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Functional amino acids stimulate muscle development and improve fillet texture of Atlantic salmon

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Abstract

Sufficient firmness is essential for consumer appreciation and the suitability for processing of fish fillets. The objective of this study was to investigate the effect of functional amino acids (AA) on fillet texture and muscle development of Atlantic salmon. Triplicate net pens of 105 g salmon were fed a standard diet, or the same diet with added 15 g/kg arginine or 15 g/kg glutamate during a 5-month rearing period. The growth rate and FCR (0.91–0.92) showed no significant dietary effects (body weight 864–887 g). Glutamate supplementation resulted in delayed postmortem glycogen degradation (pH drop) and rigour development, along with improved fillet firmness and intercellular myofibre integrity. An in vitro study with salmon myosatellite cells showed that exogenous glutamine or arginine increased the expression of muscle growth markers (*myog*, *tnnl2*, *myl*) at both 8 and 16°C culture temperature. The expression of a marker for proteolysis (*ctsb*), *myl* and *myog* were highest for the glutamine treatment at 16°C. Significant interaction between exogenous AA and temperature indicated elevated AA requirement when growth is accelerated. It is concluded that AA from the glutamate family are vital for fillet firmness. The dispensable glutamine and glutamate appear more critical compared to arginine, particularly during high-performance periods.

KEYWORDS
amino acids, Atlantic salmon, Fillet quality, gene expression, muscle

1 | INTRODUCTION

Texture perception is an important factor in consumer sensory appreciation of Atlantic salmon fillets (Hyldig & Nielsen, 2001) and is also important for the suitability for processing (Mitchie, 2012). Muscle fibre arrangement, density and integrity are among antemortem factors that influence fillet texture (Fauconneau et al., 1993; Hatae, Yoshimatsu, & Matsumoto, 1984; Hurling, Rodell, & Hunt, 1996; Kiessling, Stien, Torslett, Suontarna, & Slinde, 2006; Mørkøre, Ruohonen, & Kiessling, 2009; Taylor, Fjaera, & Skjervold, 2002; Torgersen et al., 2014) and metabolic properties (Ingebrigtsen et al., 2014; Larsson et al., 2012, 2014; Taylor et al., 2002).

It has been assumed that animals and humans can synthesize sufficient amounts of dispensable amino acids. Growing evidence suggests that certain traditionally defined dispensable amino acids (e.g., glutamine and glutamate) play important roles in multiple signalling pathways. Hence, amino acids (AA), defined as regulators of key metabolic pathways, are now referred to as functional AA, including glutamate (Glu), glutamine (Gln) and arginine (Arg) that belong to the glutamate family (Wu, 2010). A deficiency of functional AA (either indispensable or dispensable) impairs not only protein synthesis, but also whole-body homoeostasis. Notably, supplementing Glu or Arg to a conventional diet (traditionally considered to provide adequate amounts of these AA) can improve growth potential in young land-living animals

(Wu, 2009) and salmon (Oehme et al., 2010). Furthermore, a recent study showed stimulated aerobic metabolism in salmon fed a Glu-supplemented diet that coincided with improved firmness and health-related parameters (Larsson et al., 2014).

Glu and Gln (considered as dispensable) have central metabolic roles and are interconvertible (Neu, Shenoy, & Chakrabarti, 1996; Tapiero, Mathe, Couvreur, & Tew, 2002b; Young & Ajami, 2001). In fish, this interconversion is important for controlling ammonia concentration, especially in the brain (Anderson, 2001; Terjesen, 2008). Glu functions as a neurotransmitter and as a precursor for γ aminobutyrate synthesis (Trudeau et al., 2000). Glu and Gln are indispensable for purine and pyrimidine synthesis (Li, Mai, Trushenski, & Wu, 2009) and are important energy sources in the intestine (Neu et al., 1996). A strong positive correlation was found between firmness and genes involved in responses to oxidative stress in salmon (Larsson et al., 2012). Glutamine is involved in the formation and function of the cytoskeleton and is a component of the antioxidant glutathione (Wu, 2010). Additionally, Ashraf and Foolad (2007) suggested that proline contributes to improved stress tolerance by stabilizing subcellular structures, scavenging free radicals and buffering cellular redox potential under stress conditions. Recent reports have also shown positive effect of L-ornithine in stress models (Miyake et al., 2014).

Arg belongs to the nutritionally semi-indispensable AA in fish, depending on the size or age of the fish (Li et al., 2009). Arg functions as a precursor for the synthesis of protein, nitric oxide, urea, polyamines, proline, Glu, creatine and agmatine and is important in cell proliferation, signalling and growth regulation in animals (Andersen et al., 2013; Ball, Urschel, & Pencharz, 2007; Flynn, Meininger, Haynes, & Wu, 2002; Morris, 2007; Wu & Morris, 1998). An Arg requirement of 21.2–21.6 g/kg dietary dry weight has been indicated for Atlantic salmon (Berge, Lied, & Sveier, 1997).

The overall objective of this study was to investigate the effect of dietary supplementation of Arg or Glu on texture, morphology of skeletal muscle cells, cathepsin activity, pH development and rigour contraction rate of Atlantic salmon fillets. Secondly, an in vitro model system was set up to study muscle cell growth pattern, metabolism and expression of genes related to growth, extracellular matrix and proteolysis in muscle cells cultured in a growth media supplemented with Arg or Gln. The cells were cultured at two different temperatures to simulate different rearing temperatures.

2 | MATERIALS AND METHODS

2.1 | In vivo study

2.1.1 | Fish feeding trial

The feeding trial was performed at Nofima Averøy Research Station. The fish were treated as production fish up to sacrifice. The feeding trial was conducted in net pens in sea water from 15th of April to 15th of September 2009. The water temperature at 3-m depth averaged 12°C during the trial (in the range 6–16.3°C). The day before commencing the experiment, Atlantic salmon smolts (*Salmo salar* L.) with an average weight of 105 g were transferred to nine net pens with a volume of 125 m^3 (33 ppt. salinity), with 500 smolts per net pen. Three experimental diets were fed to randomly distributed cages in triplicate per treatment.

2.1.2 | Feed and feed composition

The fish were fed a commercial extruded dry feed (3- and 7-mm pellets) manufactured by Skretting AS Stavanger, Norway (Control group), or the same feed coated with 15 g/kg L-arginine (Arginine group) or 15 g/kg L-glutamate (Glutamate group; Table 1). The experimental diets were prepared by coating the Control diet with arginine (Fenchem Biotek Ltd, Nanjing, China) or glutamate (Meihua Holdings Group Co., Ltd, Hebei, China), respectively, by dissolving these enantiomer forms of the AA in 36 g/kg distilled water (70°C, 0.43 kg/L). The Control diet was coated with the same amount of distilled water (36 g/kg, 70°C). Each diet (Control, Glutamate and Arginine) was then dried on a tray for 1 day and thereafter coated with rapeseed oil (20 ml/kg, 70°C) to avoid possible taste differences and leakage of the coated AA. The stability of pellets has been quality assured by Dessen, Arge, Thomassen, and Rørvik (2016). The fish were fed in excess of recorded feed intake with automatic feeders. Uneaten pellets were collected after each meal as described by Einen, Mørkøre, Rora, and Thomassen (1999), and feed recovery was tested according to Helland, GrisdaleHelland, and Nerland (1996).

2.1.3 | Chemical analysis of the feed

The pellets were grinded before analysis of AA using a Biochrom 30 AA analyser (Biochrom LTD Cambridge, UK). Free AA and nitrogenous compounds were analysed after deproteinizing the samples with sulphosalicylic acid and filtering (0.22 μm Ultrafree CL; Davis, 2002). Prior to total AA analysis of the feeds, tryptophan and tyrosine were hydrolysed with 4.2 ~~mol/L~~ NaOH (Hugli & Moore, 1972), and the remaining AA were hydrolysed with 6 ~~mol/L~~ HCl (Davis, 2002). Analyses of total AA in the feed and free AA were performed to confirm added levels. It appeared that glutamate quantification was unreliable using only total AA analysis. Presumably, glutamate was partially degraded during acid hydrolysis, so the gentler sample treatment involved in the free AA analysis resulted in higher glutamate recovery. The feeds were analysed for dry matter (105°C until constant weight), ash (550°C until constant weight) and nitrogen (Kjeltec Auto System, Moline, Illinois, USA). Crude fat was determined by the Folch method (Folch, Lees, & Sloane Stanley, 1957), and starch was analysed as glucose after enzymatic hydrolysis employing a commercial kit (K-TSTA 05/06, Megazyme, Australia).

2.1.4 | Fish sampling

At harvest in September 2009 (average body weight 0.86 kg), six fish from each net pen (18 fish per dietary treatment) were killed by percussive stunning, gill cut, bled in a tank with running sea water

TABLE 1 Nutritional composition of the experimental diets (3- and 7-mm pellets)

	3 mm			7 mm		
	Control	Glutamate	Arginine	Control	Glutamate	Arginine
Proximate composition (dry matter basis)						
Dry matter (g/kg)	912	912	915	889	897	895
Crude lipid (g/kg)	275	274	269	374	370	369
Crude protein[a] (g/kg)	514	518	529	435	438	459
Ash (g/kg)	72	76	75	56	65	52
Starch (g/kg)	59	57	60	82	79	77
Energy (MJ/kg)	25.2	25.1	25.2	27.2	27.0	27.1
Astaxanthin (mg/kg)	63	65	66	45	45	46
Total dispensable amino acids[b] (g/kg)						
Ala	23.3	23.9	23.8	19.0	18.7	18.5
Ammonium[c]	29.0	29.4	29.8	25.4	24.5	25
Asx[d]	37.7	38.3	38.3	31.5	31.1	30.8
Cys	4.1	4.1	4.2	3.7	3.6	3.7
Glx[e]	64.8	75.7	65.6	57.2	66.4	55.7
Gly	21.8	22.3	22.2	18.5	18.1	18.2
Pro	16.8	17.8	17.5	18.8	19.9	19.2
Ser	17.2	17.5	17.4	15.3	15	14.8
Tyr	14.0	13.9	13.9	11.6	11.6	11.4
Total indispensable amino acids[b] (g/kg)						
Arg	28.2	28.6	39.6	24.2	23.8	34.9
His	9.5	9.7	9.7	8.4	8.2	8.2
Ile	18.1	18.6	18.6	16.1	15.5	15.6
Leu	30.4	31.0	31.2	26.9	26.3	26.3
Lys	30.3	31.0	31.0	24.9	24.3	24.2
Met	11.8	11.8	12.0	9.4	9.2	9.2
Phe	18.4	18.7	18.7	17	16.8	16.5
Thr	16.9	17.1	17.0	14.2	14.0	13.8
Trp	4.9	4.7	4.7	4.0	4.1	4.1
Val	21.1	22.2	22.4	19.1	18.4	18.3
Free dispensable amino acids[b] (g/kg)						
Ala	1.47	1.57	1.55	2.02	2.02	2.02
Ammonia[c]	0.04	0.04	0.04	0.05	0.05	0.05
Anserine[c]	0.40	0.38	0.38	0.00	0.00	0.00
Asn	0.00	0.00	0.00	0.43	0.43	0.46
Asp	0.31	0.34	0.34	0.74	0.70	0.75
Glu	1.24	14.78	1.50	1.77	16.34	1.70
Gly	0.97	1.02	1.02	0.97	0.95	0.98
Ornithine[c]	0.08	0.08	0.11	0.25	0.26	0.26
Phosphoethanolamine[c]	0.00	0.00	0.00	0.00	0.00	0.00
Phosphoserine[c]	0.13	0.14	0.15	0.18	0.18	0.17
Pro	0.38	0.31	0.42	0.32	0.34	0.35
Ser	0.36	0.39	0.41	0.59	0.59	0.61
Taurine[c]	4.05	4.27	4.22	4.75	4.63	4.68
Tyr	0.37	0.37	0.37	0.78	0.74	0.76

(Continues)

TABLE 1 (Continued)

	3 mm			7 mm		
	Control	Glutamate	Arginine	Control	Glutamate	Arginine
Free indispensable amino acids[b] (g/kg)						
Arg	0.59	0.62	11.37	1.91	1.53	17.59
His	0.64	0.70	0.70	0.63	0.64	0.69
Ile	0.45	0.49	0.50	0.76	0.82	0.78
Leu	1.07	1.26	1.25	2.02	1.96	1.97
Lys	0.96	1.02	1.01	1.59	1.50	1.55
Met	0.45	0.51	0.55	0.67	0.64	0.65
Phe	0.55	0.67	0.63	1.02	0.96	0.99
Thr	0.51	0.53	0.55	0.92	0.86	0.91
Val	0.57	0.62	0.60	1.06	1.03	1.07

[a]Nx6.25.
[b]Amino acids given as dehydrated residues.
[c]Not corrected for water molecule, given as hydrated residues.
[d]Asx represents Asp and Asn.
[e]Glx represents Glu and Gln.

and hand-filleted within 0.5 hr postmortem. The leftmost fillet sides were placed on solid trays on ice in closed styrofoam boxes. The fillet length was recorded immediately after filleting and after 1, 3, 6, 9, 12, 24, 48 and 72 hr postmortem, to monitor rigour contraction of fillets (length decrease, cm). Muscle was sampled 1 hr postmortem for histology and enzyme activity analyses, anterior to the dorsal fin above the lateral line, 1 hr postmortem, and muscle pH was analysed in the same area at 1, 6, 12, 24, 48 and 72 hr postmortem. The rightmost fillet sides were packed individually in sealed plastic bags and stored on ice until texture analyses 72 hr postmortem. The muscle samples for enzyme activity measurements were quickly frozen in liquid nitrogen before storing at −80°C until further analyses.

2.1.5 | Fat content and muscle pH

Fillet fat content was analysed in the cutlet between the posterior part of the dorsal fin and the gut by image analysis (Folkestad et al., 2008). pH analyses were performed in the dorsal fillet part using a pH meter, 330i SET (Wissenschaftlich-Technische-Werkstätten GmbH, Weilheim, Germany), connected to an BlueLine 21 electrode (Schott Instruments Electrode, SI Analytics GmbH, Mainz, Germany) and TFK 325 temperature compensator (Wissenschaftlich-Technische-Werkstätten GmbH, Weilheim, Germany).

2.1.6 | Instrumental texture analyses

The instrument used was a texture analyser TA-XT2 (Stable Micro Systems Ltd, Surrey, UK) equipped with a flat-ended cylindrical probe (12.5 mm diameter, type p/0.5) and a 30-kg load cell. The test speed was 1 mm/s. Firmness was recorded as the force required to puncture the fillet surface (breaking force) below the dorsal fin (average of triplicate analyses).

2.1.7 | Cathepsin B (CTSB), cathepsin L (CTSL) and CTSB+CTSL activity

Analyses were determined according to Bahuaud et al. (2009) by homogenizing 300 mg of muscle in 900-ml extraction buffer (100 mmol/L sodium acetate in 2 ml/L Triton X-100, pH 5.5) in a Precellys 24 homogenizer (Bertin Technologies, France) at 5,500 rpm two times 20 s. The homogenates were centrifuged at 16,000 × *g* for 30 min, and the cathepsin activity was analysed fluorimetrically in the supernatants (Kirschke, Wood, Roisen, & Bird, 1983). N-CBZ-L-phenylalanyl-L-arginine-7- amido-4-methylcoumarin (Z-Phe-Arg-Nmec) was used as a substrate for CTSB + CTSL, and N-CBZ-L-arginyl-L-arginine-7-amido-4-methylcoumarin (Z-Arg-Arg-Nmec), for CTSB. CTSL activity was obtained by subtracting the result of CTSB activity from the result of CTSB + CTSL activity. All samples were analysed in triplicates.

2.1.8 | Histology

Histological samples were prepared as described by Bahuaud et al. (2009). A Leica CTR 6000B (Leica Microsystems, Nusscloch, Germany) with a digital camera (Evolution MP Color, Media Cybernetics Inc., Silver Spring, MD) was used to take micrographs for counting the relative number of myofibres. All image acquisitions were controlled by Image Pro Plus 4.0 software (Media Cybernetics, Silver Spring, MD). The relative number of myofibres and myofibre–myofibre detachments in muscle samples from fish fed the different diets were counted.

2.2 | In vitro study

2.2.1 | Muscle cell isolation

Myosatellite cells were isolated and cultured as previously described by Vegusdal, Østbye, Tran, Gjøen, and Ruyter (2004). After 2 days of

culturing, the cells were trypsinated by washing twice in PBS, before trypsin–EDTA (Sigma-Aldrich, St. Louis, USA) was added to loosen the cells. The cells were then resuspended in growth media (GM; 900 ml/L L15-Glutamax; Invitrogen, Carlsbad, USA) containing 10% FBS (Sigma-Aldrich), 0.01 mol/L HEPES (Sigma-Aldrich), 10 ml/L antibiotic–antimycotic solution (10 mg/ml streptomycin and 25 μg/ml amphotericin B [Sigma-Aldrich]) and reseeded in laminin-coated cell flasks for gene expression analysis and on coverslips for immunocytochemistry.

2.2.2 | Incubation with AA

At confluent stage, the muscle cells were transferred to either 8 or 16°C and added one of three GM: (i) Control: arginine at 0.3 g/L and glutamine at 0.5 g/L; (ii) Arginine: 0.6 g/L arginine and 0.5 g/L glutamine; and (iii) Glutamine: 0.3 g/L arginine and 1 g/L glutamine. Standard L-15 medium contains 0.5 g/L of glutamine (and no glutamate). The cells were cultured at two temperatures (either 8 or 16°C) to study temperature effect (simulated different rearing temperatures). Temperature effects were investigated per se and also interactions between culturing temperature and exogenous AA concentrations in the GM. All treatment groups consisted of four parallels (n = 4). The initial temperature of 12°C was gradually raised or reduced over a period of 4 hr. The cells cultured at 16°C were harvested after 4 days (64 day degrees; temperature × days), whereas the cells cultured at 8°C were harvested after 8 days (64 day degrees). Media samples were immediately frozen at −80°C after harvesting, whereas the cells were first washed twice in PBS, homogenized using Qiashredder Spin Column (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol and frozen at −80°C.

2.2.3 | Metabolite profile in growth media

Samples of media from cells (1.5 ml) were freeze-dried and dissolved in 1.0 ml PBS (1 mmol/L, pH7.4) with 0.5 mmol/L 3-(trimethylsilyl) propanesulfonic acid (DSS) as the chemical shift standard (0 ppm) and transferred to 5 mm NMR tubes. High-resolution ^{1}H-NMR spectra were recorded at room temperature on a Bruker Avance DRX500 spectrometer (Bruker GmbH, Bremen, Germany), using water presaturation in the relaxation delay. A sweep width of 6 kHz was collected into 48 k points. Number of scans was set to 512. The raw data were multiplied with a 0.5-Hz exponential line broadening factor before Fourier transformation into 32 k data points. All spectra were phased and baseline-corrected. Identification and quantification of metabolites were performed in Chenomx NMR software suite version 7.0 (Chenomx, Alberta, Canada). Prior to quantification, the native DSS line width (ca 4 Hz) was adjusted to reflect the narrower line width of other metabolites (ca 1.7 Hz). Comparison of composition of the different media was made by normalizing the spectra to phenylalanine (according to the product information at a concentration of 0.125 g/L [corresponding to 0.76 mmol/L] in L-15).

2.2.4 | Gene expression studies

The cells were washed twice in PBS, and RNA was isolated using RNeasy Mini Kit and DNaseI (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. Quantity and quality of RNA were evaluated using NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Delaware, USA). cDNA was synthesized from 0.25 μg RNA in a total volume of 25 μl using Taqman® Reverse Transcriptase Reagents (Applied Biosystems, Foster City, CA, USA) and oligod(T) primers according to the manufacturer's protocol. Quantitative PCR was performed in a LightCycler 480 Instrument (Roche Applied Science, Mannheim, Germany) with gene-specific primers (Table 2). Annealing temperature for all primers was 60°C. *rpol2* and *eif1a* were evaluated as reference genes using geNorm (Vandesompele et al., 2002), and *eif1a* was identified as the most stable. Quantitative PCR was performed according to Thomassen, Rein, Berge, Østbye, &

TABLE 2 Primers for gene expression analysis. *RNA polymerase 2 (rpol2)*, *elongation initiation factor 1a (eif1a)*, *myosin light chain (myl)*, *myogenin* (*myog*), *myogenic differentiation 1* (*myod1b*), *myogenic factor 6* (*myf6*), *troponinI2* (*tnnI2*), *actinin* (*actn*), *heparan sulphate proteoglycan 2* (*hspg2*), *collagen1a1* (*col1a1*), *periostin* (*postn*), *microfibrillar-associated protein 2* (*mfap2*), *filaminA* (flnA), *cathepsinB* (*ctsb*), *cathepsinL* (*ctsl*)

	GenBank accession number	Forward (5′–3′)	Forward (5′–3′)
rpol2	ca049789	taacgcctgcctcttcacgttga	tgagggaccttgtagccagcaa
eif1a	AF321836	caccaccggccatctgatctacaa	tcagcagcctccttctcgaacttc
myl	nm_001123716	ccatcaacttcaccgtcttcctcac	cagcccacaggttcttcatctcc
myog	DQ294029	attgagaggctgcaggcacttg	gtgcggtagtgtaagccctgtgtt
myod1b	aj557150	ccgcaacacgaagcaactattacagc	ggaaccctcctggcctgataacac
myf6	dq479952	cctttgtaccacgggaatgacagc	tgtcggtcggtgcagactttctt
tnnI2	dv670595	tccataccggccttgtcgtcaat	ggtgaccatggatttgagatccaacc
actn	aj534876	tgggctcaagctcatgttgctg	tccagggccttgttcacgttg
hspg2	FJ825137	tgcataccctcctctgacca	ttcatcttcttgcccacgct
col1a1	fj195608	gtgctattggtgagactgga	accatcgttaccagcagag
postn	bt071890	ccacgacatctcacgtccaa	cttggtgacggacggtactc
mfap2	nm_001141303	ttgaagagacgggtgctgac	aggtcaccagccaatgtagc
flnA	bt072731	tgagcctgaatggagcgaag	ccagggatgtgactgccatt
ctsb	dr696159	agggggaactccttactggct	cgatgccacagtggtccttacct
ctsl	cb502996	gtatagtgaaatgtgtgacc	aaccagagcaataattcaag

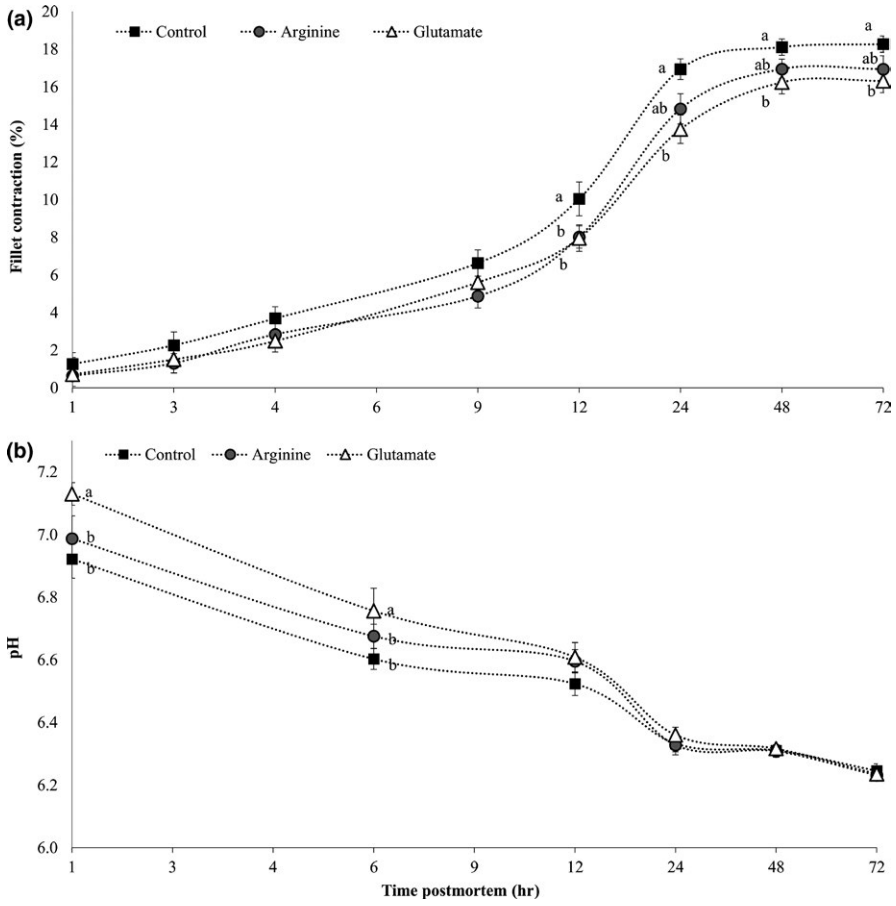

FIGURE 1 Fillet contraction (a) and pH development (b) during ice storage of Atlantic salmon. Results are given as mean ± *SEM* for salmon fed a basis diet (Control) or the same diet with added arginine (Arginine) or glutamate (Glutamate). Different letters indicate significant differences between dietary treatments within time point (n = 24)

Ruyter (2012). The relative gene expression level was calculated according to the ΔΔCt method with adjustment for differences in primer efficiency (Pfaffl, 2004).

2.2.5 | Cell proliferation assay

Assessment of cell proliferation was carried out by immunocytochemical detection of proliferating cell nuclear antigen (PCNA; ZYMED® Laboratories Inc, California, USA). PCNA staining was performed according to the manufacturer's protocol except for two additional steps; cells were washed with PBS and treated with 1 ml/L Triton X-100 (Merck Chemicals, Darmstadt, Germany) for 10 min to disrupt the cell membrane after blocking the endogenous peroxidase activity. Digital RGB images were taken (as described in the Histology section) of four discrete areas in each sample. The proliferating cells appeared as brown on a white background in the images, while the stable non-proliferating cells were stained blue. A MATLAB script (The Math-Works Inc., Massachusetts, USA) for automatic counting of the number of proliferating and non-proliferating cells in each image could therefore be constructed using the MATLAB® Image Processing Toolbox. In short, this automatic script consisted of seven succeeding steps: (i) flat-field correction (40) of the G-colour-layer to remove vignetting, (ii) median filtering (Gonzalez, Woods, & Eddins, 2004; MATLAB function medfilt2) to remove minor noise, (iii) extended-minima transform (Soille, 1999; MATLAB function imextendedmin) to segment the dark cell regions from the white background, (iv) morphological operations (Gonzalez et al., 2004; MATLAB function imopen and bwdist) to remove very small objects and very narrow objects from the segmentation, (v) labelling remaining connected components (Gonzalez et al., 2004; MATLAB function bwlabel) as discrete cells, (vi) classifying segmented cells with higher intensity values in the R-layer than in the B-layer as brown and the others as blue, (vii) counting the number of cells in each class (MATLAB function regionprops).

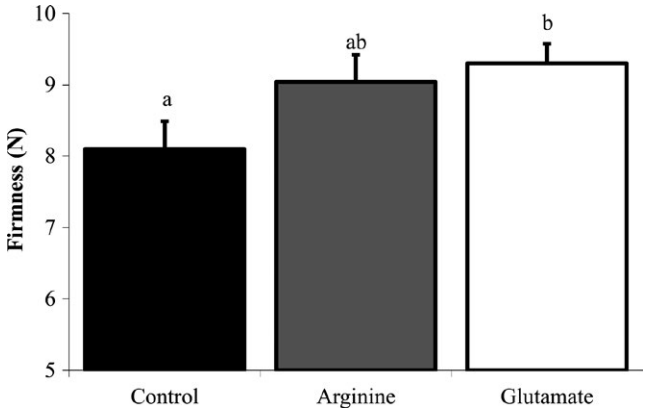

FIGURE 2 Firmness (N) of Atlantic salmon fillets. The fillets were stored on ice for 72 hr. Results are given as mean ± *SEM* for salmon fed a basis diet (Control) or the same diet with added arginine (Arginine) or glutamate (Glutamate). Different letters indicate significant differences between dietary treatments (n = 24)

2.2.6 | Statistics

Statistical analyses were performed using SAS, version 9.3 for Windows (SAS Institute Inc., Cary, NC, USA). Exogenous nutritional effects were analysed by one-way analysis of variance (ANOVA) using the GLM procedure. The dependent variables fillet contraction, pH, firmness and percentage detachment were tested using this method, followed by Duncan's multiple comparison test. Relative gene expression, cell number count and media concentration of AA were tested by the use of two-way ANOVA, with AA treatment and incubation temperature as fixed effects and their interaction. Tests of simple effect of AA treatment within each incubation temperature were also conducted. The level of significance was chosen at $p \leq .05$, and the results are presented as means ± standard error of the mean (*SEM*).

3 | RESULTS

3.1 | In vivo study

3.1.1 | Growth

The average body weight increased from 105 to 876 g during the experimental period of 5 months. The body weight of the Control group (863.6 ± 29.7 g) did not differ significantly from the Glutamate (878.0 ± 9.9 g) or Arginine group (887.3 ± 21.5 g).

3.1.2 | Fillet contraction

The fillets contracted rapidly during the initial 24 hr of storage, averaging 16.9%, 14.8% and 13.7% for the Control, Arginine and Glutamate groups, respectively (Figure 1a). Thereafter, the contraction rate decreased, reaching a final contraction after 72-hr storage of 18.3%, 16.9% and 16.3% for the Control, Arginine and Glutamate groups, respectively. The Glutamate group had a significantly lower fillet contraction than the Control group after 12-hr ($p = .03$), 24-hr ($p = .002$), 48-hr ($p = .02$) and 72-hr ($p = .02$) postmortem. The Arginine group only showed a significantly lower contraction ($p = .03$) compared to the Control group after 12 hr postmortem.

3.1.3 | Fat content and muscle pH

The fillet fat content was 139 ± 5 g/kg on average with no significant differences between the fish groups. All treatment groups showed a gradually declining pH with time, from an initial pH of 6.9–7.1 (1 hr) to a final pH of 6.2 (72 hr) on average (Figure 1b). The fillets of the Glutamate group had significantly higher muscle pH compared to the Control group at 1 hr ($p = .04$) and 6 hr ($p = .03$) postmortem. No significant difference between the Control group and the Arginine group was found.

3.1.4 | Fillet firmness

Instrumental texture analyses of fillets stored for 72 hr revealed that the glutamate supplementation resulted in significantly ($p = .02$) firmer (9.5 N) fillets, compared with the Control group (8.1 N; Figure 2). Numerically the firmness was higher in the Arginine group (9.1 N) compared with the Control group, but the difference was not statistically significant ($p = .10$).

3.1.5 | Histological analysis

The myofibre density did not differ significantly between the dietary groups (data not shown), but the frequency of myofibre–myofibre

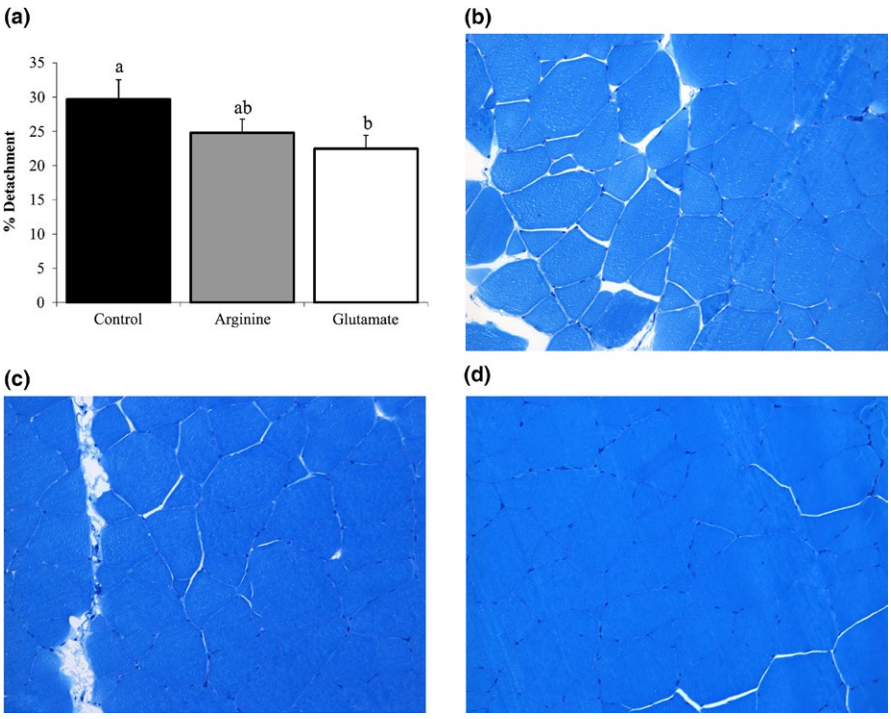

FIGURE 3 Myofibre–myofibre detachment of Atlantic salmon fillets 1 hr postmortem. Percentage of myofibre–myofibre detachment (a) and light microscopy observations of muscle samples from salmon fed a basis diet (Control) (b) or the same diet with added arginine (Arginine) (c) or glutamate (Glutamate) (d). Results are given as mean ± *SEM*. Different letters indicate significant differences between dietary treatments ($n = 24$). Magnification ×20

TABLE 3 Relative gene expression in muscle cells cultured in growth media supplemented with arginine or glutamine and cultured at two temperatures (8 and 16°C)

	8°C			16°C			Two-way ANOVA		
	Control	Arginine	Glutamine	Control	Arginine	Glutamine	Treatment	Temperature	Interaction
Markers for muscle growth									
myod1b	0.0 ± 0.4	−0.6 ± 0.5	0.3 ± 0.3	0.7 ± 0.5	0.4 ± 0.1	1.1 ± 0.2	$p \leq .05$	$p < .001$	NS
myf6	0.0 ± 0.4	−1.4 ± 0.3	−0.5 ± 0.4	−0.3 ± 0.3	−1 ± 0.3	−0.4 ± 0.3	$p < .0001$	NS	NS
myog	0.0 ± 0.1	0.6 ± 0.0	0.6 ± 0.1	0.1 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	$p < .0001$	$p \leq .05$	$p \leq .05$
actn	0.0 ± 0.9	0.3 ± 0.4	0.7 ± 1.7	1.4 ± 0.6	1.6 ± 1	1.9 ± 1	NS	$p \leq .05$	NS
tnnl2	0.0 ± 1.3	2.5 ± 0.4	2.3 ± 0.7	−0.1 ± 0.5	2.5 ± 0.3	2.4 ± 1.2	$p < .0001$	NS	NS
myl	0.0 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.9 ± 0.2	1.2 ± 0.1	$p < .0001$	$p < .0001$	$p \leq .05$
Markers for extracellular matrix									
col1a1	0.0 ± 1.1	−0.6 ± 0.6	1 ± 1.0	0.8 ± 1.1	0.1 ± 1.1	0.8 ± 1.2	$p \leq .05$	NS	NS
flnA	0.0 ± 0.6	−0.5 ± 0.2	0.2 ± 0.5	0.1 ± 0.5	−0.5 ± 0.7	−0.3 ± 0.5	NS	NS	NS
postn	0.0 ± 0.3	−0.4 ± 0.2	0.3 ± 0.3	−0.5 ± 0.4	−0.8 ± 0.6	−0.4 ± 0.3	$p \leq .05$	$p \leq .05$	NS
hspg2	0.0 ± 0.6	−0.3 ± 0.2	0.2 ± 0.4	0.7 ± 0.5	0.5 ± 0.8	0.8 ± 0.5	NS	$p \leq .05$	NS
mfap2	0.0 ± 0.8	−0.9 ± 0.6	−0.3 ± 0.5	0.2 ± 0.9	−0.7 ± 0.4	0.5 ± 0.7	$p \leq .05$	NS	NS
Markers for proteolysis									
ctsb	0.0 ± 0.4	−0.3 ± 0.2	0.2 ± 0.2	−0.2 ± 0.4	−0.2 ± 0.2	0.4 ± 0.3	$p \leq .05$	NS	NS
ctsl	0.0 ± 0.6	−0.4 ± 0.9	−0.2 ± 0.7	1.6 ± 0.3	1.3 ± 0.6	1.5 ± 0.5	NS	$p < .0001$	NS

The two-way ANOVA statistics are shown with *p*-values for the effect of exogenous amino acid treatment (glutamine and arginine), incubation temperature (8 or 16°C) and interaction between treatment and temperature. NS: not significant ($p > .05$). Data are shown as mean ± *SEM* ($n = 4$ per treatment).

detachments was significantly ($p < .05$) lower in the Glutamate group (23% ± 2) compared to the Control group (30% ± 3; Figure 3a–d). Myofibre–myofibre detachments of the Arginine group (25% ± 2) did not differ significantly from the other dietary fish groups.

3.1.6 | Cathepsin activity

Activities of CTSB (range 0.57–0.65 ± 0.06 mU/g muscle), CTSL (range 1.07–1.18 mU/g muscle) and CTSB+CTSL (range 1.71–1.72 ± 0.14 mU/g muscle) showed no significant difference between the dietary treatments.

3.2 | In vitro study

3.2.1 | Gene expression analysis

The gene expression analysis and statistical overview are shown in Table 3. The supplementation of arginine or glutamine significantly increased the gene expression levels of *myl*, *tnnl2* and *myog* at both 8 and 16°C compared with the Control group, and a significant interaction was observed between treatment and temperature for *myl* and *myog*. There was no significant differences in gene expression of *myl*, *tnnl2* and *myog* between Arginine or Glutamine at 8°C, but Glutamine showed the highest gene expression level of *myl* ($p < .05$) and *myog* ($p < .05$) at 16°C. Gene expression of *myod1b* and *myf6* was significantly higher in the Glutamine compared to the Arginine ($p < .05$) treatment, but no significant differences were detected between Control and Glutamine or Control and Arginine treatments. The expression of *actn* was not affected by treatment, but both *myod1b* and *actn* expressions were significantly affected by the temperature.

At 8°C, Glutamine had higher gene expression of *col1a1* ($p < .05$), *flnA* ($p < .05$) and *postn* ($p < .05$) compared to Arginine, but not to Control, whereas no differences in expression of these genes were found at 16°C. Gene expression of *mfap2* showed effect of treatment at 16°C with higher level in Glutamine compared to Arginine ($p < .05$). Glutamine showed highest gene expression of *ctsb* at 16°C ($p < .05$), but not at 8°C. Temperature had a significant effect on *postn* and *hspg2* gene expressions. Gene expression of *ctsl* at 16°C was higher in Glutamine compared to Arginine ($p < .05$), but not when compared to Control. Gene expression of *ctsl*, but not *ctsl*, was significantly higher at 16°C compared to 8°C culturing temperature.

3.2.2 | Muscle cell proliferation

Percentage of proliferating cells was significantly higher in muscle cells supplemented with glutamine and arginine cultivated at 16°C compared with 8°C (Figure 4), but no significant differences were observed between the treatment groups.

3.2.3 | Metabolite profile in cell culture growth media

^{1}H-NMR analyses of the cell culture growth media (GM) after 64 day degrees culturing (4–8 days) confirmed increased level of arginine and

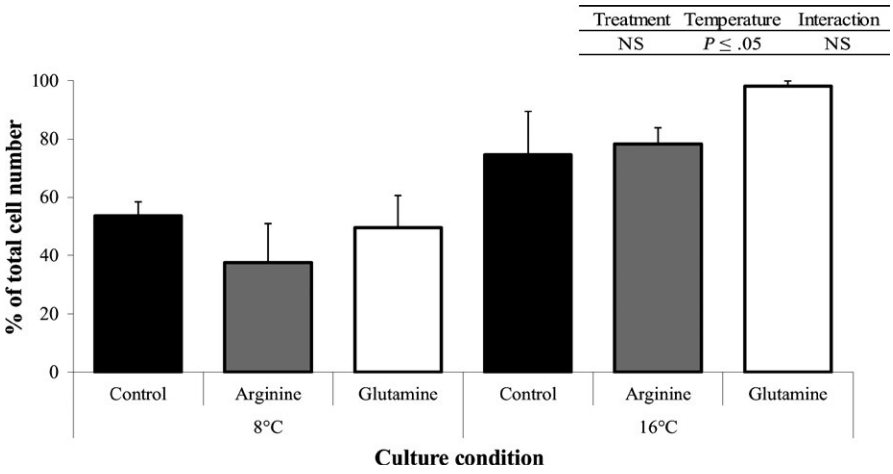

FIGURE 4 Percentage of proliferating muscle cells of total number of muscle cells cultured at either 8 or 16°C in a standard growth media (GM; Control) or the same GM supplemented with arginine (Arginine) or glutamine (Glutamine). Two-way ANOVA statistics are shown with *p*-values for the effect of exogenous amino acid treatment, incubation temperature and interaction between treatment and temperature. NS: not significant ($p > .05$). Data are shown as mean ± *SEM* ($n = 4$ per treatment)

glutamine in the GM supplemented with these AA (Table 4). The arginine level in Arginine was consistently highest ($p < .05$), while the level in the GM of the Control and Glutamine treatments was similar and independent of incubation temperature. Glutamine supplementation resulted in significantly increased glutamine level in the GM ($p < .05$), with a higher level at 16°C compared with 8°C. The glutamine level in the GM was consistently higher in the Control compared with the Arginine treatment ($p < .05$). The glutamate level in GM of the Arginine treatment was significantly lower compared with the Control treatment at 8°C ($p < .05$) and significantly lowest at 16°C. A significant overall correlation was observed between the level of glutamate and arginine in the GM ($r = -.90$; $p = .02$). The level of alanine and isoleucine in the GM was significantly highest for the Control treatment at both incubation temperatures ($p < .05$). The level of methionine in the GM was significantly lower for the Arginine treatment at 8°C compared to Control and Glutamine treatments ($p < .05$). No significant effect of exogenous AA was observed for glycine, histidine, leucine, lysine, phenylalanine, tyrosine or valine.

To examine the effect of exogenous arginine and glutamine on energy metabolism, selected metabolites from the tricarboxylic acid cycle, in addition to lactate and galactose, were investigated. Galactose was significantly lowest in the GM of the Glutamine (2.93 mmol/L) compared with the Control (3.01 mmol/L) and Arginine (3.42 mmol/L) treatments at 16°C incubation temperature. Formate, lactate, pyruvate and succinate showed no significant differences in the GM between the exogenous AA treatments (data not shown).

4 | DISCUSSION

The growth rate and feed utilization were not affected by the dietary AA composition, but dietary supplementation of glutamate

TABLE 4 Metabolite profile in growth media

	8°C			16°C			Two-way ANOVA		
	Control	Arginine	Glutamine	Control	Arginine	Glutamine	Treatment	Temperature	Interaction
Alanine	3.42 ± 0.51	2.87 ± 0.24	2.92 ± 0.07	3.75 ± 0.12	3.27 ± 0.17	3.01 ± 0.22	$p \leq .05$	$p \leq .05$	NS
Arginine	2.68 ± 0.25	5.57 ± 0.12	2.54 ± 0.22	2.44 ± 0.11	5.52 ± 0.23	2.53 ± 0.01	$p \leq .05$	NS	NS
Glutamate	0.57 ± 0.03	0.46 ± 0.02	0.51 ± 0.07	0.54 ± 0.02	0.44 ± 0.02	0.53 ± 0.00	$p \leq .05$	NS	NS
Glutamine	0.52 ± 0.07	0.13 ± 0.00	3.93 ± 0.08	0.82 ± 0.04	0.13 ± 0.01	4.50 ± 0.24	$p \leq .05$	$p \leq .05$	$p \leq .05$
Glycine	2.55 ± 0.38	2.48 ± 0.14	2.56 ± 0.12	2.46 ± 0.13	2.70 ± 0.19	2.84 ± 0.30	NS	NS	NS
Histidine	0.92 ± 0.13	0.99 ± 0.03	0.88 ± 0.05	0.95 ± 0.06	0.96 ± 0.05	0.95 ± 0.05	NS	NS	NS
Isoleucine	1.70 ± 0.16	0.94 ± 0.10	0.97 ± 0.04	1.49 ± 0.45	1.00 ± 0.04	0.92 ± 0.01	$p \leq .05$	NS	NS
Leucine	1.10 ± 0.08	1.06 ± 0.02	1.05 ± 0.08	1.01 ± 0.04	1.04 ± 0.04	1.03 ± 0.00	NS	NS	NS
Lysine	0.37 ± 0.02	0.36 ± 0.01	0.33 ± 0.05	0.35 ± 0.02	0.34 ± 0.02	0.34 ± 0.00	NS	NS	NS
Methionine	0.53 ± 0.05	0.40 ± 0.04	0.47 ± 0.03	0.45 ± 0.05	0.45 ± 0.03	0.49 ± 0.02	$p \leq .05$	NS	$p \leq .05$
Tyrosine	1.16 ± 0.06	1.18 ± 0.05	1.22 ± 0.07	1.10 ± 0.02	1.17 ± 0.01	1.14 ± 0.01	NS	NS	NS
Valine	0.74 ± 0.05	0.77 ± 0.06	0.82 ± 0.02	0.83 ± 0.05	0.88 ± 0.03	0.85 ± 0.00	NS	$p \leq .05$	NS

Concentration (mmol/L) of amino acids in muscle cell culture media 64 day degrees after incubation with the experimental growth media Control, Arginine and Glutamine. The culture media were analysed by ^{1}H NMR spectroscopy. The statistics are presented as *p*-values for exogenous AA treatment (glutamine and arginine), incubation temperature (8 or 16°C) and interaction between treatment and temperature. NS, not significant ($p > .05$). Data are shown as means ± *SEM* ($n = 4$).

significantly improved the fillet firmness. These results are in line with those of Larsson et al. (2014), who proposed that optimal dietary AA levels for growth may differ from optimal levels for good fish health and flesh quality. Furthermore, Li et al. (2009) stated that certain AA (glutamate, histidine and glycine) are associated with improved fillet taste and texture. The firmer texture of the Glutamate group coincided with higher muscle pH at slaughter, slower pH drop and rigour contraction, lower final rigour contraction and less detachments between the muscle fibres. The pattern was similar for the Arginine group, but the difference was not significantly different from the Control group.

The final pH reflects the total amount of glycogen stored in the living tissue (Love, 1988). Hence, the present results indicate no dietary effects on the in vivo glycogen concentration, as the ultimate pH was similar for all fish groups after 72-hr storage. Rapid decline in muscle pH during the first 6 hr postmortem (pH 7–6.7) suggests that most of the biochemical changes occurred immediately after death, as was also proposed by Hansen, Mørkøre, Rudi, Olsen, & Eie, (2007). The faster contraction rate and higher final contraction of the Control group probably reflect fast and continuous shortening of the sarcomeres in the myofibrils (actin and myosin). Previous studies have reported that dietary oil source (Mørkøre, 2006) and dietary vitamin E level (Ruff, FitzGerald, Cross, Teurtrie, & Kerry, 2002) significantly affect rigour contraction in fish, but no previous studies have reported effects of dietary levels of glutamate.

When salmon are subjected to exhausting exercise before slaughter, anaerobic energy production results in lactic acid accumulation in the muscle, accompanied by decreased initial postmortem pH, ATP level and accelerated rigour contraction (Mørkøre, Mazo, Tahirovic, & Einen, 2008). Several studies have reported an association between soft flesh and a rapid pH decline postmortem (Ang & Haard, 1985; Einen et al., 1999; Kiessling, Espe, Ruohonen, & Mørkøre, 2004; Sigholt et al., 1997) possibly due to decreased strength of the connective tissue (Lavety, Afolabi, & Love, 1988). Furthermore, fast drop in muscle pH should be avoided due to its association with faster development of negative odours and growth of spoilage bacteria (Hansen et al., 2012). The present dietary groups were subjected to the same harvesting procedure; hence, the higher initial pH of the Glutamate group indicates superior robustness of the fish to cope with stress during harvesting. Slower postmortem pH drop and rigour development were reported for salmon fed inclusion of krill protein, with high level of indispensable AA (Suontama, Kiessling, Melle, Waagbo, & Olsen, 2007). A higher pH may result from improved buffer capacity, and AA that constitute the "glutamate family", that is glutamine and glutamate with arginine, proline, histidine, and ornithine, have shown to be efficient in the regulation of acid–base homoeostasis (Davey, 1960; Newsholme et al., 2003; Tapiero et al., 2002b). In Atlantic cod, Førde-Skjærvik, Skjærvik, Mørkøre, Thomassen, & Rørvik, (2006) reported improved buffering postmortem and less fillet gaping in Atlantic cod fed a histidine-supplemented diet.

Torgersen et al. (2014) reported deterioration of connective tissue constituents such as collagen, perlecan and aggrecan and also large glycogen aggregates and enlarged intercellular areas in salmon with soft texture. The cyclization of glutamate produces proline, an AA that is important for the synthesis of collagen (Tapiero et al., 2002b). The in vitro cells study indicated a positive effect of glutamine over arginine supplementation on markers for connective tissue (*collagen*, *filamin A*, *periostin* at 8°C and *microfibrillar-associated-protein-2* at 16°C). Furthermore, temperature had a significant effect on selected connective tissue markers (*periostin* and *perlecan*). The biochemistry of the "glutamate family" AA has been relatively less studied in fish than in mammals (Tapiero et al., 2002b), but Dabrowski, Terjesen, Zhang, Phang, and Lee (2005) demonstrated proline synthesis from pyrroline-5-carboxylate in trout, and earlier 14C-tracer studies suggest that proline may be synthesized from glutamate in fish (Cowey, Adron, & Blair, 1970). However, further studies will be necessary to elucidate the exact biochemical pathways involved.

Fish muscle softening has been associated with higher degree of enlargement of existing fibres (hypertrophy) relative to recruitment of new fibres (hyperplasia; Zimmerman & Lowery, 1999) and degradation of myofibres and connective tissue by proteases like lysosomal cathepsin (Aoki & Ueno, 1997; Bahuaud et al., 2010; Yamashita & Konagaya, 1990, 1991). The present in vivo study showed no dietary effect on fibre size distribution or cathepsin activity, but the in vitro study showed positive effects of both AA and temperature on genes related to muscle growth regulation (*myod1b* and *myf6*) and structure (*myosin* and *troponin*). In addition, the Glutamine treatment resulted in significantly higher expression of the *cathepsin B* gene.

Mammalian studies have indicated that muscle growth due to arginine and glutamine supplementation is associated with their roles as precursors for several other AA as well as their direct effects on growth and gene expression regulation (Li et al., 2009; Meijer, 2003; Mommsen, 2001; Watford, 2008; Wu, 2009). Further, arginine and the metabolic products of arginine are greatly responsible for muscle growth (Blachier, Boutry, Bos, & Tome, 2009; Mommsen, 2001; Tapiero, Mathe, Couvreur, & Tew, 2002a) and particularly synthesis of proline (via ornithine and pyrroline-5-carboxylate) and polyamines (Tapiero et al., 2002a). In fish, arginine activates the release of glucagon, glucagon-like peptide-I, growth hormone, insulin-like factor I and somatostatins, which are growth-regulating molecules. Furthermore, the cyclic process of arginine results in the formation of polyamines, which are important for muscle growth regulators (Mommsen, 2001). Glutamine has been identified as an indispensable nutrient for cell survival and growth and in vitro cell proliferation (Eagle, Oyama, Levy, Horton, & Fleischman, 1956; Ehrensvard, Fisher, & Stjernholm, 1949; Newsholme et al., 2003; Watford, 2008; Wu, 2009). Larsson et al. (2012) reported both negative (argininosuccinate synthase, branched-chain AA aminotransferase, L-arginine:glycine amidinotransferase) and positive (pyrroline-5-carboxylate reductase) correlations between firmness of salmon fillets and expression of genes involved in glutamate, arginine and proline metabolism. The statistical analyses in the present study revealed that exogenous factors (AA treatment, incubation temperature) had a pronounced impact on the gene expression. The higher expression of *myl*, *tnnI2* and *myog* of the Glutamine treatment at 16°C, the significant interaction between AA treatment and temperature, and the higher percentage of proliferating cells may indicate that dietary in vivo supplementation of glutamine is superior in stimulating muscle growth during periods of high body growth. Expression of *cts*

also responded differently to exogenous treatment, where expression of *ctsb* was significantly increased in the glutamine-treated cells kept at 16°C, while incubation temperature at 8°C had no significant effect. *ctsl* on the contrary was only significantly affected by incubation temperature. Higher expression of *ctsb* of the glutamine-treated cells may seem unexpected because of the documented association with fillet softening postmortem (Bahuaud et al., 2010). However, Larsson et al. (2012) suggested that increased expression of *cts* in firm salmon fillets reflected rapid removal of abnormal proteins and thus contributed to maintenance of tissues in a good condition. Thus, the overall effect of the supplementations to the media may have been an increased protein synthesis (*myl* and *tnnl* upregulation), without a concomitant increase in degradation (stable *cts* expression), resulting in net protein accretion. Glutamate was not included in the in vitro cell trial. As glutamine might have different effects than glutamate, improved fillet texture by glutamate supplementation cannot be directly explained by the in vitro trial. New in vitro studies including both glutamine and glutamate supplementation are needed to confirm ~~in vivo~~ findings.

^{1}H-NMR analyses revealed significantly lower level of alanine and isoleucine in muscle cell GM of both the Arginine treatment and Glutamine treatment, compared to the Control treatment. The GM of the Arginine treatment also showed lower methionine level than the other groups. *myog*, *myl* and *tnnl* gene expressions were higher in the AA-supplemented groups, suggesting increased protein synthesis, and thereby increased incorporation and consumption of AA, leading to the lower levels of the indispensable isoleucine and methionine. The percentage of proliferating cells was not different between the groups at either of the two temperatures. This could indicate hypertrophic growth rather than hyperplastic growth. The level of other measured metabolites was, however, not significantly different between the three groups.

5 | CONCLUSION

The skeletal muscle development was stimulated and firmness was improved by selected exogenous functional AA from the glutamate family. Fish growth was not affected, suggesting that optimal dietary amino acid level for growth is not necessary optimal level for good flesh quality. It is concluded that satisfactory dietary availability of the functional AA glutamate/glutamine is required to obtain acceptable firmness of salmon fillets.

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AUTHOR CONTRIBUTIONS

T-K.K. Østbye and T. Mørkøre wrote the manuscript. T. Mørkøre, K-A. Rørvik, B. Terjesen and B. Ruyter designed the study. T-K.K. Østbye, T. Mørkøre, I. Standal, L. Stien, J-E. Dessen, D. Bahuaud and M. Latif did the analyses.

REFERENCES

Andersen, S. M., Holen, E., Aksnes, A., Ronnestad, I., Zerrahn, J. E., & Espe, M. (2013). Dietary arginine affects energy metabolism through polyamine turnover in juvenile Atlantic salmon (*Salmo salar*). *British Journal of Nutrition*, *110*, 1968–1977.

Anderson, P. M. (2001). Urea and glutamine synthesis: Environmental influences on nitrogen excretion. In P. A. Wright & P. M. Anderson (Eds.), *Fish physiology*, Vol. *20* (pp. 239–277). London, UK and New York, NY: Academic Press.

Ang, J. F., & Haard, N. F. (1985). Chemical composition and postmortem changes in soft textured muscle from intensely feeding Atlantic cod (*Gadius Morhua*, L). *Journal of Food Biochemistry*, *9*, 49–64.

Aoki, T., & Ueno, R. (1997). Involvement of cathepsins B and L in the postmortem autolysis of mackerel muscle. *Food Research International*, *30*, 585–591.

Ashraf, M., & Foolad, M. R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany*, *59*, 206–216.

Bahuaud, D., Mørkøre, T., Østbye, T. K., Torstensen, B., Veiseth-Kent, E., Ruyter, B., … Thomassen, M. S. (2010). Atlantic salmon (*Salmo salar* L.) flesh quality - role of lysosomal cathepsins B and L in muscle degradation. In P. Lacopo & M. Riemma (Eds.), *Salmon: Biology, nutrition and consumption* (pp. 1–60). Hauppauge, NY, USA: Nova Publishers.

Bahuaud, D., Ostbye, T. K., Torstensen, B. E., Rora, M. B., Ofstad, R., Veiseth, E., … Ruyter, B. (2009). Atlantic salmon (*Salmo salar*) muscle structure integrity and lysosomal cathepsins B and L influenced by dietary n-6 and n-3 fatty acids. *Food Chemistry*, *114*, 1421–1432.

Ball, R. O., Urschel, K. L., & Pencharz, P. B. (2007). Nutritional consequences of interspecies differences in arginine and lysine metabolism. *Journal of Nutrition*, *137*, 1626S–1641S.

Berge, G. E., Lied, E., & Sveier, H. (1997). Nutrition of Atlantic salmon (*Salmo salar*): The requirement and metabolism of arginine. *Comparative Biochemistry and Physiology Part A: Physiology*, *117*, 501–509.

Blachier, F., Boutry, C., Bos, C., & Tome, D. (2009). Metabolism and functions of L-glutamate in the epithelial cells of the small and large intestines. *American Journal of Clinical Nutrition*, *90*, 814S–821S.

Cowey, C. B., Adron, J., & Blair, A. (1970). Studies on the nutrition of marine flatfish the essential amino acid requirements of plaice and sole. *Journal of the Marine Biological Association of the United Kingdom*, *50*, 87–95.

Dabrowski, K., Terjesen, B. F., Zhang, Y., Phang, J. M., & Lee, K. J. (2005). A concept of dietary dipeptides: A step to resolve the problem of amino acid availability in the early life of vertebrates. *Journal of Experimental Biology*, *208*, 2885–2894.

Davey, C. L. (1960). The significance of carnosine and anserine in striated skeletal muscle. *Archives of Biochemistry and Biophysics*, *89*, 303–308.

Davis, M. (2002). *The Biochrom handbook of amino acids* (85 pp.). Cambridge: Biochrom.

Dessen, J. E., Arge, R., Thomassen, M. S., & Rørvik, K. A. (2016). Differences in fat accumulation between immature male and female Atlantic salmon *Salmo salar* after dietary administration of tetradecylthioacetic acid. *Journal of Fish Biology*, *89*, 2085–2097.

Eagle, H., Oyama, V. I., Levy, M., Horton, C. L., & Fleischman, R. (1956). The growth response of mammalian cells in tissue culture to L-glutamine and L-glutamic acid. *Journal of Biological Chemistry*, *218*, 607–616.

Ehrensvard, G., Fisher, A., & Stjernholm, R. (1949). Protein metabolism of tissue cells in vitro; the chemical nature of some obligate factors of tissue cell nutrition. *Acta Physiologica Scandinavica*, *18*, 218–230.

Einen, O., Mørkøre, T., Rora, A. M. B., & Thomassen, M. S. (1999). Feed ration prior to slaughter—a potential tool for managing product quality of Atlantic salmon (*Salmo salar*). *Aquaculture*, *178*, 149–169.

Fauconneau, B., Chmaitilly, J., Andre, S., Cardinal, M., Cornet, J., Vallet, J. L., … Laroche, M. (1993). Characteristics of Rainbow-Trout Flesh. 2. Physical and Sensory Aspects. *Sciences des Aliments*, *13*, 189–199.

Flynn, N. E., Meininger, C. J., Haynes, T. E., & Wu, G. (2002). The metabolic basis of arginine nutrition and pharmacotherapy. *Biomedicine & Pharmacotherapy*, *56*, 427–438.

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry*, *226*, 497–509.

Folkestad, A., Wold, J. P., Rorvik, K. A., Tschudi, J., Haugholt, K. H., Kolstad, K., & Morkore, T. (2008). Rapid and non-invasive measurements of fat and pigment concentrations in live and slaughtered Atlantic salmon (*Salmo salar* L.). *Aquaculture*, *280*, 129–135.

Førde-Skjærvik, O., Skjærvik, O., Mørkøre, T., Thomassen, M. S., & Rørvik, K.-A. (2006). Dietary influence on quality of farmed Atlantic cod (*Gadus morhua*): Effect on glycolysis and buffering capacity in white muscle. *Aquaculture*, *252*, 409–420.

Gonzalez, R., Woods, R., & Eddins, S. (2004) *Digital image processing using Matlab*. Upper Saddle River, NJ: Pearson/Prentice Hall.

Hansen, A. A., Mørkøre, T., Rudi, K., Olsen, E., & Eie, T. (2007). Quality changes during refrigerated storage of MA-packaged pre-rigor fillets of farmed Atlantic cod (*Gadus morhua* L.) using traditional MAP, CO2 emitter, and vacuum. *Journal of Food Science*, *72*, M423–M430.

Hansen, A. A., Rodbotten, M., Eie, T., Lea, P., Rudi, K., & Morkore, T. (2012). The effect of crowding stress on bacterial growth and sensory properties of chilled Atlantic salmon fillets. *Journal of Food Science*, *77*, S84–S90.

Hatae, K., Yoshimatsu, F., & Matsumoto, J. J. (1984). Discriminative characterization of different texture profiles of various cooked fish muscles. *Journal of Food Science*, *49*, 721–726.

Helland, S. J., GrisdaleHelland, B., & Nerland, S. (1996). A simple method for the measurement of daily feed intake of groups of fish in tanks. *Aquaculture*, *139*, 157–163.

Hugli, T. E., & Moore, S. (1972). Determination of tryptophan content of proteins by ion-exchange chromatography of alkaline hydrolysates. *Journal of Biological Chemistry*, *247*, 2828.

Hurling, R., Rodell, J. B., & Hunt, H. D. (1996). Fiber diameter and fish texture. *Journal of Texture Studies*, *27*, 679–685.

Hyldig, G., & Nielsen, D. (2001). A review of sensory and instrumental methods used to evaluate the texture of fish muscle. *Journal of Texture Studies*, *32*, 219–242.

Ingebrigtsen, I. A., Berge, G. M., Ruyter, B., Kjær, M. A., Mørkøre, T., Sørensen, M., & Gjøen, T. (2014). Growth and quality of Atlantic cod (*Gadus morhua*) fed with high and low fat diets supplemented with glutamate. *Aquaculture*, *433*, 367–376.

Kiessling, A., Espe, M., Ruohonen, K., & Mørkøre, T. (2004). Texture, gaping and colour of fresh and frozen Atlantic salmon flesh as affected by preslaughter iso-eugenol or CO2 anaesthesia. *Aquaculture*, *236*, 645–657.

Kiessling, A., Stien, L. H., Torslett, V., Suontarna, J., & Slinde, E. (2006). Effect of pre- and post-mortem temperature on rigor in Atlantic salmon muscle as measured by four different techniques. *Aquaculture*, *259*, 390–402.

Kirschke, H., Wood, L., Roisen, F. J., & Bird, J. W. (1983). Activity of lysosomal cysteine proteinase during differentiation of rat skeletal muscle. *Biochemical Journal*, *214*, 871–877.

Larsson, T., Koppang, E. O., Espe, M., Terjesen, B. F., Krasnov, A., Moreno, H. M., … Morkore, T. (2014). Fillet quality and health of Atlantic salmon (*Salmo salar* L.) fed a diet supplemented with glutamate. *Aquaculture*, *426*, 288–295.

Larsson, T., Morkore, T., Kolstad, K., Ostbye, T. K., Afanasyev, S., & Krasnov, A. (2012). Gene expression profiling of soft and firm Atlantic salmon fillet. *PLoS One*, *7*, e39219.

Lavety, J., Afolabi, O. A., & Love, R. M. (1988). The connective tissues of fish. *International Journal of Food Science & Technology*, *23*, 23–30.

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

Love, R. M. (1988). *The food fishes – Their intrinsic variation and practical implications*. New York, NY and London, UK: Farrand Press and Van Nostrand Reinhold.

Meijer, A. J. (2003). Amino acids as regulators and components of nonproteinogenic pathways. *Journal of Nutrition*, *133*, 2057s–2062s.

Mitchie, I. (2012). Causes of downgrading in the salmon farming industry. In S. C. Kestin & P. D. Warris (Eds.), *Farmed fish quality* (pp. 129–136). Oxford, UK: Oxford fishing new books.

Miyake, M., Kirisako, T., Kokubo, T., Miura, Y., Morishita, K., Okamura, H., & Tsuda, A. (2014). Randomised controlled trial of the effects of L-ornithine on stress markers and sleep quality in healthy workers. *Nutrition Journal*, *13*, 53.

Mommsen, T. P. (2001). Paradigms of growth in fish. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *129*, 207–219.

Mørkøre, T. (2006). Relevance of dietary oil source for contraction and quality of pre-rigor filleted Atlantic cod, *Gadus morhua*. *Aquaculture*, *251*, 56–65.

Mørkøre, T., Mazo, P. I., Tahirovic, V., & Einen, O. (2008). Impact of starvation and handling stress on rigor development and quality of Atlantic salmon (Salmon salar L). *Aquaculture*, *277*, 231–238.

Mørkøre, T., Ruohonen, K., & Kiessling, A. (2009). Variation in Texture of Farmed Atlantic Salmon (*Salmo salar* L). Relevance of Muscle Fiber Cross-Sectional Area. *Journal of Texture Studies*, *40*, 1–15.

Morris, S. M. Jr (2007). Arginine metabolism: Boundaries of our knowledge. *Journal of Nutrition*, *137*, 1602S–1609S.

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

Newsholme, P., Lima, M. M., Procopio, J., Pithon-Curi, T. C., Doi, S. Q., Bazotte, R. B., & Curi, R. (2003). Glutamine and glutamate as vital metabolites. *Brazilian Journal of Medical and Biological Research*, *36*, 153–163.

Oehme, M., Grammes, F., Takle, H., Zambonino-Infante, J. L., Refstie, S., Thomassen, M. S., … Terjesen, B. F. (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.

Pfaffl, M. W. (2004). Quantification strategies in real-time PCR. In S. A. Bustin (Ed.), *IUL biotechnology series*, *A–Z of quantitative PCR* (pp. 87–120). La Jolla, CA: International University Line.

Ruff, N., FitzGerald, R. D., Cross, T. F., Teurtrie, G., & Kerry, J. P. (2002). Slaughtering method and dietary alpha-tocopheryl acetate supplementation affect rigor mortis and fillet shelf-life of turbot *Scophthalmus maximus* L. *Aquaculture Research*, *33*, 703–714.

Sigholt, T., Erikson, U., Rustad, T., Johansen, S., Nordtvedt, T. S., & Seland, A. (1997). Handling stress and storage temperature affect meat quality of farmed-raised Atlantic salmon (*Salmo salar*). *Journal of Food Science*, *62*, 898–905.

Soille, P. (1999). *Morphological image analysis: Principles and applications*. Heidelberg, Germany: Springer-Verlag.

Suontama, J., Kiessling, A., Melle, W., Waagbo, R., & Olsen, R. E. (2007). Protein from Northern krill (*Thysanoessa inermis*), Antarctic krill (*Euphausia superba*) and the Arctic amphipod (*Themisto libellula*) can partially replace fish meal in diets to Atlantic salmon (*Salmo salar*) without affecting product quality. *Aquaculture Nutrition*, *13*, 50–58.

Tapiero, H., Mathe, G., Couvreur, P., & Tew, K. D. (2002a). I. Arginine. *Biomedicine & Pharmacotherapy*, *56*, 439–445.

Tapiero, H., Mathe, G., Couvreur, P., & Tew, K. D. (2002b). II. Glutamine and glutamate. *Biomedicine & Pharmacotherapy*, *56*, 446–457.

Taylor, R. G., Fjaera, S. O., & Skjervold, P. O. (2002). Salmon fillet texture is determined by myofiber-myofiber and myofiber-myocommata attachment. *Journal of Food Science*, *67*, 2067–2071.

Terjesen, B. F. (2008). Nitrogen excretion. In R. N. Finn & B. G Kapoor (Eds.), *Fish larval physiology* (pp. 263–302). Enfield, NH, USA: Science Publishers, Inc., Enfield.

Thomassen, M. S., Rein, D., Berge, G. M., Østbye, T. K., & Ruyter, B. (2012). High dietary EPA does not inhibit Delta 5 and Delta 6 desaturases in Atlantic salmon (*Salmo salar* L.) fed rapeseed oil diets. *Aquaculture*, *360*, 78–85.

Torgersen, J. S., Koppang, E. O., Stien, L. H., Kohler, A., Pedersen, M. E., & Morkore, T. (2014). Soft texture of atlantic salmon fillets is associated with glycogen accumulation. *PLoS One*, *9*, e85551.

Trudeau, V. L., Spanswick, D., Fraser, E. J., Lariviere, K., Crump, D., Chiu, S., … Schulz, R. W. (2000). The role of amino acid neurotransmitters in the regulation of pituitary gonadotropin release in fish. *Biochemistry and Cell Biology*, *78*, 241–259.

Vandesompele, J., De, P. K., Pattyn, F., Poppe, B., Van, R. N., De, P. A., & Speleman, F. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology*, *3*, research0034, 1–11.

Vegusdal, A., Østbye, T. K., Tran, T. N., Gjøen, T., & Ruyter, B. (2004). beta-oxidation, esterification, and secretion of radiolabeled fatty acids in cultivated Atlantic salmon skeletal muscle cells. *Lipids*, *39*, 649–658.

Watford, M. (2008). Glutamine metabolism and function in relation to proline synthesis and the safety of glutamine and proline supplementation. *Journal of Nutrition*, *138*, 2003S–2007S.

Wu, G. Y. (2009). Amino acids: Metabolism, functions, and nutrition. *Amino Acids*, *37*, 1–17.

Wu, G. (2010). Functional amino acids in growth, reproduction, and health. *Advances in Nutrition*, *1*, 31–37.

Wu, G., & Morris, S. M. Jr (1998). Arginine metabolism: Nitric oxide and beyond. *Biochemical Journal*, *336*(Pt 1), 1–17.

Yamashita, M., & Konagaya, S. (1990). Participation of cathepsin L into extensive softening of the muscle of Chum salmon caught during spawning migration. *Nippon Suisan Gakkaishi*, *56*, 1271–1277.

Yamashita, M., & Konagaya, S. (1991). Hydrolytic action of salmon cathepsin-B and cathepsin-l to muscle structural proteins in respect of muscle softening. *Nippon Suisan Gakkaishi*, *57*, 1917–1922.

Young, V. R., & Ajami, A. M. (2001). Glutamine: The emperor or his clothes? *Journal of Nutrition*, *131*, 2449S–2459S.

Zimmerman, A. M., & Lowery, M. S. (1999). Hyperplastic development and hypertrophic growth of muscle fibers in the white seabass (*Atractoscion nobilis*). *Journal of Experimental Zoology*, *284*, 299–308.

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