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Original article

Effects of dietary glutamate and succinate on growth performance and mitochondrial respiration in heart and liver of Atlantic salmon (*Salmo salar*) smolts

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Abstract

The smolt stage of salmon has challenges in reaching adequate growth rates due to the changing environmental conditions at sea. Therefore, it is necessary to provide adequate diets to achieve sufficient growth. This study determined the impacts of glutamate and succinate (1% each) supplemented diet on the growth of Atlantic salmon smolts along with characterization of mitochondrial respiration using high-resolution respirometry technique. Results indicated that there was no significant difference in growth response between the treatment and control groups. Maximum oxidative phosphorylation (OXPHOS) was reached after addition of succinate. Analysis of heart homogenates revealed a significant difference in LEAK respiration state (P = 0.005). No significant difference was recorded between the diet groups for liver homogenates. Differences between heart and liver respiration revealed that mitochondrial activity is organ dependent.

Keywords: Atlantic salmon; mitochondria; succinate; glutamate; respiration; high resolution respirometry.

1 | INTRODUCTION

Aquaculture is the fastest growing food producing sector in the world (FAO 2018). As the intensification of aquaculture production occurs, the need for increased production efficiency is important (Cheng *et al.* 2011). Studies focusing on nutrition, in order to obtain better production efficiency, have caught more attention in the recent past (Trichet 2010).

In Norway, the aquaculture has been a success story so far. From the construction of the first sea cage in 1970s (FAO 2005) to the commercial aquaculture production, the country has shown steady development throughout its history. Today, Norway dominates the global farmed salmon production (FAO 2018). The seawater phase of Atlantic salmon (*Salmo salar*) is considered to be the most value-adding phase during grow-out production (Oehme *et al.* 2010). The challenge in successful farming of salmon during the seawater phase has been realised. The influence of external environmental conditions has dramatic

changes in the physiology and metabolism of the cultured fish species. Effects on growth rate, feed utilization and product quality have been studied in the past (Thorpe *et al.* 1989). The seawater phase has been shown to characterize reduced feed intake and decreased body energy level (Alne *et al.* 2011).

The above-mentioned challenges during grow-out production of Atlantic salmon can be overcome by provision of adequate dietary supplements (Burrells *et al.* 2001, Rørvik *et al.* 2007, Alne *et al.* 2009). Research on nutritional requirements has shown that dietary protein, especially amino acids can play significant role in immunity of fishes (Cheng *et al.* 2011). There are evidences of amino acids acting as metabolic regulators (Meijer 2003, Li *et al.* 2009).

Amino acids that belong to glutamate family have shown to perform versatile functions (Oehme *et al.* 2010). Glutamate is a precursor for gamma amino butyric acid (GABA), purine and pyrimidine nucleotides (Neu *et al.* 1996, Tapiero *et al.* 2002, Li *et al.* 2009,). Glutamate is an important energy source in the intestine (Neu *et al.* 1996). Glutamate affects the oxidation of nicotinamide adenine dinucleotide (NADH) in many cells (Minarik *et al.* 2002). It has an anaplerotic function in Kreb's cycle (Brosnan 2000). Glutamate plays a key role in ammonia detoxification in nervous tissues of salmon (Kolarevic *et al.* 2012). Increased feeding rate, growth and gut weight were observed in salmon fed a diet comprising glutamate and arginine (Oehme *et al.* 2010). However, there are few studies that investigated the effect of glutamate supplemented diet in salmon (Larsson *et al.* 2014).

Studies based on dietary succinate on fish were not found, hence lesser is known about the effect of this amino acid on fish. Dietary succinate has been known to function as a substrate for intestinal gluconeogenesis, a process known to improve glucose homeostasis (Vadder *et al.* 2016). This study concluded that succinate supplementation in mice led to improved body weight.

In this study, based on the functions of glutamate and succinate, mentioned above, a feeding trial was conducted using the amino acids as supplements for Atlantic salmon smolts. The main objective was to test the effects of glutamate and succinate on mitochondrial function in heart and liver. The experiment also tested if there is any effect of the diet on the growth of fish. The amino acids were added to a commercial diet (each amino acid at 1% of total diet). The content of glutamate was decided based on previous feed trials conducted on Atlantic salmon (Oehme *et al.* 2010, Larsson *et al.* 2014).

2 | METHODOLOGY

2.1 Diets and feeding

All diets were provided as dry pellets produced by Biomar AS, a leading fish feed producing company in Norway. Two diet types, control diet (BioMar AS, Norway) and experimental diet (BioMar AS, Denmark) consisting of glutamate and succinate at 1% each were provided during this experiment. The smolts were provided by Lerøy AS, a leading salmon farming company in Norway and they were gradually adapted to seawater to avoid stress conditions. The fish had a mean weight of approximately 90 g at the start of the feeding trial. A one-month acclimation period was followed by providing control diet for all the fish to ensure adaptation to the experimental conditions. Automatic feeders were used, and dissolved oxygen was maintained at 85–90%. Fish were kept in six fiberglass tanks (400-litre capacity each, square shaped with flat bottom and centre drain, three tanks for each diet type with 25 individuals per tank. All experiments were performed at NTNU sea lab located in Trondheim, Norway. After one month of acclimation period the initial weights of fish were measured and feeding trial was started (20 September 2017) by providing experimental diet for three tanks and control diet for the rest. Water temperature remained at 10 ± 1 °C throughout the trial. The entire feeding trial was done under indoor conditions, therefore artificial lighting was provided for all tanks (24 hours) using fluorescent lights.

Specific growth rate (SGR) was calculated to determine the growth performance of fish in each tank. It provided percentage increase per day. The feeding was performed for 37 days. SGR was calculated based on the following formula (Lugert *et al.* 2016):

$$SGR = \frac{\ln(w_f) - \ln(w_i)}{t} \times 100$$

Where w_f is the final weight of fish after feeding trial, w_i is the initial weight of fish before feeding trial and t is the number of days of feeding trial.

2.2 Sample collection and analysis

After 37 days of feeding trial the fish were weighed to obtain a final weight (26 October 2017). One fish from each tank was anesthetized with tricaine methane sulfonate (MS-222, 200 mg/L, Agent Chemical Laboratories Inc., US) and subjected to dissection on ice to obtain heart and liver homogenates for mitochondrial respiration analysis.

Mitochondrial respiration was measured using high-resolution respirometry method with oxygraph-2k (Oroboros Instruments, Innsbruck, Austria). Apex cordis por-

tion of heart and liver were used for analysis. Wet weights of 10 ± 1 mg of heart and 40 ± 1 mg of liver were measured (Precisa® 180A, Dietikon, Switzerland) and used to prepare homogenate.

The dissected tissue was placed into the PBI shredder tube (Pressure Biosciences Inc. Massachusetts, US). The shredder tube was placed in the pre-chilled shredder base and shredder was operated for 30 seconds at position 1 (weakest) followed by 10 seconds at position 2 (stronger) for heart tissue and 40 seconds at position 1 followed by 20 seconds at position 2 for liver tissue.

After homogenization, the homogenate was transferred to 50 ml Falcon tube with 500 µl of mitochondrial respiratory medium (MiR05: 0.5 mM EGTA, 3 mM $MgCl_2$ $6H_2O$, 60 mM lactobionic acid, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 110 mM D-Sucrose, 1 g/L Bovine Serum Albumine (BSA) essentially fatty acid free, pH 7.1 at 20 °C). Preparation of the respiratory medium was done based on Oroboros Instruments Inc (Fasching *et al.* 2016). Respiratory medium was used to wash the residual homogenates left in the shredder tube and a total of 3.5 ml of respiratory medium (consisting homogenate) was collected in the falcon tube. This volume was used for one O2k chamber among the two chambers in the oxygraph. The O2k oxygraphs were calibrated (Fasching and Gnaiger 2016) prior to the start of experiments.

Tissue homogenates were transferred into calibrated Oxygraph-2k (O2k, OROBOROS INSTRUMENTS, Innsbruck, Austria) chambers of 2 ml capacity. Oxygen polarography was performed at 15°C. Oxygen concentration (µM) as well as oxygen flux per tissue mass (pmol O_2 $s^{-1}mg^{-1}$) were recorded real-time using the DatLab software (OROBOROS INSTRUMENTS, Innsbruck, Austria).

A modified version of substrate-uncoupler-inhibitor titration (SUIT) protocol (Kuznetsov *et al.* 2008; Gnaiger 2009) was used to test mitochondrial function in heart and liver homogenates. The non-phosphorylating LEAK respiration was induced by addition of malate, a complex I (CI) linked substrate (0.5 mM, 25 µl). Subsequently, addition of a saturating concentration of (Adenosine diphosphate) ADP (2.5 mM, 25 µl) provided enough energy for respiration. Oxidative phosphorylation (OXPHOS) capacity of CI activity was measured after addition of ADP. Titration of cytochrome C (10 µM, 5 µl) was done to assess the intactness of outer mitochondrial membrane. Afterwards, addition of glutamate (10 mM, 50 µl), a substrate provider for CI was done. The OXPHOS capacity of CI and CII linked substrates was measured by addition of succinate (10 mM, 20 µl). A step-wise titration of carbonyl cyanide p-trifluoro-methoxyphenyl hydrazine (FCCP) was performed (0.5 µM, 3µl) which leads to proton leakage through inner membrane of mitochondria. This was used for measurement of Electron Transfer System (ETS) capacity, a noncoupled state with optimum concentration of uncoupler. Subsequent addition of rotenone (0.5 µM, 10 µl), a CI inhibitor provided measurement of CII linked ETS capacity. For the control of other oxygen consuming processes malonate (5 mM, 5 µl) and antimycin A (2.5 µM, 10 µl) were used to inhibit CII and CIII. The resultant residual oxygen consumption (ROX) reflects oxygen consumption from undefined sources. Only homogenates that managed to maintain enough oxygen concentration (>150 µM O2) in the O2k chambers were considered for analysis. Ten samples were chosen from each tissue type to analyse mitochondrial respiration.

2.3 Protein content analysis

The Lowry assay method was used for quantification of protein content in homogenate samples. The assay was performed based on the manual (Walker 2002). 100 µl of standards and samples were used with absorbance reading at 550 nm in a UV-visible spectrophotometer (Varian Cary 50 Bio UV-Visible spectrophotometer, Agilent Technologies, US). Measurements were performed in triplicates.

2.4 Citrate synthase (CS) enzyme activity analysis

CS enzyme activity was analysed by following the protocol of Oroboros (Eigentler *et al.* 2015). Whole tissue samples of heart and liver were collected (n = 10 from each diet type) and stored at –80°C in plastic cryogenic tubes (Sigma-Aldrich, US). Afterwards, the tissue samples were weighed (Precisa® 180A, Dietikon, Switzerland) and immediately transferred into glass test tubes (Fisher Scientific, US) placed on ice. A volume of 3.5 ml respiration medium was added into the tubes followed by homogenization (Ultra Turrax T10 basic homogenizer, IKA, Germany) for 30 seconds at level 4. A portion (2ml) of homogenized samples were transferred into microcentrifuge tubes (Fisherbrand™, Fisher Scientific, US) kept on ice. The samples were centrifuged (Biofuge Pico, Heraeus Company, Germany) at 13,000 rpm for 5 minutes. The supernatant was used to determine CS activity. The commercial citrate synthase was used as a standard to check the chemicals and assay conditions. The reaction components were added in the order as mentioned in the protocol (Table 1) into a 1 ml plastic cuvette (VWR International, US). The volume of sample to be added into the plastic cuvette was decided based on Table 2.

Oxaloacetate was added immediately before the measurement of enzyme activity and the cuvette was sealed with parafilm (Sigma-Aldrich), swiveled gently three times. The parafilm was then removed and the cuvette was placed in a UV Visible spectrophotometer (Varian

Cary 50 Bio UV-Visible spectrophotometer, Agilent Technologies, US). The absorbance values were measured at 412 nm wavelength. CS activity was calculated using the following formula (Eigentler *et al.* 2015),

Specific activity: $\frac{r_A}{l \cdot \varepsilon_B \cdot V_B} \cdot \frac{V_{cuvette}}{V_{sample} \cdot \rho}$

where

v, specific activity of the enzyme measured in µmol min^{-1} mg^{-1}; rA, dA/dt rate of absorbance change (min^{-1}); l, optical path length (= 1 cm); ƐB, extinction coefficient of B (TNB) at 412 nm and pH 8.1 = 13.6 mM^{-1} cm^{-1}; VB, stoichiometric number of B (TNB) in the reaction (= 1); $V_{cuvette}$, volume of solution in the cuvette (= 1000 µl); V_{sample}, volume of sample added to a cuvette (5 – 100 µl); ρ, mass concentration or density of biological material in the sample, V_{sample} (protein concentration: mg cm^{-3})

TABLE 1 Components and their volumes added in 1ml cuvette for CS activity measurement.

Component	*Volume added (µl)*	*Final concentration*
10% Triton X-100	25	~0.25 %
Acetyl CoA	25	~0.31 mM
1.01 mM DTNB	100	~0.1 mM
Vsample	See Table 2	~5mg/ml
Distilled H_2O	(800 µl - V_{sample})	-
Oxaloacetate	50	~0.5 mM

TABLE 2 Volumes of components added into 1 ml cuvette.

Sample	*V_{sample} (µl)*
CS standard	5
Medium (MiR05)	10
Heart homogenate	5
Liver homogenate	10

2.5 Statistical analysis

Independent sample *t*-test was used to compare growth differences between the two diet groups (control vs. experimental). Ten samples (*n* = 10) were selected from each diet group to perform the independent samples *t*-test. A *p*-value of < 0.05 was considered statistically significant. Flux response between the diet groups and tissue types were also analysed using *t*-test. Two-way ANOVA was used to test the interaction between tissue type and diet type on flux response. Normality of data was tested with Shapiro-Wilk test. Simple log transformation was used to transform non-normal data into normal distribution. The non-parametric test of Mann-Whitney was used in circumstances where normality of data was not possible to achieve to employ parametric test methods.

3 | RESULTS AND DISCUSSION

3.1 Fish growth analysis

Weight based comparison between the two diet groups showed no statistically significant difference (*P* > 0.05). The number of individuals weighed was 180 in total (90 from each diet group). The mean weight of control diet group showed a slightly higher value (191.1 ± 3.1 g) than experimental diet group (187.0 ± 2.8 g) having no significant difference. Tank based SGR analysis also showed no significant differences between the diet groups (Figure 1).

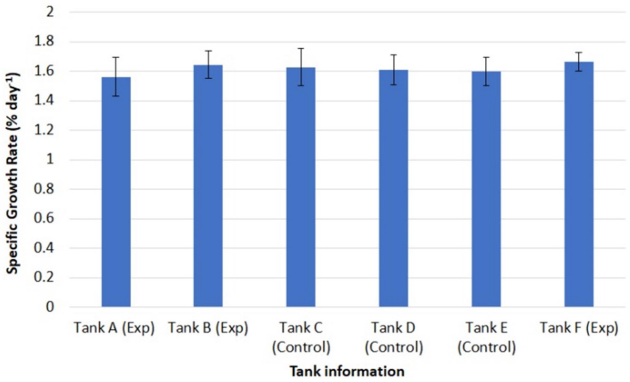

FIGURE 1 Mean specific growth rate percentage values based on each tank. Error bars represent standard errors. (Exp stands for experimental tanks)

3.2 High resolution respirometry

3.2.1 Mitochondrial respiration capacity in heart and liver

There was no significant difference in flux values of substrates/inhibitors between the two diet groups except for malate in heart mitochondria (Table 3). The flux values of malate showed a statistically significant difference (*P* = 0.005). No significant difference was observed in liver mitochondria (Table 4).

TABLE 3 The flux of added substrates and inhibitors in heart mitochondria of Atlantic salmon (Mean ± SEM). The flux values are the tissue response to substrates/inhibitors measured in pmol s^{-1} ml^{-1}) (*n* = 10).

Substrate/inhibitor	*Control diet*	*Experimental diet*	*P-value*
Malate	1.47±0.08	1.01±0.11	0.005
ADP	6.46±0.7	6.24±0.9	0.855
Cytochrome C	7.20±0.8	6.83±0.9	0.762
Glutamate	12.98±1.8	12.41±1.5	0.813
Succinate	13.30±1.9	12.90±1.6	0.872
FCCP	9.63±1.2	9.55±1.6	0.969
Rotenone	1.78±0.2	2.42±0.8	0.451
Malonate	0.54±0.2	0.38±0.1	0.454
ROX	1.08±0.3	0.53±0.09	0.212

ADP, Adenosine diphosphate;
ROX, Residual oxygen consumption

TABLE 4 Mean value ± Standard Error Mean of the flux of added substrates and inhibitors in liver mitochondria of Atlantic salmon. The flux values are the tissue response to substrates/inhibitors measured in pmol s^{-1} ml^{-1}).

Substrate/inhibitor	*Control diet*	*Experimental diet*	*P-value*
Malate	1.25±0.1	1.02±0.2	0.299
ADP	1.95±0.1	1.83±0.4	0.224
Cytochrome C	2.04±0.2	1.97±0.5	0.407
Glutamate	3.18±0.3	3.36±0.8	0.663
Succinate	4.80±0.4	5.26±1.3	0.740
FCCP	4.33±0.3	4.01±0.9	0.305
Rotenone	1.64±0.1	1.76±0.4	0.798
Malonate	0.29±0.01	0.39±0.07	0.545
ROX	1.27±0.02	0.40±0.07	0.149

ADP, Adenosine diphosphate;
ROX, Residual oxygen consumption

3.2.2 Mitochondrial respiratory states with respect to tissue mass of heart and liver homogenates

Analysis of high resolution respirometry data revealed a significant difference in the LEAK respiration state ($P < 0.05$) between the control and experimental diet groups (1.466 ± 0.267 pmol $s^{-1}mg^{-1}$ and 1.008 ± 0.373 pmol s^{-1} mg^{-1} respectively) for heart homogenates (Table 5). However, maximum OXPHOS and ETS states did not show a significant difference in diet groups.

TABLE 5 Mean O2 flux ± standard deviation at respiratory states LEAK, Max OXPHOS and ETS for heart samples (n = 10) displayed in units pmol $s^{-1}mg^{-1}$.

Respiratory states	*Control diet*	*Experimental diet*	*P-value*
LEAK state	1.466 ± 0.267	1.008 ± 0.373	0.005
OXPHOS state	13.299 ± 5.971	12.898 ± 4.923	0.872
ETS state	9.631 ± 3.843	9.553 ± 4.949	0.969

Analysis of liver homogenates showed no significant differences between the diet groups for all three respiratory states (Table 6). Stable flux response was observed after addition of cytochrome C in heart and liver homogenates therefore, confirming that the outer mitochondrial membrane was intact in the samples.

Maximum OXPHOS was achieved after succinate (CI + CII) titration and proved no significant difference in respiration. No significant difference was also observed in ETS respiration (after addition of FCCP) between the diet groups. Similar results were observed in liver mitochondrial respiration with no significant differences ($P > 0.05$) in liver homogenates. Similar to heart homogenates, the maximum OXPHOS was observed after addition of succinate. A slightly higher OXPHOS was observed in experimental diet group than control group after addition of succinate although not significant. Comparison of heart mitochondrial respiration with liver respiration shows higher activity (higher oxygen flux values) of mitochondria present in heart homogenates.

TABLE 6 Mean O2 flux (± SD) at respiratory states LEAK, Max OXPHOS and ETS for liver samples (n = 10) displayed in units pmol $s^{-1}mg^{-1}$.

Respiratory states	*Control diet*	*Experimental diet*	*p-value*
LEAK state	1.253±0.394	1.023±0.555	0.299
OXPHOS state	4.801±1.344	5.263±3.958	0.731
ETS state	4.337±1.102	4.012±2.905	0.745

As shown on Table 7, a two-way ANOVA test showed that statistically significant differences exist between heart and liver responses for substrates and inhibitors. Flux responses from malate, rotenone, malonate and ROX showed no significant differences between tissue types.

TABLE 7: Tissue based comparison (heart vs liver) of flux response values and interaction between tissue type and diet type

Heart vs. liver		*Tissue type (heart vs. liver) × diet type (control vs. experimental)*		
Substrate	***P***	***F***	***P***	***df***
Malate	0.482	0.772	0.385	1
ADP	<0.05	0.046	0.831	1
Cytochrome C	<0.05	0.054	0.818	1
Glutamate	<0.05	0.089	0.767	1
Succinate	<0.05	0.006	0.941	1
FCCP	<0.05	0.013	0.912	1
Rotenone	0.626	0.320	0.575	1
Malonate	0.903	0.366	0.549	1
ROX	<0.05	0.626	0.434	1

ADP, Adenosine diphosphate;
ROX, Residual oxygen consumption

3.3 Citrate synthase enzyme activity

Statistical analysis of CS activity in tissue slices of heart samples showed no significant difference between the diet groups based on the significance level of 5% (Figure 2). Citrate synthase enzyme activity showed relatively lower enzyme activity in liver samples (Figure 3) than heart samples. However, no statistically significant difference was found between the diet groups.

4 | DISCUSSION

The present feeding experiment incorporated glutamate and succinate supplemented diets due to the promising results reported by a previous study (Oehme *et al.* 2010) on Atlantic salmon growth. Analysis from growth results

in this experiment showed that the overall comparison of fish weight between the diet groups did not show any significant difference. Individual tank-based analysis of SGR did not show any statistically significant difference. Tank F fed with experimental diet showed the highest SGR percentage among all tanks. However, only slight differences in SGR were found among tanks regardless of the diet provided. Tank A fed with experimental diet showed the lowest growth response. Though the reason behind this is unclear, there may be an effect between the tank placement and growth response.

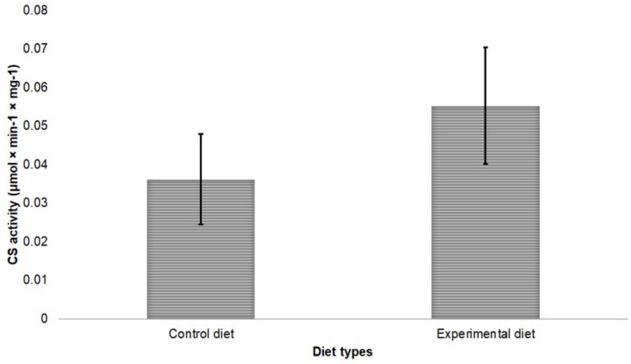

FIGURE 2 Mean values of CS activity in heart. The error bars represent standard error mean

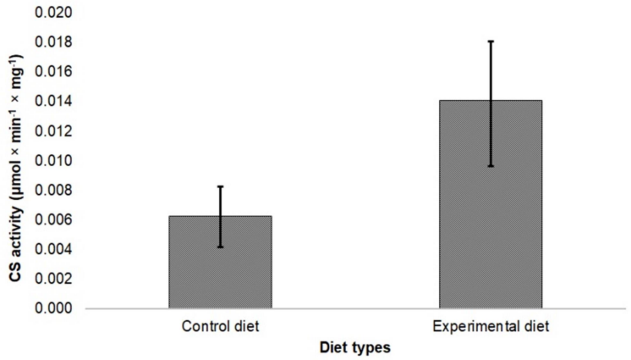

FIGURE 3 Mean values of citrate synthase activity in liver tissues. The error bars represent standard error mean.

The flux response from succinate titration showed the maximum OXPHOS in HRR results, thus it was considered for OXPHOS calculations. It was evident that HRR analysis showed lower FCCP response in heart than in liver homogenates. There can be many reasons behind this observation. Lower FCCP response was also observed from another study (Hasli 2015) conducted on Atlantic salmon. Furthermore, it was observed that the flux response of FCCP did not become stable even after several repeated titrations. Related results were found in another study (Hasli 2015). Another reason for the lack of response from FCCP could be the poor perfusion of the tissue than expected. This may have led to reduced respiration from lowered partial pressure.

Results from CS activity showed lower enzyme activity in heart and liver. The reason behind this might be the lower oxidative capacity of mitochondria. Although the tissue samples were stored at –80°C instead of liquid nitrogen, it was found that CS activity can be preserved at subzero temperatures for longer periods of time (Shepherd and Garland 1969) therefore, the effect of storage method used for the tissues in sustaining the enzyme activity was ruled out. Larger amounts of tissues for CS activity can lead to lack of reaction itself. It was revealed that the DTNB used in the protocol turns yellow after the reaction, yet an increase in absorbance will continue to occur. However, this increase in absorbance is related to post reaction changes thus, not relevant for CS activity measurement. It is important to measure the linear absorbance increase at the start of the reaction. Moreover, it is necessary to minimize the time (<10 seconds) between the addition of oxaloacetate in the cuvette and measurement in the spectrophotometer. The use of plastic cuvettes for CS activity measurement was decided due to the wavelength of 412 nm (Brandt 2010). It was reported that the amount of this enzyme is correlated with the ability of the cell to use oxygen (Moriyama and Srere 1971). The CS activity results showed higher enzyme activity in heart tissues in comparison with liver, thus proving higher mitochondrial density present in the heart.

Obtaining liver samples for homogenate preparation consistently from the same portion is necessary to achieve reliable results. Homogenate preparation from varying portions of the liver may lead to variable mitochondrial activity. Improper shredding of samples may contain tissue particles present in the homogenate that may cause some disturbance in results. The initial dissolved oxygen concentration present in the sample at the beginning of a SUIT titration can affect the execution of the protocol in the sample. Rapid oxygen depletions were observed in some samples that led to oxygen deficiency to continue the SUIT titration. Such samples had to be discarded and new samples were prepared. However, the oxygen concentration never fell below 150 µmol L^{-1} during a proper SUIT titration procedure, therefore, should not lead to limited respiration.

5 | CONCLUSIONS

This study showed no significant difference in growth of salmon smolts fed glutamate and succinate enhanced diets at 1 % each. Higher mitochondrial respiration was found in heart homogenates than liver homogenates. Maximum OXPHOS was recorded after succinate addition.

Future studies could be conducted using different temperature regimes to determine the effect on mitochondrial respiration and growth. Longer feeding periods along with studies focused on gut mitochondrial activity

will facilitate in getting a better understanding of metabolism in Atlantic salmon. Few studies have been conducted on salmonids' mitochondrial activity and therefore, there is a need for further understanding of the complex processes that take place in cells.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Alne H, Oehme M, Thomassen M, Terjesen B and Rørvik K-A (2011) Reduced growth, condition factor and body energy levels in Atlantic salmon *Salmo salar* L. during their first spring in the sea. Aquaculture Research 42(2): 248–259.

Alne H, Thomassen MS, Takle H, Terjesen BF, Grammes F, Oehme M, Refstie S, Sigholt T, Berge RK and Rørvik KA (2009) Increased survival by feeding tetradecylthioacetic acid during a natural outbreak of heart and skeletal muscle inflammation in S0 Atlantic salmon, *Salmo salar* L. Journal of Fish Diseases 32: 953–961.

Brandt M (2010) Introduction to Absorbance Spectroscopy. https://www.rose-hulman.edu/~brandt/Fluorescence/Absorbance_Spectroscopy.pdf (accessed on 24 April 2018).

Brosnan JT (2000) Glutamate, at the interface between amino acid and carbohydrate metabolism. The Journal of Nutrition 130(4S Suppl): 988s–990s.

Burrells C, Williams PD and Forno PF (2001) Dietary nucleotides: a novel supplement in fish feeds 1. Effects on resistance to disease in salmonids. Aquaculture 199: 159–169.

Cheng Z, Buentello A and Gatlin DM (2011) Effects of dietary arginine and glutamine on growth performance, immune responses and intestinal structure of red drum, *Sciaenops ocellatus*. Aquaculture 319: 247–252.

Eigentler A, Draxl A, Wiethüchter A, Kuznetsov A V, Lassing B and Gnaiger E (2015) Laboratory protocol: citrate synthase a mitochondrial marker enzyme. Mitochondrial Physiology Network 17.04(03): 1–11.

FAO (2005) National Aquaculture Sector Overview - Norway. http://www.fao.org/fishery/countrysector/naso_norway/en. Accessed on 5 September 2018.

FAO (2018) The State of World Fisheries and Aquaculture-Meeting the Sustainable Development Goals. Food and Agriculture Organization of the United Nations, Rome, Italy.

Fasching M and Gnaiger E (2016) O2k quality control 2: instrumental oxygen background correction and accuracy of oxygen flux. http://wiki.oroboros.at/index.php/MiPNet14.06_Instrumentalo2Background (accessed on 22 May 2018).

Fasching M, Fontana-Ayoub M and Gnaiger E (2016) Mitochondrial respiration medium - MiR06. www.bioblast.at/index.php/MiPNet14.13_Medium-MiR06 (accessed on 15 February 2018).

Gnaiger E (2009) Capacity of oxidative phosphorylation in human skeletal muscle: new perspectives of mitochondrial physiology. The International Journal of Biochemistry and Cell Biology 41(10): 1837–1845.

Hasli PR (2015) Characterization of mitochondrial respiration and quality differences in diploid and triploid Atlantic salmon (*Salmo salar* L.) at 5°C, 10°C and 15°C. MSc thesis, Norwegian University of Life Sciences, Ås, Norway.

Kolarevic J, Takle H, Felip O, Ytteborg E, Selset R, Good CM, Baeverfjord G, Asgard T and Terjesen BF (2012) Molecular and physiological responses to long-term sublethal ammonia exposure in Atlantic salmon (*Salmo salar*). Aquatic Toxicology 124–125: 48–57.

Kuznetsov AV, Veksler V, Gellerich FN, Saks V, Margreiter R and Kunz WS (2008) Analysis of mitochondrial function in situ in permeabilized muscle fibers, tissues and cells. Nature Protocols 3(6): 965–976.

Larsson T, Koppang EO, Espe M, Terjesen BF, Krasnov A, Moreno HM, Rørvik K-A, Thomassen M and Mørkøre T (2014) Fillet quality and health of Atlantic salmon (*Salmo salar* L.) fed a diet supplemented with glutamate. Aquaculture 426–427: 288–295.

Li P, Mai K, Trushenski J and Wu G (2009) New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. Amino Acids 37(1): 43–53.

Lugert V, Thaller G, Tetens J, Schulz C and Krieter J (2016) A review on fish growth calculation: multiple functions in fish production and their specific application. Reviews in Aquaculture 8(1): 30–42.

Meijer AJ (2003) Amino Acids as Regulators and Components of Nonproteinogenic Pathways. The Journal of Nutri-

tion 133(6): 2057S–2062S.

Minarik P, Tomaskova N, Kollarova M and Antalik M (2002) Malate dehydrogenases--structure and function. General physiology & biophysics 21(3): 257–265.

Moriyama T and Srere PA (1971) Purification of Rat Heart and Rat Liver Citrate Synthases. The Journal of Biological Chemistry 246(10): 3217–3223.

Neu J, Shenoy V and Chakrabarti R (1996) Glutamine nutrition and metabolism: where do we go from here? The FASEB Journal 10(8): 829–837.

Oehme M, Grammes F, Takle H, Zambonino-Infante J-L, Refstie S, Thomassen MS, Rørvik K-A and Terjesen BF (2010) Dietary supplementation of glutamate and arginine to Atlantic salmon (*Salmo salar* L.) increases growth during the first autumn in sea. Aquaculture 310: 156–163.

Rørvik K-A, Alne H, Gaarder M, Ruyter B, Måseide NP, Jakobsen JV, Berge RK, Sigholt T and Thomassen MS (2007) Does the capacity for energy utilization affect the survival of post-smolt Atlantic salmon, *Salmo salar* L., during natural outbreaks of infectious pancreatic necrosis. Journal of Fish Diseases 30: 399–409.

Shepherd D and Garland PB (1969) The kinetic properties of citrate synthase from rat liver mitochondria. Biochemical Journal 114(3): 597–610.

Tapiero H, Mathe G, Couvreur P and Tew KD (2002) II. Glutamine and glutamate. Biomedicine & pharmacotherapy 56(9): 446–457.

Thorpe JE, Adams CE, Miles MS and Keay DS (1989) Some influences of photoperiod and temperature on opportunity for growth in juvenile Atlantic salmon, *Salmo salar* L. Aquaculture 82(1): 119–126.

Trichet VV (2010) Nutrition and immunity: an update. Aquaculture Research 41(3): 356–372.

Vadder FD, Kovatcheva-Datchary P, Zitoun C, Duchampt A, Backhed F and Mithieux G (2016) Microbiota-produced succinate improves glucose homeostasis via intestinal gluconeogenesis. Cell Metabolism 24: 151–157.

Walker JM (2002) The protein protocols handbook. Humana Press, Totowa, NJ.

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NM data collection; **NM & BE** data analysis; **NM** manuscript preparation; **REO & BE** research supervision