottom of radial flow settlers. Samples were analyzed for carbonaceous biochemical oxygen demand (cBOD), TN, TP, and TSS; and resulting concentrations were used for determination of mass balances and waste production metrics. Other measurements required for mass balance assessment included: volumes flushed from each discharge location (L/collection period) and feed delivered (kg/collection period). The volume flushed from radial flow settlers was determined by collecting the flow in a tared bucket and subsequently weighing the solution. Drum filter backwash volume was assessed by capturing and weighing the cumulative backwash created over a 4-h collection period. Feed was weighed into calibrated feeders. Mass balance calculations, used to determine effluent waste mass per kilogram feed were performed, as follows:

$$\text{Mass}\,(\text{kgwaste}/\,\text{kgfeed}) = \frac{(C_{out})\,(\text{mg})}{\text{L}} \times \frac{\text{Total DischargeVolume}}{\text{TotalFeed}\,(\text{kg})} \times \frac{\text{kg}}{10^6\text{mg}}$$

where C_{out} = effluent concentration. Total waste production was calculated by summing the waste mass contained in the effluents of each RAS.

2.7. Fish sampling protocols

Length and weight measurements of a random sample of 60–90 fish were collected monthly for the first 4 months; thereafter, one month was skipped due to increased sensitivity of larger salmon to handling. Final length and weight measurements were collected at the conclusion of the study. Sample size was calculated as follows: $n = (Z \times (\text{stdev.}_{\text{grams}}/\text{accepted error}_{\text{grams}}))^2$, where $Z = 1.65$ (relative to a 90% confidence interval) (Kitchens, 1998). Mortalities were removed and recorded daily to assess cumulative survival. Thermal growth coefficients (TGC), condition factor (CF), and feed conversion ratios (FCR) were calculated as follows:

$$\text{TGC} = (\text{End Weight}^{1/3} - \text{StartWeight}^{1/3})/((\text{DaysBetween} \times \text{Avg.Temp.}) \times 1000)$$

$$\text{CF} = 100,000 \times \text{Weight}/(\text{Length})^3$$

$$\text{FCR} = \text{CumulativeFeedDelivered}/\text{FishBiomassGain}$$

Where weight is in grams, length is in mm, and temperature is in °C.

Fish health was evaluated through assessments of fin condition, blood chemistry, and histopathology; and microbial communities of the fish gut, biofilter, and culture environment were assessed through gene sequencing (Schmidt et al., 2016); these outcomes will be reported in detail in separate publications. A comprehensive analysis of processing attributes, product quality, and fillet yield will also be presented separately.

2.8. Statistical analysis

All parameters that were sampled during multiple events over time from the same location, including water quality parameters, growth, and waste production rates were analyzed using a mixed models approach, which assigned Tank as a random effect and Time as a random covariate (Ling and Cotter, 2003). Each data set was analyzed for normality using a Shapiro-Wilk test and non-normal data were transformed for statistical comparison. A probability level of 0.05 was used to determine significance. Statistical analyses were carried out using SYSTAT 13 software (2009).

3. Results

3.1. Growth performance and survival

The mean growth curves established per dietary treatment were nearly identical throughout the study (Fig. 2). The average weight of Atlantic salmon fed the FMF and FM diets at the conclusion of the study was 1.716 ± 0.076 and 1.720 ± 0.065 kg, respectively (Fig. 2), and average length was 51 ± 1 and 50 ± 0 cm, respectively. Salmon fed the FMF and FM diets grew 239 ± 13 and 240 ± 10 g/month, respectively. The mean thermal growth coefficient calculated for salmon fed the FMF and FM diets was 2.14 ± 0.05 and 2.12 ± 0.01, respectively, and mean condition factor (CF) was 1.25 ± 0.01 and 1.28 ± 0.02, respectively ($P > 0.05$). Condition factor was slightly lower at each sampling point for fish fed the FMF diet; albeit, it appeared that these fish began the study with a slightly lower CF (Fig. 3). Overall, diet did not affect ($P > 0.05$) growth performance parameters.

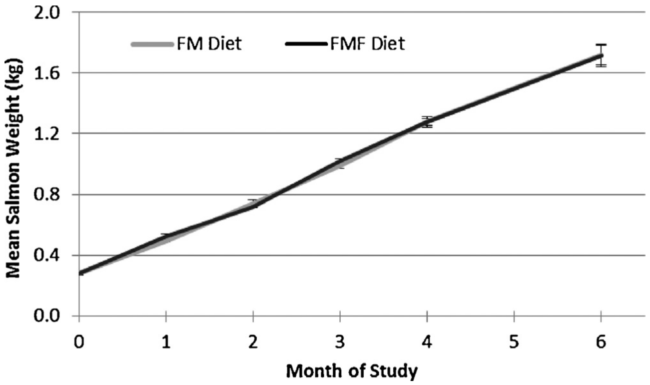

Fig. 2. Atlantic salmon weights (n = 3; mean ± 1 standard error) plotted over the 6-month study for populations fed a fishmeal-free diet v. a fish meal-based diet.

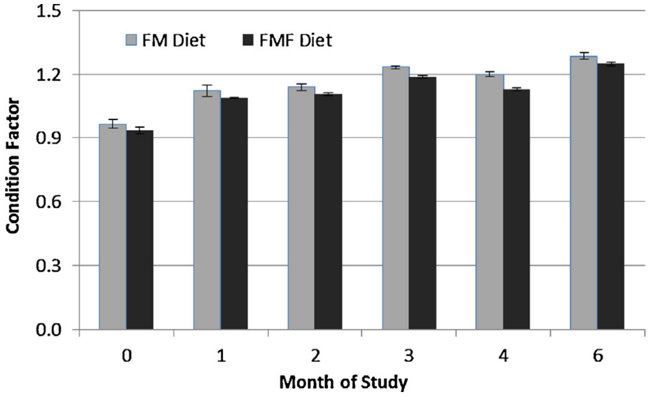

Fig. 3. Condition factor (n = 3; mean ± 1 standard error) of Atlantic salmon fed a fishmeal-free v. a fishmeal-based diet over the 6-month study duration. Note that salmon were not sampled during month 5 of the study.

Feeding rates and resulting feed conversion ratios were not affected ($P > 0.05$) by dietary treatment. Average feeding rates for the FMF and FM dietary treatments over the study duration were 1.0 ± 0.0 and 1.1 ± 0.1% of the tank biomass, respectively. Mean feed conversion ratios for the FMF and FM dietary treatments were 0.89 ± 0.03 and 0.90 ± 0.02, respectively. In addition, mean fish densities at the conclusion of the study for the FMF and FM dietary treatments were 71 ± 2 and 68 ± 5 kg/m^3, respectively. Atlantic salmon survival was not affected by dietary treatment. Mean survival, excluding jumpers and culls for sampling, was 99.8 ± 0.2 and 99.7 ± 0.3% for Atlantic salmon fed the FMF and FM diets, respectively ($P > 0.05$).

3.2. Water quality and waste production

3.2.1. Nitrogenous waste – culture water

Over the study duration, total ammonia nitrogen (TAN) measured in the culture water of RAS receiving the FMF and FM diets was 0.17 ± 0.01 and 0.13 ± 0.01 mg/L, respectively (Table 5). TAN was greater ($P = 0.001$) for the FMF dietary treatment. Nitrite nitrogen was 0.05 ± 0.04 and 0.03 ± 0.02 mg/L for the FMF and FM dietary treatments, respectively ($P > 0.05$). Nitrate nitrogen (NO_3-N) was 65 ± 2 and 57 ± 1 mg/L for the FMF and FM dietary treatments, respectively (Table 5), and was higher in RAS receiving the FMF diet ($P = 0.013$).

3.2.2. Nitrogenous waste – effluent/solids removal devices

The average of TN waste production (sum of two effluents for each RAS), as measured during sampling events at months 2, 4, and 6, was similar ($P > 0.05$) for each diet; 0.022 ± 0.002 kg TN/kg feed for the FMF diet and 0.020 ± 0.002 kg TN/kg feed for the FM diet (Table 6). Nitrogen retention efficiency for salmon sampled from the FMF and FM treatments was 44 ± 1 and 43 ± 2%, respectively, at the conclusion of the study, indicating equivalent nitrogen retention from each diet.

Table 5
Mean tank water quality concentrations (mg/L, unless otherwise noted) collected at the sidewall drain for low exchange RAS in which Atlantic salmon were fed fish meal-free (FMF) and fish meal-based (FM) diets over the study duration.

	FMF Diet	FM Diet
Alkalinity	206 ± 2	208 ± 2
Carbon Dioxide	4 ± 0	3 ± 0
cBOD$_5$	0.9 ± 0.1	0.9 ± 0.1
Dissolved Oxygen	10.0 ± 0.0	10.0 ± 0.0
Heterotroph Bacteria (CFU/mL)	437 ± 83	493 ± 121
Nitrite Nitrogen	0.05 ± 0.04	0.03 ± 0.02
Nitrate Nitrogen[a]	65 ± 2	57 ± 1
Oxidative Reduction Potential (mV)	248 ± 1	255 ± 4
pH	8.1 ± 0.0	8.1 ± 0.0
Temperature (°C)	15.2 ± 0.0	15.2 ± 0.0
Total Ammonia Nitrogen[a]	0.17 ± 0.01	0.13 ± 0.01
Total Phosphorus[a]	4.3 ± 0.1	0.9 ± 0.0
Total Suspended Solids[a]	1.3 ± 0.2	1.7 ± 0.1
True Color (Pt-Co Units)[a]	20 ± 2	25 ± 2
UV Transmittance (%)	81 ± 1	79 ± 1

[a] Indicates significant difference between treatments.

The general distribution of TN waste contained in the two discharge flows was also similar for each diet. For RAS receiving the FMF diet, 0.005 ± 0.000 kg TN/kg feed (24%) was removed at the cone bottom of the radial flow settler and 0.017 ± 0.002 kg TN/kg feed (76%) was removed with the drum filter backwash (Table 6). For RAS receiving the FM diet, 0.003 ± 0.000 kg TN/kg feed (17%) was removed at the cone bottom of the radial flow settler and 0.017 ± 0.001 kg TN/kg feed (83%) was removed with the drum filter backwash (Table 6). Total nitrogen levels measured at the inlet and outlet of the radial flow settlers were 59 ± 4 and 58 ± 2 mg/L, respectively for the FMF diet; compared to 53 ± 1 and 54 ± 4 mg/L at the inlet and outlet of RAS receiving the FM diet (Table 7). Total nitrogen measured at the inlet and outlet of the drum filters was 57 ± 1 and 59 ± 0 mg/L, respectively for the FMF diet; compared to 55 ± 1 and 52 ± 2 mg/L at the inlet and outlet of RAS receiving the FM diet (Table 7). Negligible TN removal efficiencies across these unit processes are reflective of the majority of TN existing in the dissolved form, mainly as nitrate-nitrogen.

3.2.3. Total phosphorus – culture water

Total phosphorous measured in RAS receiving the FMF and FM diets was 4.3 ± 0.1 and 0.9 ± 0.0 mg/L, respectively (Table 5); hence, the FMF diet resulted in greater TP in the culture water ($P = 0.000$). Average TP levels for each treatment began to diverge at the onset of the study (Fig. 4). After approximately one month, when systems had reached equilibrium, TP in RAS receiving the FMF diet was consistently 4 fold greater compared to TP in RAS receiving the FM diet (Fig. 4).

3.2.4. Total phosphorus – effluent/solids removal devices

The average of TP waste production (sum of the two effluents for each RAS), as measured during sampling events at months 2, 4, and 6, was greater for the FMF diet, i.e., 0.0089 ± 0.0005 kg TP/kg feed v. 0.0059 ± 0.0007 kg TP/kg feed for the FM diet ($P = 0.000$; Table 6). Distribution of TP among effluents followed a similar trend per dietary treatment; whereas, the bulk of TP was removed via the drum filter backwash (Table 6). For RAS receiving the FMF diet, 0.0035 ± 0.0001 kg TP/kg feed (41%) was removed at the cone bottom of the radial flow settler and 0.0054 ± 0.0005 kg TSS/kg feed (59%) was removed with the drum filter backwash (Table 6). For RAS receiving the FM diet, 0.0020 ± 0.0001 kg TSS/kg feed (35%)

Table 6
Total waste flushed (total nitrogen, total phosphorus, total suspended solids) from the cone-bottom of the radial flow settler and the drum filter backwash effluent, compared between RAS in which salmon were fed fish meal-free and fish meal-based diets, respectively.

Total Nitrogen

RAS Location	FMF Diet (kg TN/kg feed)	FMF Diet% Total Mass Flushed	FM Diet (kg TN/kg feed)	FM Diet% Total Mass Flushed
Radial Flow Settler	0.005 ± 0.000	24 ± 1	0.003 ± 0.000	17 ± 1
Drum Filter	0.017 ± 0.002	76 ± 1	0.017 ± 0.001	83 ± 1
Total Mass Flushed	0.022 ± 0.002	100	0.020 ± 0.002	100

Total Phosphorus

RAS Location	FMF Diet (kg TSS/kg feed)	FMF Diet% Total Mass Flushed	FM Diet (kg TSS/kg feed)	FM Diet% Total Mass Flushed
Radial Flow Settler	0.0035 ± 0.0001	41 ± 2	0.0020 ± 0.0001	35 ± 3
Drum Filter	0.0054 ± 0.0005	59 ± 2	0.0040 ± 0.0006	65 ± 3
Total Mass Flushed	[a]0.0089 ± 0.0005	100	0.0059 ± 0.0007	100

Total Suspended Solids

RAS Location	FMF Diet	FMF Diet% Total Mass Flushed	FM Diet (kg TP/kg feed)	FM Diet% Total Mass Flushed
Radial Flow Settler	0.152 ± 0.010	52 ± 3	0.071 ± 0.010	35 ± 5
Drum Filter	0.141 ± 0.021	48 ± 3	0.150 ± 0.031	65 ± 5
Total Mass Flushed	[a]0.297 ± 0.028	100	0.221 ± 0.032	100

Biochemical Oxygen Demand

RAS Location	FMF Diet	FMF Diet% Total Mass Flushed	FM Diet (kg TP/kg feed)	FM Diet% Total Mass Flushed
Radial Flow Settler	0.036 ± 0.002	47 ± 2	0.023 ± 0.002	41 ± 3
Drum Filter	0.043 ± 0.005	53 ± 3	0.034 ± 0.004	62 ± 5
Total Mass Flushed	[a]0.079 ± 0.005	100	0.056 ± 0.005	100

[a] Indicates significant difference between treatments.

Table 7
Total suspended solids, total nitrogen, and total phosphorus concentrations and removal efficiencies across the radial flow settler and drum filter in replicated RAS in which Atlantic salmon were fed fish meal-free and fish meal-based diets.

Metric	Unit Process	FMF Diet	FM Diet
TN In (mg/L)	Radial Flow Settler	59 ± 4	53 ± 1
TN Out (mg/L)	Radial Flow Settler	58 ± 2	54 ± 4
TN Removal Efficiency (%)	Radial Flow Settler	2 ± 6	−1 ± 7
TN In (mg/L)	Drum Filter	57 ± 1	55 ± 1
TN Out (mg/L)	Drum Filter	59 ± 0	52 ± 2
TN Removal Efficiency (%)	Drum Filter	−3 ± 2	4 ± 2
TP In (mg/L)	Radial Flow Settler	4.8 ± 0.3	1.0 ± 0.0
TP Out (mg/L)	Radial Flow Settler	4.5 ± 0.2	1.0 ± 0.0
TP Removal Efficiency (%)	Radial Flow Settler	6 ± 1	0 ± 2
TP In (mg/L)	Drum Filter	4.6 ± 0.2	1.0 ± 0.0
TP Out (mg/L)	Drum Filter	4.5 ± 0.3	1.0 ± 0.0
TP Removal Efficiency (%)	Drum Filter	2 ± 4	2 ± 1
TSS In (mg/L)	Radial Flow Settler	3.5 ± 0.7	3.2 ± 0.1
TSS Out (mg/L)	Radial Flow Settler	1.4 ± 0.1	1.9 ± 0.1
TSS Removal Efficiency (%)	Radial Flow Settler	60 ± 9	40 ± 4
TSS In (mg/L)	Drum Filter	1.1 ± 0.0	1.5 ± 0.2
TSS Out (mg/L)	Drum Filter	1.0 ± 0.1	1.3 ± 0.2
TSS Removal Efficiency (%)	Drum Filter	13 ± 9	13 ± 8

Note: Inlet to the radial flow settlers is equivalent to tank bottom drain outlet and inlet to the drum filters is equivalent to tank side drain outlet.

was removed at the cone bottom of the radial flow settler and 0.0040 ± 0.0006 kg TSS/kg feed (65%) was removed with the drum filter backwash (Table 6).

Total phosphorus levels measured at the inlet and outlet of radial flow settlers were 4.8 ± 0.3 and 4.5 ± 0.2 mg/L, respectively for the FMF diet; compared to 1.0 ± 0.0 and 1.0 ± 0.0 mg/L within the inlet and outlet of RAS receiving the FM diet (Table 7). Total phosphorus levels at the inlet and outlet of the drum filters were 4.6 ± 0.2 and 4.5 ± 0.3 mg/L, respectively for the FMF diet; compared to 1.0 ± 0.0 mg/L in both the inlet and outlet of RAS receiving the FM diet (Table 7). The removal efficiency of TP across the drum filter for the FMF and FM diets was 2 ± 4 and 2 ± 1%, respectively (Table 7). Negligible TP removal efficiencies across these unit processes are reflective of the majority of TP existing in the dissolved form.

3.2.5. *Total suspended solids – culture water*

Over the study duration, water exiting the tank side drains of RAS receiving the FMF and FM diets contained average TSS levels of 1.3 ± 0.2 and 1.7 ± 0.1 mg/L, respectively (Table 5). Although the magnitude of difference was small, the FM diet resulted in higher TSS ($P = 0.009$). The dual-drain culture tank fractionated TSS efficiently, with bottom drain TSS levels averaging 3.5 ± 0.7 and 3.2 ± 0.1 mg/L, respectively, for FMF and FM diets (Table 7).

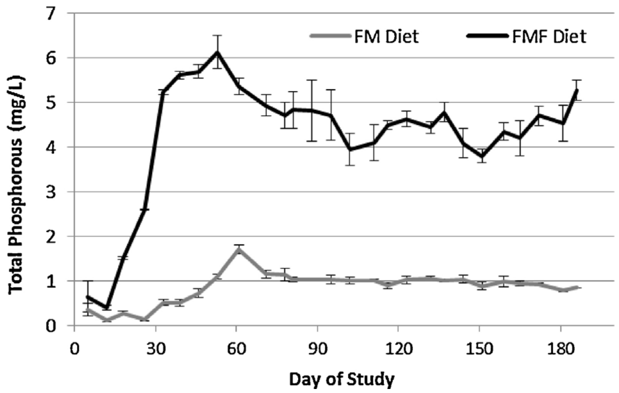

Fig. 4. Weekly total phosphorus concentrations (n = 3; mean ± 1 standard error) measured over the study duration in low exchange RAS in which Atlantic salmon were fed a fishmeal-free diet v. a fishmeal-based diet.

3.2.6. *Total suspended solids – effluent/solids removal devices*

The average of TSS waste production (sum of the two effluents for each RAS), as measured during sampling events at months 2, 4, and 6, was greater for the FMF diet, i.e., 0.297 ± 0.028 kg TSS/kg feed v. 0.221 ± 0.032 kg TSS/kg feed for the FM diet ($P = 0.040$; Table 6). For RAS receiving the FMF diet, 0.152 ± 0.010 kg TSS/kg feed (52%) was removed at the cone bottom of the radial flow settler, and 0.141 ± 0.021 kg TSS/kg feed (48%) was removed with the drum filter backwash (Table 6). For RAS associated with the FM diet, 0.071 ± 0.010 kg TSS/kg feed (35%) was removed at the cone bottom of the radial flow settler and 0.150 ± 0.031 kg TSS/kg feed (65%) was removed with the drum filter backwash (Table 6).

Total suspended solids levels measured at the inlet and outlet of the radial flow settlers were 3.5 ± 0.7 and 1.4 ± 0.1 mg/L, respectively for the FMF diet; compared to 3.2 ± 0.1 and 1.9 ± 0.1 mg/L at the inlet and outlet of radial flow settlers associated with the FM diet (Table 7). Resulting TSS removal efficiencies across the radial flow settler for the FMF and FM diets were 60 ± 9 and 40 ± 4%, respectively (Table 7).

Total suspended solids levels measured at the inlet and outlet of the drum filters were 1.1 ± 0.0 and 1.0 ± 0.1 mg/L, respectively for the FMF diet; compared to 1.5 ± 0.2 and 1.3 ± 0.2 mg/L within the inlet and outlet of RAS receiving the FM diet (Table 7). Resulting TSS removal efficiencies across the drum filter for the FMF and FM diets were 13 ± 9 and 13 ± 8%, respectively (Table 7).

3.2.7. *Carbonaceous biochemical oxygen demand*

Over the study duration, average cBOD levels in the culture water of RAS receiving the FMF and FM diets were identical, 0.9 ± 0.1 v. 0.9 ± 0.1 mg/L, respectively (Table 5). The average of cBOD waste production (sum of the two effluents in each RAS), as measured during sampling events at months 2, 4, and 6, was greater ($P = 0.000$) for the FMF diet, 0.079 ± 0.005 kg cBOD/kg feed v. 0.056 ± 0.005 kg cBOD/kg feed for the FM diet (Table 6). Distribution of cBOD among effluents followed a similar trend for each diet. Slightly more cBOD was removed via the drum filter backwash compared to the radial flow settler (Table 6). For RAS receiving the FMF diet, 0.036 ± 0.002 kg cBOD/kg feed (47%) was removed at the cone bottom of the radial flow settler and 0.043 ± 0.005 kg cBOD/kg feed (53%) was removed with the drum filter backwash (Table 6). For RAS receiving the FM diet, 0.023 ± 0.002 kg cBOD/kg feed (41%) was removed at the cone bottom of the radial flow settler and 0.034 ± 0.004 kg cBOD/kg feed (62%) was removed with the drum filter backwash (Table 6).

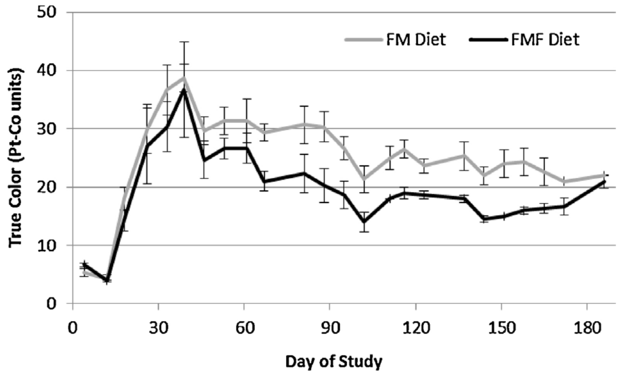

Fig. 5. Weekly true color levels (n = 3; mean ± 1 standard error) measured over the study duration in low exchange RAS in which Atlantic salmon were fed a fishmeal-free diet v. a fishmeal-based diet.

Table 8
Mean dissolved metals/trace element concentrations (mg/L) measured in the culture water of RAS associated with the fish meal-free and fish meal-based diets.

Parameter	FMF Diet	FM Diet	MDL
Barium	0.18 ± 0.02	0.23 ± 0.02	0.002
Calcium[a]	93 ± 0	99 ± 1	0.495
Copper	0.030 ± 0.003	0.031 ± 0.001	0.005
Magnesium	11.8 ± 0.1	11.9 ± 0.1	0.031
Phosphorus[a]	4.8 ± 0.2	1.0 ± 0.1	0.019
Potassium[a]	7.6 ± 0.3	3.9 ± 0.1	0.332
Silicon	4.8 ± 0.2	4.9 ± 0.2	0.286
Sodium[a]	53 ± 3	36 ± 4	0.097
Strontium	0.8 ± 0.00	0.8 ± 0.0	0.002
Sulfur[a]	17.6 ± 0.9	10.6 ± 0.2	0.130
Zinc	0.050 ± 0.003	0.050 ± 0.007	0.016

[a] Indicates significant difference between treatments; MDL = Minimum Detection Limit.

3.2.8. *Color and ultraviolet transmittance*

True color of the culture water of RAS receiving the FMF and FM diets was 20 ± 2 and 25 ± 2 Platinum Cobalt (Pt-Co) units, respectively ($P = 0.000$; Table 5). True color was consistently lower in RAS associated with the FMF diet from approximately Day 50 until the conclusion of the study (Fig. 5). Ultraviolet transmittance (UVT) was 81 ± 1 and 79 ± 1% in the culture water of RAS receiving the FMF and FM diets, respectively ($P = 0.124$; Table 5). These data are indicative of greater water clarity in RAS that received the FMF diet.

3.2.9. *Heterotrophic bacteria*

Mean, total heterotrophic bacteria counts for the FMF and FM diets were 437 ± 83 and 493 ± 121 colony forming units/mL, respectively, over the study duration (Table 5), and were not affected by dietary treatment ($P > 0.05$).

3.2.10. *Dissolved metals/trace elements*

Of the 25 dissolved metals/trace elements analyzed, 14 were measured at levels less than minimum detection limit (MDL) in the culture water for both dietary treatments, including: aluminum, arsenic, boron, cadmium, chromium, cobalt, iron, lead, manganese, molybdenum, nickel, selenium, titanium, and vanadium. Metals/trace elements measured at levels >MDL for both treatments included: barium, calcium, copper, magnesium, phosphorus, potassium, sodium, silicon, strontium, sulfur, and zinc (Table 8). Dissolved calcium was lower ($P = 0.005$) in RAS receiving the FMF diet, 93 ± 0 mg/L v. 99 ± 1 mg/L for the FM diet (Table 8). Diet also affected potassium levels in the culture water ($P = 0.000$). Dissolved potassium was 7.6 ± 0.3 mg/L for the FMF diet v. 3.9 ± 0.1 mg/L for the FM diet (Table 8). Dissolved phosphorus (DP) levels mirrored TP results for each treatment. Average DP

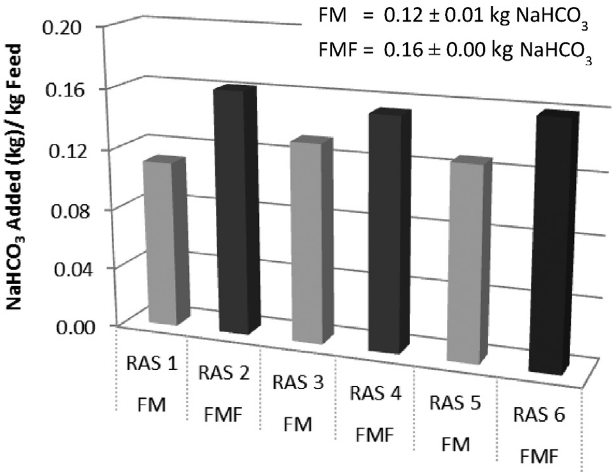

Fig. 6. Cumulative sodium bicarbonate amounts added to each recirculation aquaculture system to maintain alkalinity levels near 200 mg/L.

was 4.8 ± 0.2 mg/L in RAS receiving the FMF diet and 1.0 ± 0.1 mg/L in RAS receiving the FM diet ($P = 0.000$). In addition, dissolved sodium was greater ($P = 0.002$) in RAS that received the FMF diet, 53 ± 3 mg/L v. 36 ± 4 mg/L for the FM diet (Table 8). Dissolved sulfur was also greater ($P = 0.004$) in RAS receiving the FMF diet, 17.6 ± 0.9 mg/L v. 10.6 ± 0.2 mg/L for the FM diet. Detectable levels of barium, copper, magnesium, silicon, strontium, and zinc were similar ($P > 0.05$) between diets.

3.2.11. *Controlled water quality*

Dissolved oxygen, temperature, alkalinity, and pH were equally controlled among RAS (Table 5). Average dissolved oxygen in the culture water for each dietary treatment was 10.0 mg/L, equaling 100% oxygen saturation at 15.2 °C, the mean water temperature for both treatments. Mean alkalinity, which was controlled by sodium bicarbonate addition, was 206 ± 2 and 208 ± 2 mg/L for the FMF and FM diets, respectively (Table 5). Sodium bicarbonate was added at a mean rate of 0.16 ± 0.00 and 0.12 ± 0.01 kg $NaHCO_3$/kg feed for the FMF and FM diets, respectively ($P = 0.008$). More sodium bicarbonate addition was required in RAS receiving the FMF diet to maintain alkalinity at ≥200 mg/L (Fig. 6). Average culture water pH was 8.1 mg/L for each treatment and corresponded with relatively low carbon dioxide (CO_2) levels of 4 ± 0 and 3 ± 0 mg/L in RAS receiving the FMF and FM diets, respectively (Table 5).

4. Discussion

4.1. *Diet background*

Complete replacement of fishmeal with plant-derived proteins, thus far, has been challenging for Atlantic salmon diets due to generally reduced growth and antinutritional effects such as gut enteritis (Baeverfjord and Krogdahl, 1996; Francis et al., 2001; Krogdahl et al., 2003). Therefore, the present study evaluated a diet that was not as reliant on plant-based proteins that used practical ingredients such as blended mixed nut meal, poultry meal, wheat flour, and corn protein concentrate to replace fishmeal.

The use of terrestrial animal byproducts in aquaculture diets is common practice in North America but has been relatively controversial in other parts of the world, particularly Europe, due to concerns over the transmission of spongiform encephalopathies (prion diseases such as "Mad Cow"); however, EU Regulation 56/2013 was recently passed nullifying previous regulations that prohibited the use of such ingredients (EU, 2013), thereby adding a variety of non-ruminant animal byproducts to the list of ingredients available for European salmon diets. Several experiments, including the present study, have shown that poultry by-product meal is an effective alternate protein for salmonid diets (Fowler, 1991; Steffens, 1994; Alexis et al., 1985; Sugiura et al., 1998; Sealey and Hardy, 2011; Burr et al., 2012). Recent research also indicates that the inclusion of poultry meal and porcine blood meal in Atlantic salmon diets can result in health benefits such as reduced liver triacylglycerols and a trend towards improved gut health (Liland et al., 2015). The protein digestibility of poultry meal varies depending on source (Cheng and Hardy, 2002; Sealey and Hardy, 2011), but high quality poultry by-product has been found to have similar (Cheng and Hardy, 2002; Cheng et al., 2004) or in some cases better (Sugiura et al., 1998) digestibility coefficients compared to fishmeal. Fowler (1991) found that 20% of fishmeal could be replaced with poultry by-product meal in diets fed to juvenile Chinook salmon *Oncorhynchus tshawytscha* without compromising growth and feed conversion; and Burr et al. (2012) reported comparable growth of juvenile Atlantic salmon when feeding diets that contained poultry by-product blended with other proteins to completely replace fishmeal.

Nut meal has also been found to be a potentially effective protein replacement for salmonid diets, but has not been extensively evaluated. Barrows and Frost (2014) reported that diets containing blends of pistachio and almond meal with only 5% fishmeal resulted in comparable rainbow trout growth compared to a standard fishmeal-based diet. The same study found that almond and pistachio meals had apparent digestibility coefficients for protein, fat, and amino acids similar to fishmeal (Barrows and Frost, 2014), further implying that nut meal is a suitable alternate protein for salmonid diets. The results of the present study showed that mixed nut meal is a viable replacement protein for post-smolt Atlantic salmon diets when blended with poultry byproduct, wheat flour, and corn protein concentrate proteins to completely replace fishmeal and when fed to salmon under the conditions of this trial.

When examining the potential of new protein sources for aquafeeds, ingredient availability and cost must be considered. The US tree nut industry, which is based in California, New Mexico, and Arizona (Agricultural Marketing Service, 2012), has a portion of product that does not meet USDA certification and is therefore graded out and sold for "non-human consumption" (Agricultural Marketing Service, 2003). Adaptive Bio-Resources, LLC (Escalon, CA, USA) is currently making approximately 40 tons of nut meal per month with potential to increase production. The quantity of nut meal co-product available is not large enough to justify its use by the global salmon industry (Barrows and Frost, 2014); however, nut meal could be useful as a niche ingredient in diets used for a smaller, but growing industry utilizing land-based RAS to raise Atlantic salmon, rainbow trout, or other salmonids. In addition, nut meal could serve as a useful feed ingredient locally or regionally in areas close to production plants.

4.1.1. *Fish in: fish out ratio*

The Monterey Bay Aquarium Seafood Watch (MBASW) evaluates the environmental footprint of seafood based on a specific scoring system and provides a public recommendation that describes the sustainability of seafood products (Monterey Bay Aquarium, 2011). The MBASW considers the amount and sustainability of wild fish used to produce food fish and promotes the post-harvest use of by-products from processed fish. An important measure of the MBASW scoring system is the ratio of wild fisheries inputs to farmed fish outputs, or fish in: fish out (FIFO) ratio. During this study, both diets used oils derived from fish; however, the FMF diet was formulated with oil acquired from whiting fish processing wastes and the FM diet contained oil from wild-caught menhaden. The use of whiting fish trimmings to extract the lipid used in the FMF diet resulted in a wild fisheries in: farmed fish out ratio of

0:1 and was therefore considered highly sustainable, thus meeting the MBASW feed requirement for green labeling (Monterey Bay Aquarium, 2011). It is important to note that other certifying organizations may not discount fisheries byproduct in calculating FIFO. Achievement of a 0:1 FIFO ratio for Atlantic salmon is a dramatic improvement compared to reported ratios for commercially farmed Atlantic salmon, which not so long ago were as high as 5:1 (Naylor et al., 2009). However, the Norwegian salmon farming industry has rapidly improved the efficiency of marine resource use over the last decade. Ytrestøyl et al. (2015) reported that the Norwegian salmon industry became a net producer of fish protein, as of 2013, citing achievement of a FIFO ratio of 0.7.

4.2. Fish performance

4.2.1. Growth

The measurement of equal growth performance and feed conversion for post-smolt Atlantic salmon fed a fishmeal-free diet compared to a standard fishmeal-based diet during the present study is notable. Many studies have attempted to replace a portion of or in some cases all fishmeal with alternate protein ingredients in Atlantic salmon diets. Some of these trials resulted in less than optimal growth, health, feed conversion, and/or other performance metrics (Mundheim et al., 2004; Kraugerud et al., 2007; Reftsie et al., 2010; Pratoomyot et al., 2011; Burr et al., 2013). A few studies that evaluated partial fishmeal replacement in Atlantic salmon diets reported equal growth, health, and other key performance metrics compared to diets with full fishmeal inclusion (Reftsie et al., 2001; Reftsie and Tiekstra, 2003; Aas et al., 2006; Torstensen et al., 2008; Øverland et al., 2009; Bendikson et al., 2011; Burr et al., 2012). For example, Torstensen et al. (2008) reported that growth rates of post-smolt Atlantic salmon grown from 0.3 to approximately 4 kg were not affected by replacing 40% fishmeal with plant proteins and krill meal and 70% fish oil with vegetable oil. However, Atlantic salmon fed diets with 80% fishmeal replacement by plant proteins and krill meal grew significantly slower compared to a control group fed a diet with full fishmeal inclusion. In addition, Øverland et al. (2009) found that pea protein concentrate could replace 20% fishmeal without compromising Atlantic salmon growth performance, nutrient digestibility, or gut health. In one of few studies that have attempted to feed diets void of fishmeal to Atlantic salmon, Espe et al. (2006) reported slower growth, but similar FCRs for salmon fed plant-based diets compared to a fishmeal-based diet. Salmon were grown from approximately 0.3–0.7 kg during this study (Espe et al., 2006). To the authors' knowledge, only one study has demonstrated that Atlantic salmon growth performance was not compromised when feeding a diet devoid of fishmeal. Burr et al. (2012) found that juvenile (31.5 g) Atlantic salmon fed diets containing various blends of soy, corn, wheat, algae, and poultry byproduct proteins grew at equal rates, to a mean size of approximately 250 g, compared to salmon fed a fishmeal-based diet. Achievement of equal growth when feeding fishmeal-free diets has been largely attributed to proper supplementation of essential amino acids, vitamins, and minerals that otherwise would be made available by fishmeal or other ingredients (Barrows et al., 2010; Burr et al., 2012). A similar approach was used during the present trial to ensure that essential amino acids, vitamins, and minerals were supplemented, thus a specific balance of these components is likely critical to achieving optimal Atlantic salmon performance when feeding fishmeal-free diets. The present study could be the first published work of its kind to demonstrate uncompromised performance of post-smolt Atlantic salmon of this size (0.28–1.72 kg) when feeding a fishmeal-free diet.

4.2.2. Survival

Dietary treatment did not have an effect on Atlantic salmon survival, which was >99% for both treatments over the relatively long, 6-month study. Apart from a few salmon that jumped out of tanks and those that were culled for sampling purposes, the highest cumulative mortality recorded for an individual RAS was two fish, from a tank receiving the FM diet. On average, just one mortality was recorded per RAS for each dietary treatment. The high survival rate indicated that Atlantic salmon fed each diet were in good health.

4.3. Water quality

Measurement of equal Atlantic salmon performance and survival indicated that water quality concentrations did not reach thresholds that negatively impacted fish for either dietary treatment. However, the FMF diet produced greater amounts of TP, TSS, and cBOD per kg feed in the effluent. Potential explanations for differences in waste production and implications for waste discharge are discussed in the following sections.

4.3.1. Phosphorus

Total and dissolved phosphorus was approximately four times greater in the culture water of RAS associated with the FMF diet. Nearly all TP was in the dissolved form. TP was also greater ($P < 0.05$) in the effluent of RAS receiving the FMF diet compared to the FM diet (Fig. 4; Tables 5 and 8). The FMF and FM diets produced 0.009 ± 0.001 v. 0.006 ± 0.001 kg TP/kg feed, respectively. Elevated phosphorus (P) levels measured for RAS receiving the FMF diet likely resulted from P that was not biologically required by the salmon and therefore excreted into the water. The FMF diet was formulated with a slightly greater amount of P (1.41%) compared to the FM diet (1.30%; Table 2) to adjust for the predetermined apparent digestibility coefficient (ADC) of phosphorous provided by ingredients (Barrows et al., 2014). Phosphorus digestibility for poultry meal, which was used in the FMF diet, varies depending on quality and source (Cheng and Hardy, 2002), but has been found to be comparable to fishmeal (Cheng and Hardy, 2002) or in some cases higher (Sugiura et al., 1998). The poultry meal used during the present study was known to have a phosphorous ADC of approximately 78% compared to 40% for fishmeal (Barrows et al., 2014). However, pistachio meal, which was part of the mixed nut meal blend used in the FMF diet, was known to have a low phosphorous ADC (27%) compared to fishmeal (40%) (Barrows et al., 2014; Barrows and Frost, 2014). The relatively low P digestibility of nut meal could, in part, explain the elevated P levels in RAS receiving the FMF diet. To adjust for the lower P digestibility of nut meal, and considering the Atlantic salmon's low affinity to retain phosphorous (Ketola and Harland, 1993), 32.5 g/kg of dicalcium phosphate was added to the FMF, while only 5.0 g/kg of dicalcium phosphate was added to the FM diet (Table 1). Åsgård and Shearer (1997) suggested a minimum dietary P requirement of 11 g/kg for juvenile Atlantic salmon; while other studies indicated that the dietary P requirement for Atlantic salmon is 6 g/kg or 0.6% of the diet (Ketola, 1975; Lall and Bishop, 1977). Therefore, it is also possible that dicalcium phosphate was oversupplemented in the FMF diet.

Elevated P levels contained in aquaculture effluents could impact environmental health (Bureau and Hua, 2010), challenge compliance with pollution discharge standards (Bergheim and Brinker, 2003; United States Environmental Protection Agency, 2004), and require costly treatment technologies for mitigation (Sharrer et al., 2010a). Thus, the elevated P levels produced by the FMF diet would likely be a disadvantage for most RAS operations. However, possible value-added advantages could be gained through its reuse. Recirculation aquaculture systems are capable of concentrating phosphorus and other nutrients in relatively small backwash flows (as demonstrated during this study) or dilute system overflows (Davidson et al., 2013) that can be recycled for use in aquaponics systems (Rakocy et al., 2006). Biosolids dewatering technologies (Sharrer et al., 2010a,b) can also be used to further concentrate solids from RAS backwash water and thereby produce nutrient-rich soil amendments for use as fertilizers or for other agriculture applications.

As this was, to the authors' knowledge, the first attempt to feed a fishmeal-free diet with this ingredient profile to Atlantic salmon, the excess P waste is not entirely surprising. The results suggest that dicalcium phosphate inclusion should be substantially reduced in the FMF formulation, which is a relatively simple adjustment. With this change, the FMF diet could be a promising diet for Atlantic salmon cultured in RAS.

4.3.2. *Nitrogen*

The culture water of RAS receiving the FMF diet contained greater ($P < 0.05$) concentrations of TAN and NO_3-N (Table 5). A hypothesis for the slightly increased nitrogen levels in RAS receiving the FMF diet was greater excretion of TAN or urea by the fish or hydrolysis of proteins contained in fecal material. While some aspect of the diets could have influenced culture tank nitrogen, the total nitrogen waste flushed from RAS per kg feed was similar ($P > 0.05$) between diets (Table 6). The majority of TN waste produced by each diet (>75%) was removed via the drum filter backwash. Equal TN waste loads per kg feed were expected, because the experimental diets were formulated with equal levels of crude protein and balanced for lysine, methionine, and threonine (Table 3), thus an amino acid imbalance is considered an unlikely cause for the minor differences in nitrogen measured in the culture water. A proper balance of protein and amino acids in each experimental diet was corroborated by calculation of equal nitrogen retention efficiency for salmon sampled at the conclusion of the study from each dietary treatment.

The differences in nitrogen levels in the culture water between treatments were relatively small in magnitude and not of practical significance to fish health or performance. However, elevated TAN in RAS culture water could result in increased demand on nitrifying bacteria, which in turn consume alkalinity through the biochemical conversion of ammonia nitrogen to nitrite- and nitrate nitrogen. In RAS that use hard, alkaline make-up water, a significant portion of the alkalinity requirement is supplied via new water addition; however, in systems that utilize soft/low-alkalinity water as make-up and/or are operated with low water exchange rates, nearly all alkalinity must be supplemented with sodium bicarbonate or other compounds to maintain nitrification efficiency (Loyless and Malone, 1997; Summerfelt et al., 2015b).

Davidson et al. (2013) correlated greater inorganic nitrogen in RAS receiving a fishmeal-free diet with increased demand for sodium bicarbonate addition. Another on-site study (unpublished) also found that greater sodium bicarbonate addition was required when feeding a fishmeal-free diet compared to a fishmeal-based diet in the same replicated RAS; however, during that trial inorganic nitrogen levels were lower in RAS receiving the fishmeal-free diet (unpublished, author's personal experience). Hence, the trend for increased sodium bicarbonate addition in RAS receiving fishmeal-free diets tends to be consistent regardless of differences in nitrogen loading. Therefore, the increased requirement for sodium bicarbonate addition could be related to diet composition. The authors' hypothesize that fishmeal-based diets could have inherently greater alkaline base inclusion, of which a portion becomes dissolved in RAS through fish excretion, thus providing a minor, but unintended, benefit.

Table 9
Average drum filter backwash, radial flow setter, and system flushing metrics for RAS receiving a fishmeal-free diet v. a fishmeal-based diet. Samples for determination of effluent TSS values were taken at approximately 2, 4, and 6 months of the study.

Drum Filter Backwash	FMF Diet	FM Diet
Number of Daily Backwashes	274 ± 12	260 ± 6
TSS (mg/L)	469 ± 43	450 ± 53
Flow (L/day)	431 ± 55	429 ± 16
Flow (% of Recycle Flow)	0.08 ± 0.01	0.08 ± 0.00
Radial Flow Settler	**FMF Diet**	**FM Diet**
Number of Daily Flushes[a]	1–3	1–3
TSS (mg/L)	6414 ± 442	2918 ± 249
Flushing Flow (% of Recycle Flow)	0.01	0.01
System Flushing Metrics	**FMF Diet**	**FM Diet**
Continuous Makeup Water Flow (L/day)	0	0
Water Exchange Created by RFS Flush (m^3/day)	0.469 ± 0.013	0.472 ± 0.008
Daily Water Exchange (% of System Volume)	4.9 ± 0.1	5.0 ± 0.1
System Hydraulic Retention Time (days)	20.3 ± 0.6	20.1 ± 0.3

[a] Radial flow settler volume of each RAS (265 L) was completely emptied to equally control water exchange between experimental units. Number of daily flushes was dependent on controlling nitrate-nitrogen ≤75 mg/L.

4.3.3. *Total suspended solids*

Mass balance calculations indicated that total solids production was greater ($P < 0.05$) in the effluent of RAS that received the FMF diet (Table 6). Total suspended solids levels were lower in the culture water of RAS that received the FMF diet; however, the difference in magnitude was small, and average TSS levels were relatively low for both treatments, <2 mg/L. The higher culture water TSS is of interest when considering the division of TSS among effluent sites. For example, 52% of solids produced in RAS that received the FMF diet were removed by the radial flow settler, while only 35% of solids were removed by the radial flow settler in RAS in which salmon were fed the FM diet (Table 6). Furthermore, twice as much TSS mass per kg feed was collected by the radial flow settlers in RAS receiving the FMF diet. This data points to a difference in fecal characteristics produced between diets; whereas, fecal settleability appeared to be better for salmon fed the FMF diet. When flushing radial flow settlers to check for wasted feed, fecal matter from RAS receiving the FMF diet was observed to be intact and viscous and at times clogged the catchment baskets. Solids flushed from settlers of RAS receiving the FM diet were less viscous and rarely clogged the baskets. It is important to note that the FM diet contained 128.5 g/kg feed soy protein concentrate (Table 1), and the inclusion of soy ingredients in the diets of salmonids cause less stable fecal material and an increase in fine solids due to potential disintegration of fecal particulates (Brinker and Friedrich, 2012; Davidson et al., 2013).

The greater TSS concentrations associated with the FM diet did not appear to cause adverse effects to salmon during the present study. However, increased levels of fine solids and particulates have the potential to negatively impact unit process removal efficiencies, increase biochemical oxygen demand, diminish nitrification efficiency, and increase heterotrophic bacteria populations (Cripps and Bergheim, 2000; Zhu and Chen, 2001). In this light, the FMF diet appears to offer a minor water quality benefit in RAS, due to the likelihood of increased fecal settleability and associated reduction of TSS in the culture water.

Although, the FM diet resulted in a minor increase in TSS in the culture water, the drum filters backwashed at similar frequency for each diet, i.e., 274 ± 12 and 260 ± 6 daily backwashes for the FMF and FM treatments, respectively (Table 9). The backwash was relatively dilute for each diet treatment, containing 469 ± 43 and 450 ± 53 mg/L TSS for the FMF and FM diets, respectively, in a flow that was equivalent to 0.1% of the total recycle flow for each treatment (Table 9). These results imply that there was no difference in the wash requirement for solids filtration between dietary treatments.

The radial flow settlers captured and flushed a high concentration of TSS within a relatively small flushing flow, discharged once daily, which was equivalent to 0.01% of the total recycle flow (almost ten times less than the drum filter backwash flow). Total suspended solids levels measured in the radial flow settler discharge were 6400 v. 2900 mg/L for the FMF and FM diet treatments, respectively (Table 9). Resulting solids removal efficiency across the radial flow settlers associated with the FMF and FM diets equated to 60% and 40%, respectively. The increased efficacy of solids removal across settlers associated with the FMF diet was likely related to greater fecal settleability, as previously discussed.

4.3.4. Carbonaceous biochemical oxygen demand

Although cBOD was identical in the culture water of RAS associated with each dietary treatment, cBOD mass was greater ($P < 0.05$) per kg feed in the effluent of RAS that received the FMF diet. This finding is consistent with other research (Zhu and Chen, 2001) that has shown that cBOD levels generally correlate with solids concentration, because TSS was also significantly greater per kg feed in the effluent of RAS that received the FMF diet.

4.3.5. Water clarity

True color of the culture water was affected by dietary treatment and was lower ($P < 0.05$) in RAS that received the FMF diet, indicating greater water clarity. During the first 2 months of the study, higher and more variable true color was measured in all RAS (Fig. 5), likely resulting from clouding by calcium carbonate, which precipitates out of hard water when the acid/base equilibrium is disrupted and pH shifts above a given threshold (Snoeyink and Jenkins, 1980). This phenomenon is common in on-site RAS operated with relatively low fish biomass and feeding rates and subsequently low carbon dioxide and elevated pH levels ≥8.0. Aside from this peak, however; color was consistently lower in RAS receiving the FMF diet during the last four months of the trial (Fig. 5).

These water clarity results are consistent with other studies that have evaluated alternate ingredient diets versus fish-meal based diets. Schuster (1994) found that the fishmeal content of rainbow trout diets contributed to the intensity of brown colored water in a closed RAS, while plant-based diets did not stain the water. During a study comparing the effects of feeding rainbow trout a grain-based diet versus a fish meal-based diet in low exchange RAS, Davidson et al. (2013) reported lower true color values in the culture water of RAS receiving the grain-based diet. Colored water is not necessarily a disadvantage for fish performance. However, clear water could enhance the ability of fish to capture feed and could therefore lead to enhanced growth and improved FCR (Sigler et al., 1984); and allows the farmer to observe fish health, behavior, and feeding activity (Christensen et al., 2000).

4.3.6. Metals/elements

Assessment of dissolved metals and element levels in RAS operated with low water exchange rates is important because these concentrations can accumulate to potentially dangerous levels for fish when RAS are operated with low water exchange rates (Davidson et al., 2009). Therefore, vitamin and mineral supplementation that meets, but does not exceed, the biological requirement of fish is critical when formulating diets for use in RAS. Only a few minor differences in metals/element concentrations were measured between dietary treatments, but these concentrations did not appear to negatively impact salmon from either treatment based on growth and survival results. Greater levels ($P < 0.05$) of dissolved sodium measured in RAS receiving the FMF diet were associated with the requirement for greater addition of sodium bicarbonate (Fig. 6). The relatively small differences ($P < 0.05$) in dissolved calcium, potassium, and sulfur levels could have been related to micro-additives in the diets or sodium bicarbonate. This sampling data also revealed that dissolved phosphorus levels mirrored total phosphorus concentrations, whereas average DP was also approximately four times greater in RAS that received the FMF diet. This indicates that almost all of the total phosphorus in the culture water of RAS receiving the FMF diet was present in the dissolved form.

5. Conclusions

This study is one of few published works to show that Atlantic salmon are capable of achieving equal growth performance, feed conversion, and survival when fed a diet with complete fishmeal replacement. The present study is also the first (to the author's knowledge) to evaluate a fishmeal-free diet for Atlantic salmon with this particular ingredient profile, blending mixed nut meal, poultry meal, and other protein sources. The successful use of mixed nut meal in this study adds to the diversity of ingredients available to feed suppliers and Atlantic salmon producers interested in using sustainable diets. The fishmeal-free diet produced significantly greater mass of three key wastes (total phosphorous, totals suspended solids, and carbonaceous biochemical oxygen demand); however, culture system water quality did not negatively impact fish performance. The 4-fold increase in total phosphorous dissolved in the culture water of RAS associated with the fishmeal-free diet requires further investigation; albeit, the authors are confident that phosphorus excreta can be substantially reduced by lowering the inclusion level of dicalcium phosphate in the diet formulation. The results from this study can be used by nutritionists to refine future formulations and by engineers and operators of RAS intending to use similar diets.

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