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Fillet quality of Atlantic salmon (Salmo salar L.). Relevance of dietary amino acid supplementation and acclimation temperature before slaughter.

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by

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SUMMARY

The aim of the present study was to investigate the impact of dietary composition and acclimation temperature before slaughter on fillet quality development of farmed Atlantic salmon (*Salmo salar* L.). The fish were fed a commercial extruded diets or the same diet supplemented with arginine or glutamate for one year before they were harvested in May 2010 (average body weight was 3 kg). The fish were transferred from net pens in seawater (8℃) to small tanks on land with different seawater temperature (2, 8, 16℃) for 210 minutes before they were killed with a blow to the head. The rate of fillet contraction was analyzed during the first 24 hours after slaughtering, and fillet gaping, colour, fillet texture, muscle pH, cathepsins and ATP degradation products were analyzed at regular intervals during 12 days ice storage.

Acclimation temperatures had an overall significant effect on the fillet contraction from 3-12 hours storage with the highest contraction for the salmon acclimated at 16℃. The salmon fed the arginine diet had the significantly lowest contraction rate when the fish were kept at 8℃ before slaughtering, but no significant effect of dietary treatment were observed for salmon kept at 2 or 16℃. Neither acclimation temperatures nor the dietary treatment had any significant effect on the gaping and colour score. Within the 2℃ and 16℃ temperature treatment, a significantly firmer texture (higher Fb) was observed for the glutamate diet. 16℃ acclimation temperature resulted in a significantly lower pH both at 3 and 24 hours of storage, but not after 5 and 12 days of storage. The pH of the glutamate group acclimated to 2℃ had the highest muscle pH three and 24 hours post-mortem, and the reduction rate of the pH was slower for the arginine group kept at 8℃ before slaughter as compared with the control group. Acclimation temperatures had an overall significant effect at 24 hours storage for the activity of cathepsin B (lowest at 2℃), and there was also found a significant effect at 0 hour for total activity of cathepsin B+L (highest at 8℃). The cathepsin B activity was lowest at 0 hour for salmon fed the arginine or glutamate diet at 2℃ acclimation temperature; whereas an inverse relationship was observed between the acclimation temperature and the cathepsin L activity at 24 hours for the salmon fed the arginine diet. High acclimation temperature accelerated ATP degradation after slaughtering, but the content of hypoxanthine (Hx) was lowest for salmon acclimated to 16℃ and highest for salmon acclimated to 2℃ after one week storage. It is concluded that the dietary supplementation of arginine and glutamate had positive effects on fillet contraction and texture, respectively. High acclimation temperature reduced the accumulation of hypoxanthine during storage, indicating prolonged freshness.

Keywords: *Fish quality, Acclimation temperature, Amino acids, Rigor mortis, Gaping, Colour, pH, Texture, Cathepsins, ATP breakdown products.*

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1. INTRODUCTION

Aquaculture is the fastest growing animal food producing sector in the world, with a per capita supply from aquaculture increasing from 0.7 kg in 1970 to 7.8 kg in 2008. It is documented that aquaculture accounted for 46 percent of total food fish supply in 2008 (FAO, 2010).

Atlantic salmon is the dominating specie farmed in Norway. Production in 2009 was reported to increase to 8.6 million tons, compared to a production of below 1.5 million tons in the year of 1990 (Statistics Norway, 2010). Among this, 6.9 million tons were exported from Norway to 96 countries with a value of 23.7 billion in 2009 (Statistics Norway, 2010). Gutted salmon was the largest export product. However, export of salmon fillets has increased year by year, for example, in 2009, export of Norwegian Salmon fillets increased to NOK 4.5 billion, and this means that the share increased from 13.7% in 2008 to 19% in 2009. The industry may face new challenges if the fillets will take over the export of gutted salmon, for example soft texture, fillet gaping and deviating appearance are the main problems when it comes to fillets.

Fillet quality can be affected by several factors, such as breeding, diet, slaughter procedure, storage etc. To improve the meat quality of salmon, several dietary supplementations are usually added in the normal feeds. Some amino acids and their metabolites were reported to be crucial for maintenance, growth, feed intake, nutrient utilization, immunity, behavior, reproduction, as well as resistance to environmental stressors and pathogenic organisms in various fishes (Chen et al., 1992; Li et al., 2009; Lin and Xiao, 2006). However, no information is available on the effect on fish quality by supplementing arginine and glutamate in diets for slaughter sized Atlantic salmon.

Nowadays, live salmon are usually transferred to a chilling tank in 1℃ refrigerated seawater prior to anaesthesia. Chilling prior to slaughter will produce a beneficial low muscle temperature and remove substantial thermal energy. The low temperature at slaughter has positive effect on rigor development and is reported to be delay time to onset of rigor (Lowe et al., 1993; Skjervold et al., 2001a). However, no previous studies have reported the effect on rigor development and subsequent fillet quality of salmon kept at temperature above their rearing temperature and the interaction between dietary composition and acclimation temperature.

The aim of the present study was to investigate the impact of dietary composition and acclimation temperature before slaughter on fillet quality development of farmed Atlantic salmon (*Salmo salar* L.). Arginine and glutamate were separately added to a standard extruded diet to a level above normal requirements. The fish were transferred from net pens in seawater to small tanks on land at different temperature (2, 8, 16℃) for 210 minutes before slaughtering. Fillet contraction, gaping, colour, fillet texture, muscle pH, cathepsins and ATP degradation products were analyzed at regular intervals during cold storage, up to 12 days.

2. THEORETICAL BACKGROUND

The chapter consists of three main sections. The first section gives a general introduction and discussion of fish quality. The second section focuses mainly on the amino acids: arginine and glutamate, whereas the third section includes live-chilling and pre-slaughter stress.

2.1 Fish quality

According to the standard ISO 8402:1994, quality is defined as "the totality of features and characteristics of a product or service that bear on its ability to satisfy stated or implied needs". A similar definition was also given by a book on the knowledge status of Norwegian aquaculture research: "supply of a product according to the demand and specifications of the customer" (Thomassen et al., 2007).

The term quality in fish is frequently categorized into five subcategories: sensory quality, nutritional quality, hygienic quality, technological quality, and ethical quality. Main quality parameters for raw salmon are slaughter and filleting yield, fillet fat content, colour, texture, and gaping. Several methods are used to determine the quality of fish. These can be classified into sensory (Borderias et al., 2007; Sveinsdottir et al., 2003), instrumental (Casas et al., 2006; Macagnano et al., 2005) and chemical methods (Kent et al., 2004; Rodríguez-Jérez et al., 2000).

Fillet texture is one of the most important quality parameters in fish. It is a sensory characteristic for the consumer and also an important attribute for the mechanical processing of fillets. Consumers generally prefer firm and elastic fish fillets. Hence, in contrast to mammalian meat where tenderization is a main focus, soft flesh is a challenge with fish meat because of the low content of connective tissue in the muscle (Hall, 1997).

Atlantic salmon fillet quality can be influenced by numerous factors and is affected throughout the entire value chain. These include breeding (Gjedrem, 1997), genetics (Gjøen, 1997; Gjedrem and Skjervold, 1978), feed composition (Aksnes, 1995; Einen, 1999; Hillestad and Johnsen, 1994; Øverland et al., 2009; Refstie, 1998), feeding regime (Sveier, 1998; Young et al., 2005), seasonal variations (Mørkøre, 2001), transportation, handling and pre-slaughter stress (Erikson et al., 1997a; Sigholt et al., 1997), storage time and temperature post-mortem (Erikson et al., 1997a; Sigholt et al., 1997; Skjervold et al., 2001a), processing (Kong et al., 2007), and cooking (Yagiza et al., 2009).

2.2 Arginine and Glutamate

Arginine was first isolated from a lupin seedling extract by a Swiss chemist named Ernst Schultze in 1886, and the presence of arginine as a part of animal protein was identified by Hedin in 1895. The chemical structure of arginine is presented below in Fig. 2.1.

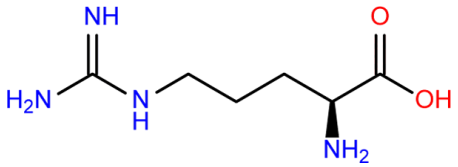


Fig.2.1. Chemical structure of arginine (PubChem Database, 2011a).

Arginine is classified as an essential amino acid for birds, carnivores and young mammals, and a semi-essential or conditionally essential amino acid for adults (Flynn, 2002), especially in case of illness and stress. Fish has low urea cycle and is believed to synthesize enough arginine to drive metabolism (Anderson, 1995).

Arginine is an amino acid of versatile functions and it is involved in many metabolic pathways in animal cells such as synthesis of proteins, nitric oxide, ornithine, polyamines, urea, glycine, creatine, glutamine, glutamate and proline (Fig.2.2).

Arginine is identified as a potential immunomodulator and is useful in severe sepsis and stress. This is related to the breakdown of arginine, synthesized polyamines which can lead to lymphocyte mitogenesis and production of nitric oxide, which is important for, among other things, the immune system, digestive tract and coagulation of the blood (Efron and Barbul, 2000; Evoy et al., 1998). Besides these features, arginine also plays an important role in lowering blood pressure (Gokce, 2004).

It is known that certain amino acids can stimulate muscle growth in fish (Brown and Cameron, 1991), but the mechanisms involved are fairly unknown. However it is known that metabolism of arginine leads to the production of ornithine, the precursor of putrescine, which is important for the synthesis of polyamines which are essential for muscle growth (Mommsen, 2001). Metabolism of arginine and its products of great importance for muscle growth are shown in Fig.2.2.

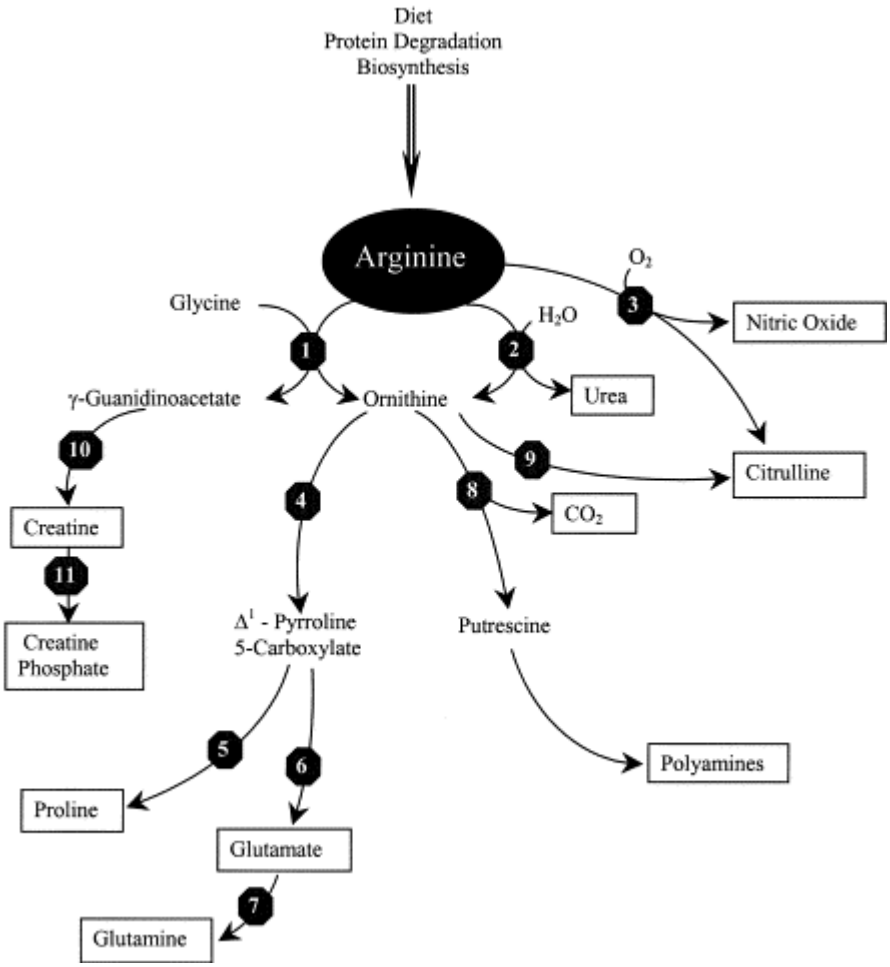


Fig.2.2. Metabolism of the versatile arginine. Products with important roles in muscle growth are in boxes (Mommsen, 2001).

Glutamate

Glutamate was discovered and identified by the German chemist Karl Heinrich Leopold Ritthausen in 1866, by the hydrolysis of gluten from wheat. The chemical structure of glutamate is presented in Fig.2.3.

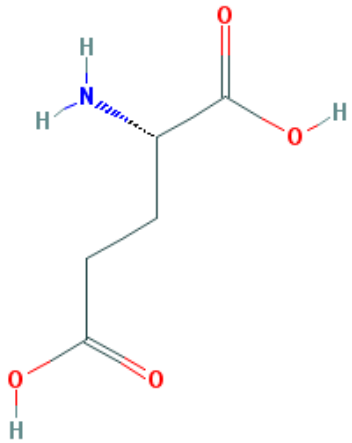


Fig.2.3. Chemical structure of glutamate (PubChem Database, 2011b).

Glutamate is considered as a non-essential amino acid, meaning that the body can synthesize sufficient quantities from other sources. Like other amino acids, glutamate is absorbed and metabolized in the small intestine (Burrin and Stoll, 2009). Glutamate is converted first to alanine in the intestine, then to glucose and eventually to lactate in the liver. Glutamate is an important bridge between the urea cycle and the Krebs cycle in mammals (Wu, 2009), and it is also the precursor for several other amino acids including alanine, aspartate, ornithine, arginine, proline and biologically active molecules such as glutathione (Fig. 2.4). Of amino acids formed from glutamate, proline is important in the synthesis of collagen and connective tissue (Tapiero et al., 2002).

Glutamate also has a number of important functions in living cells. The dominant role of glutamate is as an oxidative fuel in the intestinal mucosa and is responsible for maintenance and protection of the mucus layer (Blachier et al., 2009; Blachier et al., 1995; Burrin and Stoll, 2009). Glutamate is also related to the function of the central nervous system as a key neurotransmitter (Meldrum, 2000; Nedergaard et al., 2002). Relatively few studies have been done about glutamate and its effects in fish, but glutamate has been shown to affect steroidogenesis in rainbow trout (Leatherland et al., 2004; Leatherland et al., 2005). In addition, glutamate is essential for control of ammonia in plasma and tissues of fish - glutamine is formed from glutamate and ammonia by an increase in ammonia, for example, brain tissue (Vedel et al., 1998; Wicks and Randall, 2002). Furthermore, studies have shown that glutamate is involved in contraction and depolarisation in crustacean muscle (Colton, 1975; Robbins, 1959).

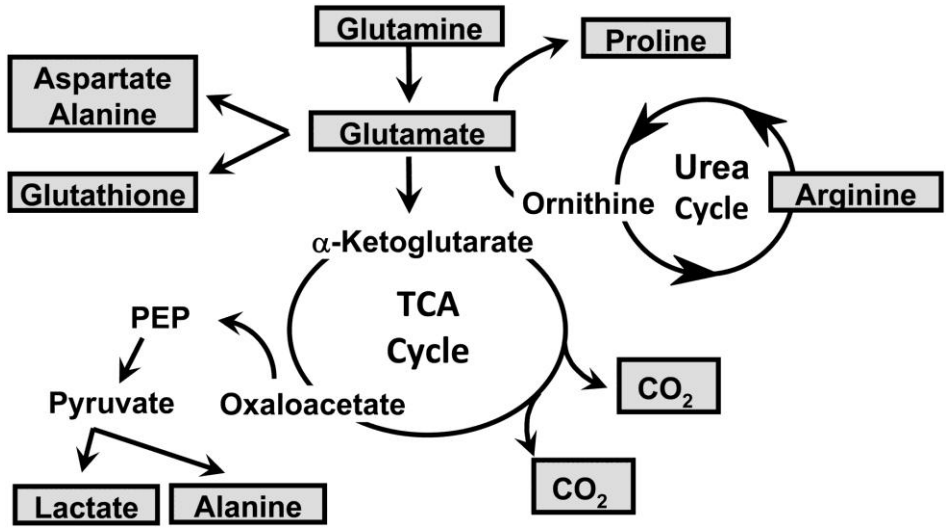


Fig.2.4. Metabolic fates of dietary glutamate in the intestine (Burrin and Stoll, 2009).

2.3 Live-chilling and pre-slaughter stress

The rearing temperature has large effects on the metabolic rates in the living fish (Brett, 1971; Guderley, 2004), the enzyme activity (Matschak et al., 1998) and also

the fluidity of biological membranes (Hazel, 1995; McElhaney, 1976; Wodtke, 1981). Furthermore, the storage temperature is accepted as being the major factor influencing the rate of post-mortem deterioration and shelf life of fish products (Erikson et al., 1997a; Himelbloom et al., 1994; Kiessling et al., 2006; Stien et al., 2005).

In Norwegian aquaculture, live salmon are often transferred to a chilling tank with refrigerated seawater of approximately 1℃ for 40-60 minutes prior to anaesthesia (Fig. 2.5). The chilled temperature effect will to some extent depend on the rearing temperature (Δ℃), but in general the refrigerated seawater temperature results in less vigorous body movements and also removal of substantial thermal energy from the fish body (Crapo and Elliot, 1987; Skjervold et al., 2001a).

Stress might be defined as a biochemical and physiological process associated with maintaining homeostasis. A stress response will normally consist of a primary release of several hormones into the bloodstream (Einarsdóttir and Nilssen, 1996; Rotllant and Tort, 1997). Furthermore, a wide range of secondary changes will be evoked in behavior, metabolism, respiration, osmotic regulation, the immune system, and in other elements of the endocrine system (Einarsdóttir and Nilssen, 1996).

Handling, transportation and crowding before slaughter of farmed salmon is known to be a significant stressor affecting blood plasma levels of for example cortisol, glucose, lactate, and osmolality (Einarsdóttir and Nilssen, 1996).

The effects of pre-slaughter stress on meat properties are mainly depending on the extent to which muscle metabolites are depleted before death. The majority of salmon flesh is white muscle, adapted to anaerobic metabolism. The effect of anaerobic metabolism is a depletion of glycogen reserves and an accumulation of lactic acid with a corresponding pH reduction. Low muscle pH is known to have several negative effects on fillet quality characteristics such as texture and water holding capacity (Hufflonergan and Lonergan, 2005; Kramer and Peters, 2007).

Progression of rigor mortis in fish meat is used as an indicator of pre-mortem stress. The pre-slaughter stress due to for example crowding results in earlier onset and a stronger maximum rigor contraction (Skjervold, 1999).

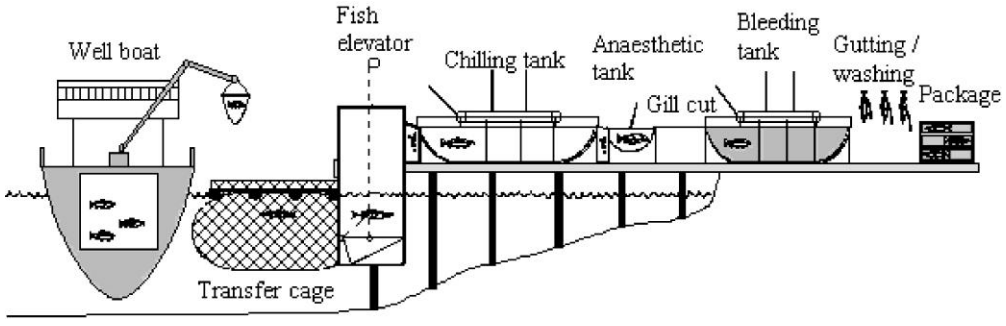


Fig.2.5. Commercial slaughter line involving live-chilling (Skjervold et al., 2002).

3. MATERIALS AND METHODS

3.1 Fish and facilities

The fish used in this experiment were Atlantic salmon (*Salmo salar* L.), obtained from Salmar ASA. The feeding trial was done at Nofima Marine seawater research station at Averøy at the northwest coast of Norway. All fish with initial weight of 105 g were randomly distributed to 9 net-pens (triplicate cages each with 500 fish) of 125 m^3 each in May 2009.

3.2 Diets and feeding regimes

The dietary treatments consisted of a control diet and two experimental diets supplemented with 1.5% arginine (Fenchem Biotech Ltd., Nanjing, China) or 1.5% L-glutamate (Meihua Holding Group Co, Ltd., Hebei, China) separately. The formulation and composition of diets are given in table 3.1. The commercial extruded dry feed was produced at Skretting (Stavanger, Norway). The three diets were fed to triplicate cages of fish during the period from May 2009 to May 2010 when the fish were harvested for this experiment. The fish were fed in excess of recorded feed intake four times per day (uneaten feed pellets were collected and weighed).

Table 3.1. Feed formulation and chemical composition.

Diet	
Formulation,%	
Fish meal (Superprime)	37
Hi Pro Soy (48%)	3.5
Soy protein concentrate (Imcopa SPC)	10
Sunflower expeller	1.6
Beans	17
Standard fish oil	15.5
Rapeseed Oil	14
Vitamin and mineral premix	0.32
Chemical composition,%	
Dry matter,%	92.9
Fat,%	35.1
Protein,%	35.3
Starch,%	7.6
Ash,%	5.4
Indigestible carbohydrate,%	9.5
Astaxanthin, mg / kg	40
Energy content, MJ	25.1

3.3 Handling and Slaughtering

Three fish from each net-pen were tagged and transferred to three 1000L tanks for further treatment. The tanks were variable in temperature (2, 8, 16℃) and each tank had 9 fish from three diets (three fish per net pen). The salmon were kept for 210 minutes in the tanks before slaughtering. The oxygen level (9 mg/L) and temperature were monitored at a constant level.

The fish were killed by a sharp blow to the head. Thereafter the fish were gill cut and bled in seawater for 20 minutes prior to being gutted and filleted. Fillets were kept on ice for further analysis. An overview of the whole experimental design is given in table 3.2.

Table 3.2. An overview of the whole experimental design

			Diet					
		n	Control	Glutamate	Arginine		0 day	5 days	12 days
Temp.	2℃	9	3	3	3	→→	Rigor	Analysis	Analysis
	8℃	9	3	3	3	→→	contraction		
	16℃	9	3	3	3	→→			
Number of fish		27	9	9	9				

3.4 Rigor development (right fillet side)

Rigor development was recorded on 27 right side pre-rigor fillets during 24 hours. Fillets were placed on smooth plastic trays (to allow free contraction) and kept on ice in Styrofoam boxes in a cold room (5℃). The rigor contraction rate was evaluated by measuring the change in fillet length (cm).

3.5 Fillet colour, texture and gaping (left fillet side)

The area above the lateral line was photographed by a digital camera (Dolphin F145C, Allied Vision Technologies, Stadtroda, Germany) in a box with standard illumination. The camera was placed on the top of the box and operated through a computer. Each pixel within the image was represented by RGB signals from red (R), green (G) and blue (B) channels of the camera with the RGB signals recording values between 0 (dark) and 255 (light). Results were transcribed to correspond to visual colour score (Roche Salmo Colour Fan TM), pigment content and fillet fat content, as described by Folkestad (Folkestad et al., 2008).

Texture was analysed by a Texture Analyzer, model TA-XT2 (SMS Stable Micro Systems, Surrey, England), equipped with a 12.5 mm diameter cylindrical plunger (type P/0.5). The cylinder was pressed into the fillets at a constant speed of 1 mm/s until it reached 60% of the sample height. The resistance force (N) of the fillets was recorded at the first breakpoint of the curve (F breakpoint, Fb) obtained during compression (when the probe punctured the fillet surface). The measurements were performed in triplicate above the lateral line (A:four cm posterior to head, B:below the dorsal fin and C:in the middle of the Norwegian Quality Cut, (NS 9401 1994). The force–time graphs were recorded and analysed using the computer software Texture Expert for Windows (version 1.15, Stable Micro Systems). Because the texture instrument was not available at the processing plant, texture evaluation was only performed in the laboratory 5 days (position A, B, C) and 12 days (position B) after slaughtering. For the statistical analyses, the mean breaking force for the three measurements was used for each fillet on day 5.

Fillet gaping was recorded according to Andersen (Andersen et al., 1994) on a scale from 0 to 5, where 0 is no gaping and 5 represent extreme gaping (the fillet falls apart).

3.6 Muscle pH

The muscle pH was measured with a 330i SET pH-meter (Wissens-chaftlich-Technische-Werkstätten GmbH & Co.KG WTW, Weilheim, Germany) equipped with a muscle-electrode (Schott pH-electrode, Blueline 21 pH, WTW, Weilheim, Germany) and a temperature probe (TFK 325, WTW, Weilheim, Germany) that were directly inserted into fillets. The pH measurements were performed at the same time and on the same fillets as texture measurement, position B. Temperature and pH was recorded simultaneously by the pH-meter.

3.7 Cathepsins

Samples were taken from the same sections of fillets as the pH measurements were done, after that the muscle pieces were quickly frozen in liquid nitrogen before being stored at -80 ℃ until further analysis. Cathepsin B, cathepsin L and Cathepsin B + L activities were determined by homogenizing 300 mg of muscle in 900 ml extraction buffer (100 mM Na-acetate in 0.2% Triton X-100, pH 5.5) in Precellys tubes, with a Precellys24 homogenizer (Bertin Technologies, France) (2 cycles of 20s at 5500 rpm, separated by a 10s break).

The obtained homogenates were centrifuged at 16,000 G for 30 minutes and the supernatants were used to determine enzyme activities. Cathepsin B+L activity was

measured fluorimetrically, according to the method of Kirschke (Kirschke et al., 1983).N-CBZ-Lphenylalanyl-L-arginine-7-amido-4-methylcoumarin(Z-Phe-Arg-Nmec) was used as a substrate for cathepsin B+L and N-CBZ-L-arginyl-L-arginine-7-amido-4-methylcoumarin (Z-Arg-Arg-Nmec) for cathepsin B. Cathepsin L activity was obtained subtracting the result of cathepsin B activity from the result of cathepsin B + L activity. In all cases, the assays were run in triplicates for all cathepsin measurements.

3.8 ATP breakdown products

Muscle sections were sampled and frozen at -80℃ until further analyses of adenosine-5-triphosphate(ATP),adenosine-5-diphos-phate(ADP),adenosine-5-monophosphate (AMP), inosine mono-phosphate (IMP), and inosine and hypoxanthine (Hx) were conducted. The samples (0.200±0.005 g) were extracted by mixing freeze-dried tissue in ice-cold 8% $HClO_4$ (2.5ml) for 30 minutes before centrifugation. Tubes were centrifuged for 10 minutes at 4000 rpm and 10℃. The supernatant were then transferred to 2 ml micro tube and centrifuged in eppendorf centrifuge at 14 000 rpm and 10℃. The supernatant (1ml) was added with 3M K_2CO_3 (0.3ml) for neutralization, centrifuged for 10 minutes, and finally filtrated (0.45μm filter) through syringe filter. ATP and degradation products were analysed by HPLC in a Waters Alliance liquid chromatograph system (2695) equipped with a photodiode array detector (2996). The analysis were performed on a silica column xTerra^{TM} MS $C_{18,5}$ 4.6 mm×250 mm) with guard column XTerra $MC_{18,5}$ 3.9 mm×20 mm. The injection volume was 10μl. The mobile phase used for the separation of nucleotides consisted of two eluents. A: acetonitrile and B: 50 mm phosphate buffer with 10 mm CH_3 $(CH_2)_3]_4$ NBr (pH 7.0) as a gradient. The flow rate and temperature were 1.5 ml min/l and 30℃, respectively.

3.9 Statistical Analysis

Data were analysed by ANOVA using the SAS program (Version 9.2; SAS Institute Inc., Cary, USA). The alpha level was set to 5% ($P < 0.05$).

4. RESULTS

The results are presented for the following parameters: body measurements, free amino acids and metabolites in skeletal muscle, fillet contraction, gaping score, colour, texture, muscle pH, cathepsins activity and also ATP breakdown products. Results for the contraction rate and fillet quality parameters are in general presented first with regard to the overall effect of diet and temperature, and thereafter with regard to the effect of temperature and diet separately.

4.1 Body measurements

The average final body weight of the Atlantic salmon was 3.03 kg, 3.07 kg and 3.03 kg for the control, arginine and glutamate group, respectively. The liver comprised 0.88-0.99% of the body weight. The average pigment content was 7 mg/kg and the fillet fat content was 17-17.1% on average. The slaughter parameters showed no significant variation between the dietary groups (Table 4.1).

Table 4.1. Biometric parameters, pigment content and fat content in skeletal muscle of farmed Atlantic salmon fed a commercial diet (Con) or the same diet supplemented with arginine (Arg) or glutamate (Glu).

				ANOVA	
Sampling	Con	Arg	Glu	SEM	P-value
Body weight, kg	3.03	3.07	3.03	0.08	0.889
Gutted body weight, kg	2.67	2.70	2.66	0.07	0.898
Body length, cm	60.3	60.6	59.8	0.66	0.698
Condition factor [1]	1.22	1.22	1.24	0.05	0.748
Fillet weight, kg	0.95	0.94	0.93	0.03	0.466
Fillet length, cm	35.2	34.3	34.3	0.50	0.883
Liver index [2]	0.99[a]	0.88[b]	0.91[b]	0.03	0.014
Pigment content, mg/kg	7.0	7.0	7.0	0.30	0.982
Fat content, %	17.1	17.1	17.0	0.30	0.851

Results are shown as mean per 3 net pens (n=10 per net pen). SEM refers to Standard Error of Mean.

[1] Condition factor = 100 × gutted body weight (g) × body length (cm)$^{-3}$
[2] Liver index = 100 × liver weight (g) × whole body weight (g)$^{-1}$

4.2 Selected free amino acids and metabolites

The arginine and urea content were significantly highest in the muscle of the Atlantic salmon fed with the diet supplemented with arginine. Glutamate, glutamine and taurine showed no significant differences between the dietary treatments.

Table 4.2.Content of selected free amino acids and metabolites in muscle of farmed Atlantic salmon fed a commercial diet (Con) or the same diet supplemented with arginine (Arg) or glutamate (Glu).

				ANOVA	
Sampling	Con	Arg	Glu	SEM	P-value
Arginine	14.3[b]	23.4[a]	11.2[b]	2.97	0.016
Glutamate	91.3	92.2	93.7	2.87	0.840
Glutamine	45.5	46.6	38.1	3.59	0.202
Taurin	80.8	68.2	74.4	4.87	0.203
Urea	50.9[b]	67.0[a]	48.5[b]	3.54	0.001

Results are shown as mean values for each diet from triplicate net pens, 10 salmon from each net pen. SEM referres to Standard Error of Mean.

Different letters denote significant differences ($p<0.05$) between different dietary treatments.

4.3 Fillet contraction

Analyses of variance (ANOVA) of the overall effect of diet and temperature on the fillet contraction rate (% of initial fillet length) showed that acclimation temperatures had a significant effect on the fillet contraction from 3-12 hours storage, whereas the dietary treatment had a significant effect on the fillet contraction at 24 hours storage (Table 4.3). The acclimation temperature also tended to influence the contraction at one hour storage (P=0.068) and at 24 hour storage (P=0.093). The interaction between temperature treatment prior to slaughtering and dietary treatment was also tested, but was removed from the model as no significant effects were observed.

Temperature

A contraction of 5-6% was reached after 1 hour of storage for salmon kept at 16℃ before slaughter, whereas the same contraction was reached after 3 and 6 hours for the salmon kept at 8℃ and 2℃ acclimation temperature, respectively. At 6 hours of storage time, the contraction of the 16℃ group averaged 15% which was 1.7 times higher than the 8℃ group (9.1% contraction) and 2.4 times higher compared with the 2℃ group (6.2% contraction). The maximum contraction at 24 hours of storage was 17.1, 19.1 and 19.9% for the 2℃, 8℃ and 16℃ group, respectively.

Diet

Significant effect of diet was observed only for fillets from salmon kept at acclimation temperature of 8 ℃. The salmon fed the arginine diets had significantly lowest contraction at 3, 6, 12 and 24 hours of storage, which was 5.2, 9.0, 13.5, 16.9%, respectively (Fig. 4.1).

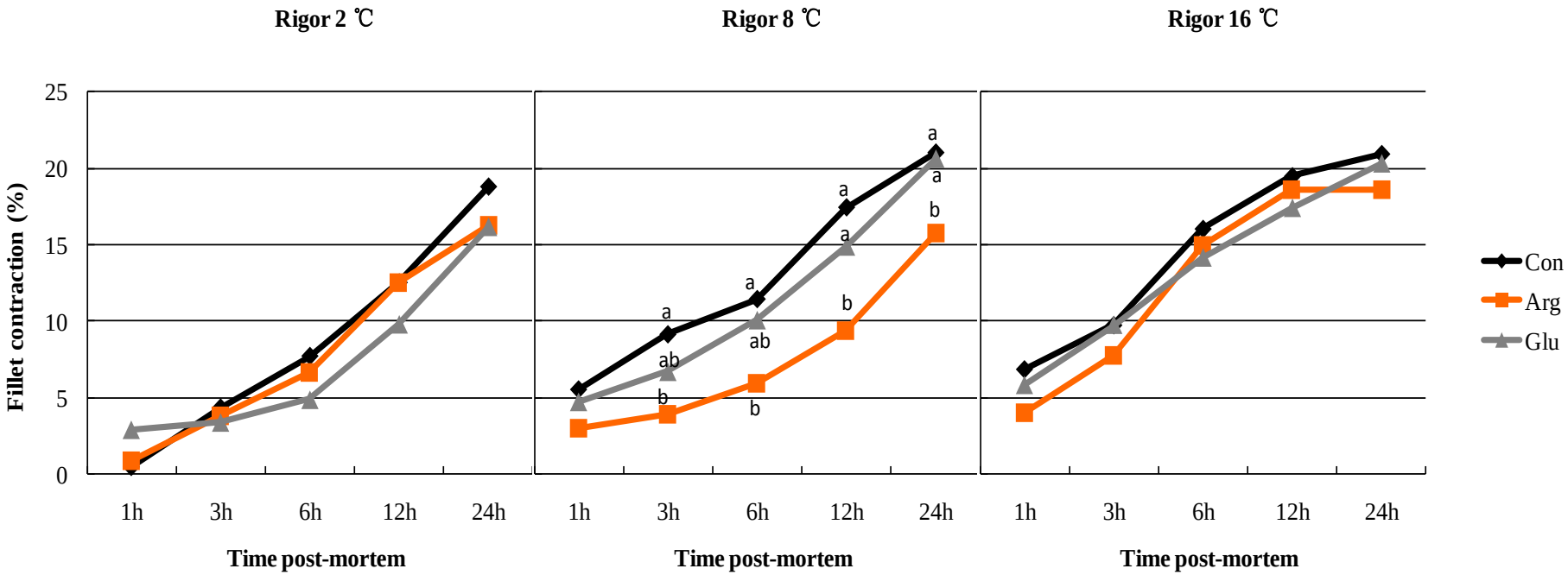


Fig.4.1. Development in rigor contraction (% of initial fillet length) during 24 hours storage of farmed Atlantic salmon fed a commercial diet (Con) or the same diet supplemented with arginine (Arg) or glutamate (Glu). The fish were transferred from the cages in seawater (8 ℃) to tanks with seawater of 2 ℃ (A), 8 ℃ (B) or 16 ℃ (C) for 3.5 hours before they were slaughtered. Different letters denote significant differences ($p<0.05$) between dietary treatments within temperature treatment.

Table 4.3. Development in rigor contraction (% of initial fillet length) during 24 hours storage of farmed Atlantic salmon fed a commercial diet (Con) or the same diet supplemented with arginine (Arg) or glutamate (Glu). The fish were transferred from the cages in seawater (8 ℃) to tanks with seawater of 2 ℃, 8 ℃ or 16 ℃ for 3.5 hours before they were slaughtered. The statistical model used were Y=diet+temperature+ε, so P-diet, P-temp and P-model are given below in addition to Standard Error of Mean (SEM).

						ANOVA			
1h		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	1.4	0.5	0.9	2.9	1.2	0.502	0.068	0.150
	8℃	4.4	5.6	3.0	4.7				
	16℃	5.5	6.9	4.0	5.8				
	Average		4.3	2.6	4.5				
3h		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	3.9^{B}	4.3	3.8	3.4^{B}	1.1	0.247	0.008	0.018
	8℃	6.6^{AB}	9.2^{a}	4.0^{b}	6.7^{ABab}				
	16℃	9.1^{A}	9.7	7.7	9.7^{A}				
	Average		7.7	5.2	6.6				
6h		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	6.2^{B}	7.7^{B}	6.7^{A}	4.9^{B}	1.1	0.236	<0.0001	0.0003
	8℃	9.1^{AB}	11.4^{ABa}	5.9^{Bb}	10.1^{ABab}				
	16℃	15.0^{A}	17.4^{A}	15.0^{A}	14.1^{A}				
	Average		11.7	9.0	9.7				
12h		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	11.6^{B}	12.5^{B}	12.5^{AB}	9.8^{B}	1.4	0.296	0.007	0.0176
	8℃	13.9^{B}	17.4^{ABa}	9.3^{Bb}	14.9^{ABa}				
	16℃	18.5^{A}	19.5^{A}	18.6^{A}	17.4^{A}				
	Average		16.5	13.5	14.0				
24h		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	17.1	18.7	16.3	16.2	0.9	0.048	0.093	0.038
	8℃	19.1	21.0^{a}	15.8^{b}	20.6^{a}				
	16℃	19.9	21.0	18.6	20.3				
	Average		20.2^{a}	16.9^{b}	19.0^{ab}				

Significant differences ($p<0.05$) between dietary treatments within temperature treatments are given by different small letters (horizontal), and significant differences ($p<0.05$) between different temperature treatments within dietary treatments are given by different capital letters (vertical).

4.4 Gaping score

Analyses of variance (ANOVA) of the overall effect of diet and temperature on the degree of gaping showed that neither acclimation temperatures nor the dietary treatment had any significant effect on the gaping (Table 4.4). The interaction between temperature treatment and diet was also tested, but was removed from the model as no significant effects were observed. The gaping score of the Con group kept at 2 ℃ increased from score 0 after 24 hours storage to score 2 after 5 days of storage, but no significant increase was observed for the other groups.

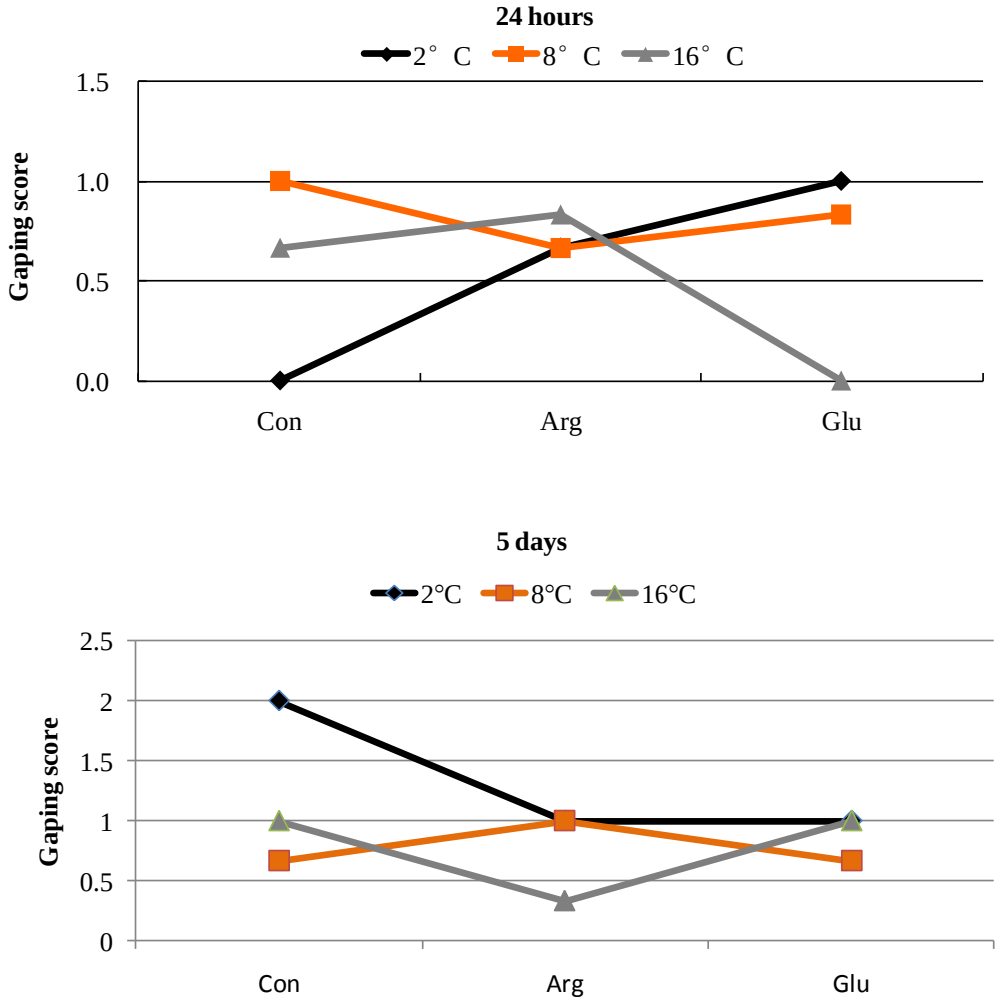


Fig.4.2. Gaping scores (0-5) during cold storage of fillets of farmed Atlantic salmon fed a commercial diet (Con) or the same diet supplemented with arginine (Arg) or glutamate (Glu). The fish were transferred from the cages in seawater (8 ℃) to tanks with seawater of 2 ℃, 8 ℃ or 16 ℃ for 3.5 hours before they were slaughtered.

4.5 Fillet colour

Although there were some minor numerical differences between diets and temperature treatments, analyses of variance (ANOVA) showed that acclimation temperatures and diet had no overall effect on the fillet colour score analyzed after 5 or 12 days of storage (Table 4.4). The interaction between temperature treatment and diet was also tested, but was removed from the model as no significant effects were observed.

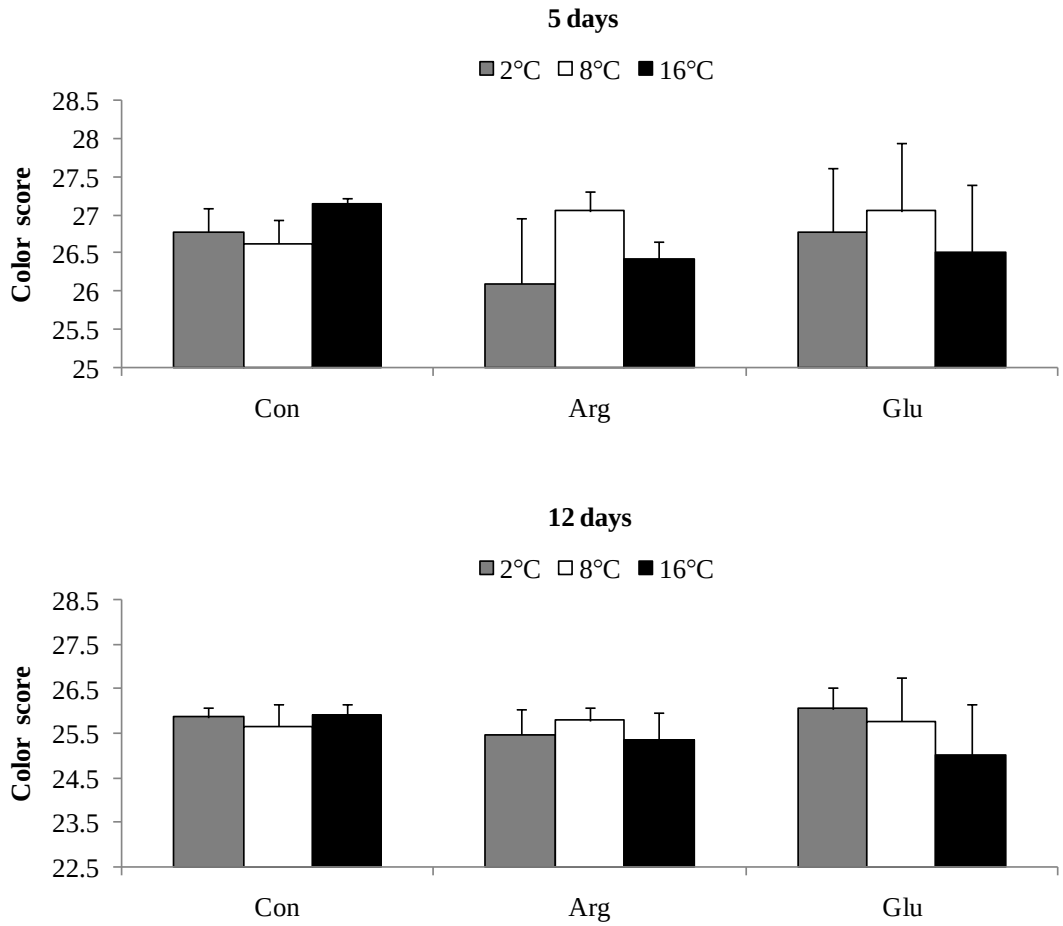


Fig.4.3. SalmoFan colour score (mean±SE) analysed 5 and 12 days post-mortem of farmed Atlantic salmon fed a commercial diet (Con) or the same diet supplemented with arginine (Arg) or glutamate (Glu). The fish were transferred from the cages in seawater (8 ℃) to tanks with 2 ℃, 8 ℃ or 16 ℃ seawater for 3.5 hours before they were slaughtered.

4.6 Texture analysis

During the whole storage period, the numerically highest break force was observed at 2℃ for the Glu diet. Break force during storage from 5 to 12 days did not change significantly for any of diets or temperature treatments.

Analyses of variance (ANOVA) of the overall effect of diet and temperature on the texture showed that neither acclimation temperatures nor dietary treatment had a significant effect on the texture (Table 4.4). However, within the 2℃ and 16℃ temperature treatment, a significantly firmer texture (higher Fb) was observed for the Glu diet. For the salmon kept at 8℃, no significant differences were observed between the dietary groups. The interaction between temperature treatment and diet was also tested, but was removed from the model as no significant effects were observed.

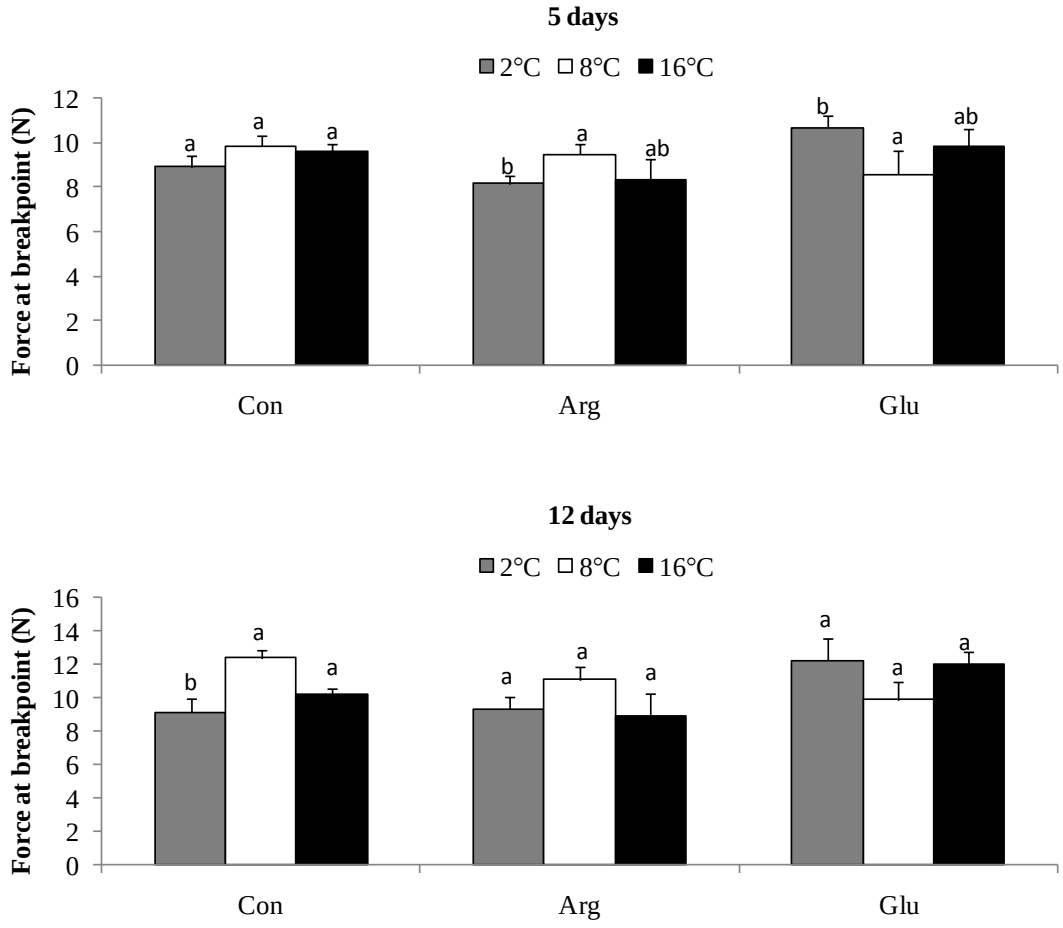


Fig.4.4. Fillet firmness measured as breaking force (N) 5 and 12 days post-mortem of farmed Atlantic salmon fed a commercial diet (Con) or the same diet supplemented with arginine (Arg) or glutamate (Glu). The fish were transferred from the cages in seawater (8 ℃) to tanks with 2 ℃, 8 ℃ or 16 ℃ seawater for 3.5 hours before they were slaughtered. Different letters denote significant differences ($p<0.05$) between dietary treatments within temperature treatment.

4.7 Muscle pH

The ultimate muscle pH reflects the glycogen stores of the muscle; i.e. reduction of pH is caused by formation of lactate from breakdown of muscle glycogen. The muscle pH decreased after 5 days of storage period for all dietary fish groups; but an increase was observed for the Arg group at 2℃ and 8℃ acclimation temperature after 12 days of storage.

Analyses of variance (ANOVA) of the overall effect of diet and temperature on the pH showed that acclimation temperature had a significant effect on the pH both at 3 and 24 hours storage, but not 5 days and 12 days post mortem. The dietary treatment had no overall significant effect on pH (Table 4.4), although there was a tendency as the P-value was 0.058 at 12 days storage. The interaction between temperature treatment and diet was also tested, but was removed from the model as no significant effects were observed.

Temperature

An average pH of 6.4 was reached after 3h storage for salmon kept at 16℃ before slaughter, whereas the same pH was reached after 24h for the salmon kept at 2℃ and 8℃ acclimation temperature, respectively. At 3h storage time, the pH of the 16℃ group averaged 7.0 which was similar compared with the 8℃ group (pH 6.9) and 1.1 times higher compared with the 2℃ group (pH 6.2). The minimum pH at 24 hours storage was 6.4, 6.3 and 6.2 for the 2℃, 8℃ and 16℃ group, respectively.

Diet

No overall significant effect was observed between dietary treatments, but within the 2℃ acclimation temperature treatment a significantly highest pH was observed at 3 and 24 hours of storage for the Glu group. Also the rate of pH reduction during the first 24 hours post-mortem was slower for the Arg and Glu groups compared with the Con group.

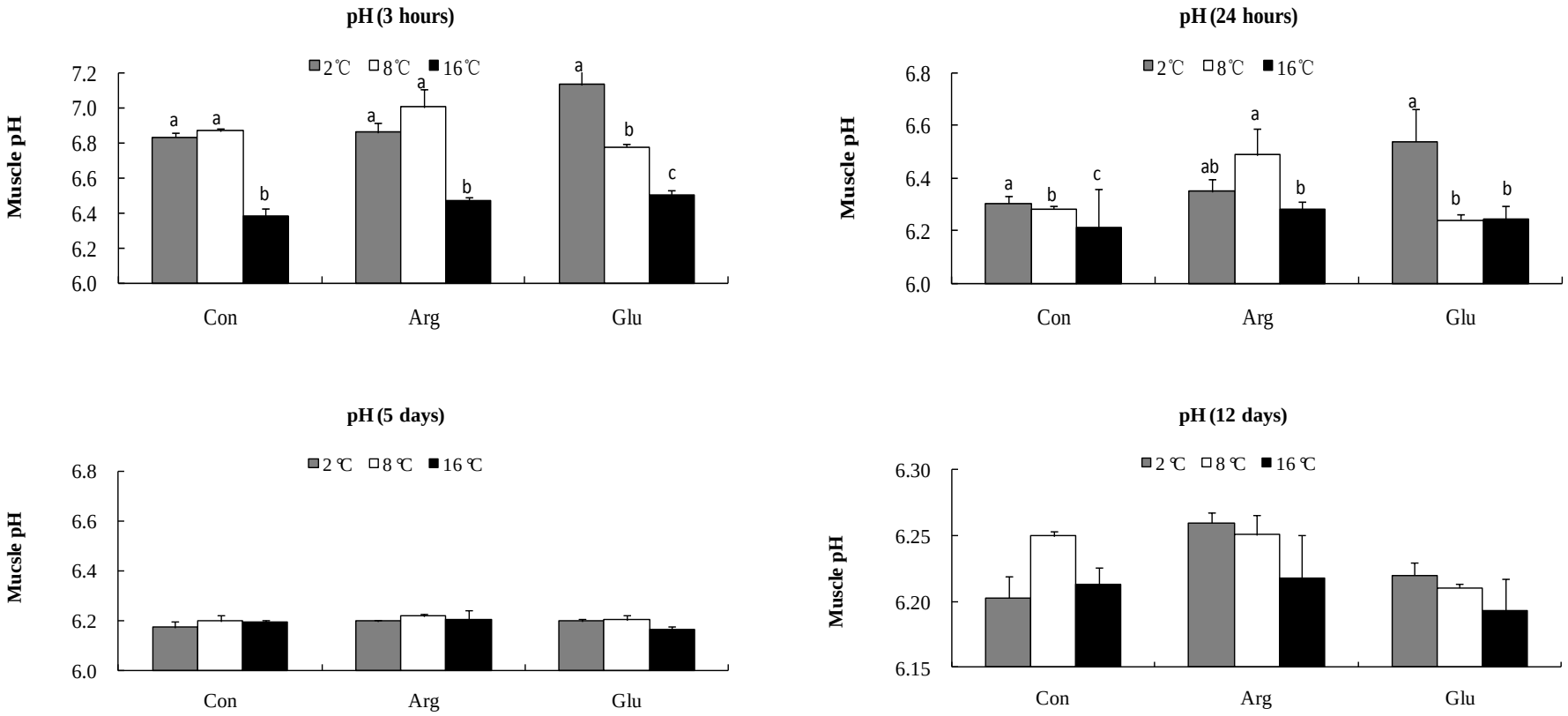


Fig.4.5. Development of pH (mean±SE) during cold storage of pre-rigor fillets of farmed Atlantic salmon fed a commercial diet (Con) or the same diet supplemented with arginine (Arg) or glutamate (Glu). The fish were transferred from cages in seawater (8 ℃) to tanks with 2 ℃, 8 ℃ or 16 ℃ seawater for 3.5 hours before they were slaughtered. Different letters denote significant differences ($p<0.05$) between temperatures within dietary treatments.

Table 4.4. Fillet gaping analysed 24 hours and 5 days post-mortem, and colour and texture, analysed 5 and 12 days post-mortem of farmed Atlantic salmon fed a commercial diet (Con) or the same diet supplemented with arginine (Arg) or glutamate (Glu). The fish were transferred from cages in seawater (8 ℃) to tanks with either 2 ℃, 8 ℃ or 16 ℃ seawater for 3.5 hours before harvesting. Results from ANOVA (model Y=diet+temperature+ε) are presented as P-values for each parameter and for the total model. SEM referres to Standard Error of Mean.

						ANOVA			
Gaping24h		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	0.6	0	0.7	2.91	0.4	0.944	0.776	0.958
	8℃	0.8	1	0.7	4.73				
	16℃	0.5	0.7	0	5.79				
	Average		0.6	0.7	0.6				
Gaping5d		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	1.3	1	2	1	0.3	0.567	0.343	0.507
	8℃	0.8	1	0.7	0.7				
	16℃	0.8	0.3	1	1				
	Average		0.8	1.2	0.9				
Colour5d		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	26.6	26.1	26.8	26.8	0.3	0.765	0.743	0.884
	8℃	26.9	27.1	26.6	27.1				
	16℃	26.7	26.4	27.2	26.5				
	Average		26.5	26.9	26.8				
Colour12d		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	25.8	25.5	25.9	26.1	0.3	0.212	0.932	0.498
	8℃	25.7	25.8	25.7	25.8				
	16℃	25.4	25.4	25.9	25.0				
	Average		25.5	25.8	25.6				
Fb5d		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	9.3	8.9^{b}	8.2^{b}	10.7^{a}	0.4	0.854	0.718	0.908
	8℃	9.4	9.9	9.9	8.6				
	16℃	9.3	9.6^{ab}	8.4^{b}	9.9^{a}				
	Average		9.5	8.8	9.7				
Fb12d		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	10.2	9.1^{b}	9.4^{b}	12.2^{a}	0.6	0.216	0.556	0.371
	8℃	11.1	12.4	11.1	9.9				
	16℃	10.4	10.2^{b}	8.9^{b}	12.0^{a}				
	Average		10.6	9.8	11.4				

Significant differences ($p<0.05$) between dietary treatments within temperature treatments are given by different small letters (horizontal).

Table 4.5. pH analysed after 3 and 24 hours, 5 and 12 days post-mortem of farmed Atlantic salmon fed a commercial diet (Con) or the same diet supplemented with arginine (Arg) or glutamate (Glu). The fish were transferred from the cages in seawater (8 ℃) to tanks with either 2 ℃, 8 ℃ or 16 ℃ seawater for 3.5 hours before harvesting. Results from ANOVA (model Y=diet+temperature+ε) are presented as P-values for each parameter and for the total model. SEM referres to Standard Error of Mean.

							ANOVA		
pH 3h		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	7.0^{A}	6.8	6.9	7.1	0.1	0.562	0.0002	0.0012
	8℃	6.9^{A}	6.9	7.0	6.8				
	16℃	6.4^{B}	6.4	6.5	6.5				
	Average		6.7	6.8	6.8				
pH 24h		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	6.4^{A}	6.3	6.3	6.5	0.04	0.195	0.055	0.070
	8℃	6.3^{AB}	6.3	6.5	6.2				
	16℃	6.2^{B}	6.2	6.3	6.2				
	Average		6.3	6.4	6.3				
pH 5d		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	6.2	6.2	6.2	6.2	0.01	0.450	0.424	0.503
	8℃	6.2	6.2	6.2	6.2				
	16℃	6.2	6.2	6.2	6.2				
	Average		6.2	6.2	6.2				
pH 12d		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	6.2	6.2	6.3	6.2	0.01	0.058	0.123	0.052
	8℃	6.2	6.3	6.3	6.2				
	16℃	6.2	6.2	6.2	6.2				
	Average		6.2	6.2	6.2				

Significant differences ($p<0.05$) between acclimation temperature within dietary treatments are given by different capital letters (vertical).

4.8 Cathepsins analysis

Analyses of variance (ANOVA) of the overall effect of diet and temperature on the activity of cathepsin B showed that acclimation temperatures had a significant effect at 24 hours of storage, and there was also found a significant effect at 0 hour for total activity of cathepsin B+L. The dietary treatment had a significant effect on the activity of cathepsin L at 0 hour (Table 4.3).

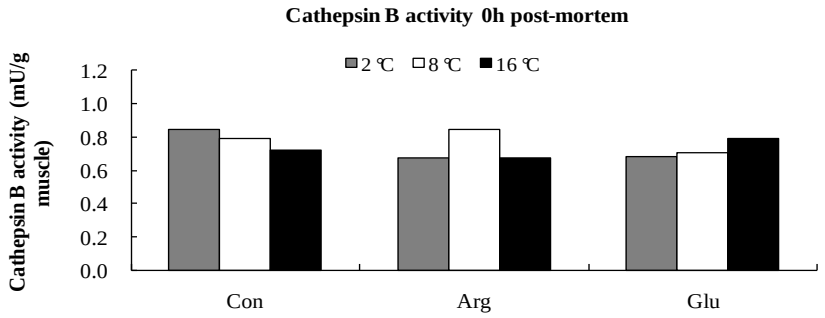
Temperature

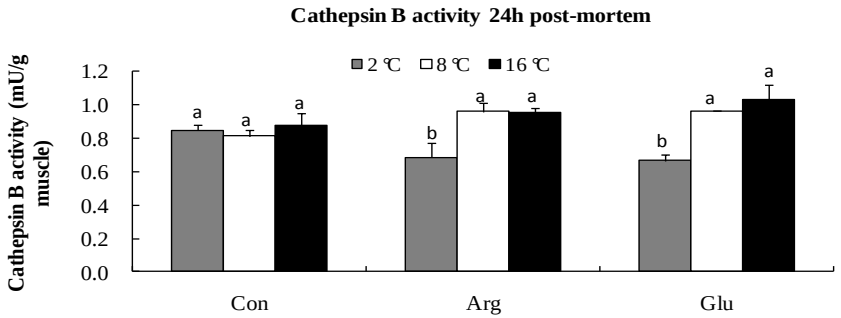
The average activity of cathepsin B at 0 hour was similar for the 2℃, 8℃ and 16℃ group, so was cathepsin L, while an average activity of cathepsin B+L of 2.4 mU/g were reached for salmon kept at 8℃, which was 0.20 mU/g higher than salmon kept at 2℃ and 16℃.

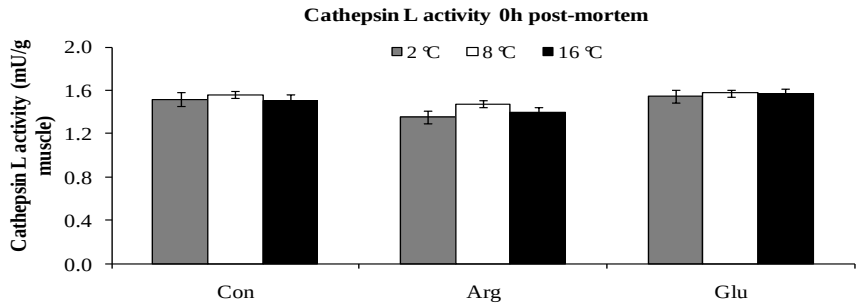
At 24 hours of storage time, the average activity of cathepsin B of the 2℃ group averaged 0.72 mU/g and cathepsin L of the 2℃ group averaged 1.60 mU/g, were both higher than the 8℃ group and the 16℃ group. The total activity of cathepsin B and L at 24 hours of storage was 2.32, 2.46 and 2.37 mU/g for the 2℃, 8℃ and 16℃ group, respectively.

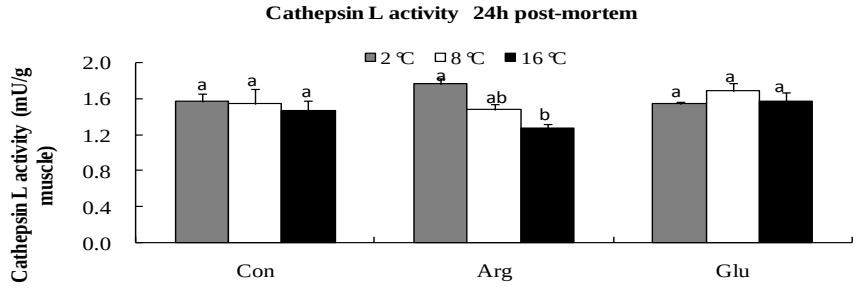
Diet

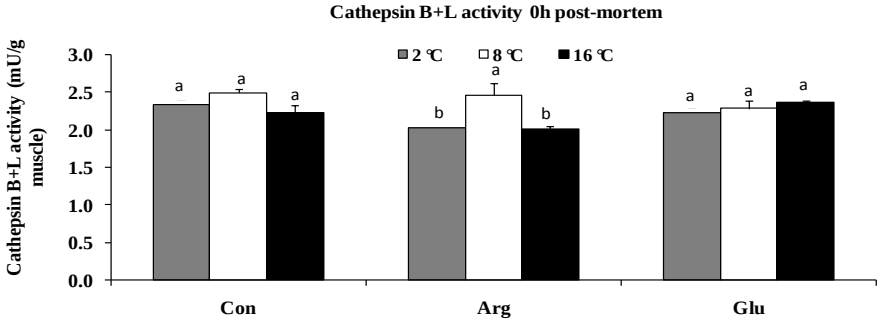
Significant effect of diet was observed for salmon kept at acclimation temperature of 2℃ and 16℃ for cathepsin L at 0 hour. The salmon fed the arginine diet had significantly lowest activity of cathepsin L at 0 hour, which was a 0.12-0.15 mU/g unit lower compared with the salmon fed the control or glutamate diet.


Cathepsin B activity 0h post-mortem
Cathepsin B activity (mU/g muscle)
2 ℃ 8 ℃ 16 ℃
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Con
Arg
Glu


Cathepsin B activity 24h post-mortem
Cathepsin B activity (mU/g muscle)
2 ℃ 8 ℃ 16 ℃
a a a
b a a
b a a
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Con
Arg
Glu


Cathepsin L activity 0h post-mortem
Cathepsin L activity (mU/g muscle)
2 ℃ 8 ℃ 16 ℃
2.0
1.6
1.2
0.8
0.4
0.0
Con
Arg
Glu


Cathepsin L activity 24h post-mortem
Cathepsin L activity (mU/g muscle)
2 ℃ 8 ℃ 16 ℃
a a a
a ab b
a a a
2.0
1.6
1.2
0.8
0.4
0.0
Con
Arg
Glu


Cathepsin B+L activity 0h post-mortem
Cathepsin B+L activity (mU/g muscle)
2 ℃ 8 ℃ 16 ℃
a a a
b a b
a a a
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Con
Arg
Glu

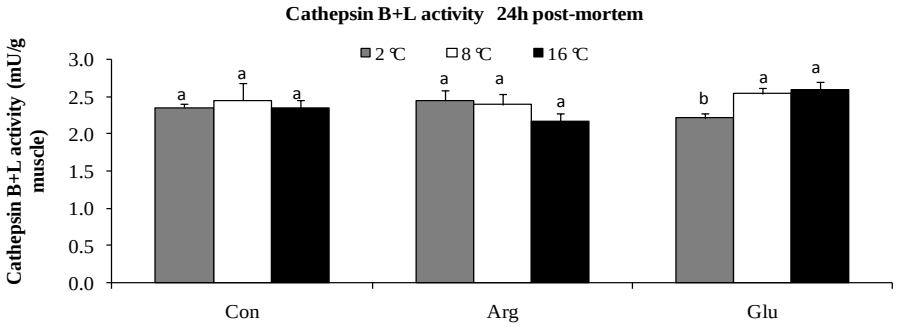

Cathepsin B+L activity 24h post-mortem
Cathepsin B+L activity (mU/g muscle)
2 ℃ 8 ℃ 16 ℃
a a a
a a a
b a a
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Con
Arg
Glu

Fig.4.6 Activity of cathepsin B, L and B+L (mean ± SE) 1h and 24hpost-mortem in muscle of farmed Atlantic salmon fed a commercial diet (Con) or the same diet supplemented with arginine (Arg) or glutamate (Glu). The fish were transferred from cages in seawater (8 ℃) to tanks with 2 ℃, 8 ℃ or 16 ℃ seawater for 3.5 hours before they were slaughtered. Different letters denote significant differences ($p<0.05$) between temperature within diet.

Table 4.6 Development of activity of cathepsin B, L and B+L during 24 hours storage of farmed Atlantic salmon fed a commercial diet (Con) or the same diet supplemented with arginine (Arg) or glutamate (Glu). The fish were transferred from cages in seawater (8 ℃) to tanks with either 2 ℃, 8 ℃ or 16 ℃ seawater for 3.5 hours before harvesting. Results from ANOVA (model Y=diet+temperature+ε) are presented as P-values for each parameter and for the total model.

							ANOVA			
Cathepsin 0h	B		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
		2℃	0.72	0.84	0.67	0.65	0.04	0.460	0.645	0.626
		8℃	0.77	0.79	0.85	0.71				
		16℃	0.74	0.72	0.67	0.79				
		Average		0.78	0.73	0.72				
Cathepsin 0h	L		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
		2℃	1.48	1.52^{a}	1.36^{b}	1.55^{b}	0.03	0.001	0.441	0.031
		8℃	1.54	1.56	1.47	1.58				
		16℃	1.49	1.51^{a}	1.40^{b}	1.57^{a}				
		Average		1.53^{a}	1.41^{b}	1.57^{a}				
Cathepsin 24h	B		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
		2℃	0.72^{B}	0.85	0.69^{B}	0.67^{B}	0.05	0.761	0.004	0.021
		8℃	0.91^{A}	0.81	0.96^{A}	0.96^{A}				
		16℃	0.95^{A}	0.88	0.95^{A}	1.03^{A}				
		Average		0.84	0.86	0.88				
Cathepsin 24h	L		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
		2℃	1.60^{A}	1.57	1.76^{A}	1.54	0.05	0.327	0.111	0.150
		8℃	1.57^{AB}	1.55	1.48^{AB}	1.69				
		16℃	1.44^{B}	1.48	1.28^{B}	1.57				
		Average		1.53	1.48	1.60				
Cathepsin B+L 0h			Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
		2℃	2.21^{B}	2.34	2.03^{B}	2.24	0.06	0.131	0.038	0.049
		8℃	2.41^{A}	2.50	2.47^{A}	2.29				
		16℃	2.20^{B}	2.23	2.01^{B}	2.36				
		Average		2.36	2.18	2.29				
Cathepsin B+L 24h			Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
		2℃	2.32	2.35	2.45	2.21	0.07	0.456	0.392	0.477
		8℃	2.46	2.45	2.39	2.54				
		16℃	2.37	2.35	2.17	2.60				
		Average		2.38	2.31	2.45				

Significant differences ($p<0.05$) between dietary treatments within temperature treatments are given by different small letters (horizontal), and significant differences ($p<0.05$) between temperature treatments within dietary treatments are given by different capital letters (vertical).

4.9 ATP breakdown products

Analyses of variance (ANOVA) of the overall effect of diet and temperature on the content of ATP breakdown products showed that acclimation temperatures had a significant effect on the content of ATP, IMP and Ino after 3 hours storage. There was a significant temperature effect on the content of Hx after one week storage, whereas the dietary treatment only had a significant effect on the content of Hx after 1 week storage (Table 4.6). The effect of temperature on the content of Ino corresponded with a P-value of 0.096 after 1 week storage. The effect of diet on the content of AMP, Hx corresponded with a P-value of 0.083 and 0.078 after 3 hours of storage, respectively.

Temperature

After 3h post-mortem, the average activity of IMP of the 16℃ group averaged 18.02 μm/g, which was 3.3 times higher than the 2℃ group (5.39 μm/g) and 2.9 times higher compared with the 8℃ group (6.32 μm/g). The activity of Hx one week post-mortem was 14.8, 14.2 and 12.9μm/g for the 2℃, 8℃ and 16℃ group, respectively.

Diet

Significant effect of diet was observed only for fillets from salmon kept at acclimation temperature of 8℃, and only for Hx. The salmon fed the arginine diets had significantly highest activity of Hx one week post-mortem.

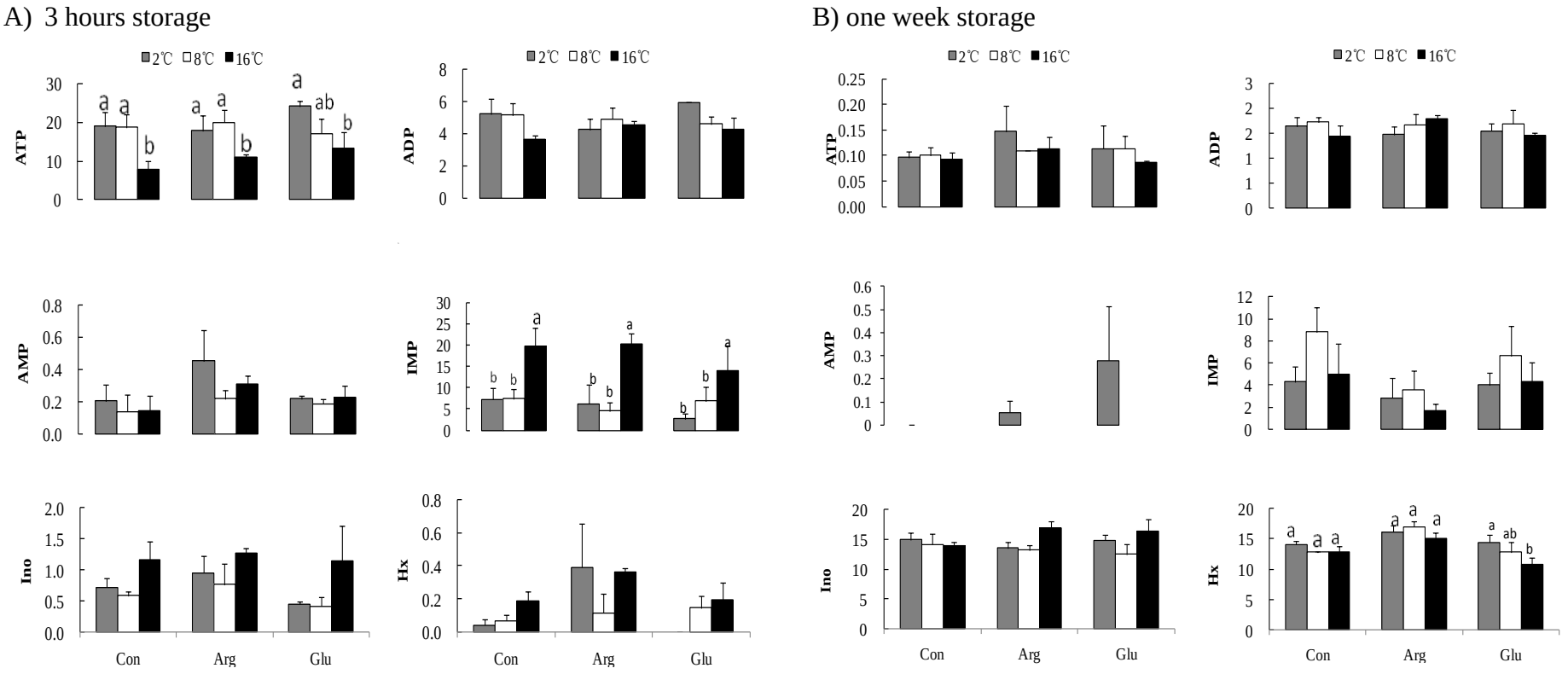


Fig.4.7 Development in adenosine-5'-triphosphate (ATP), adenosine-5'-diphos-phate (ADP), adenosine-5'-monophosphate (AMP), inosine mono-phosphate (IMP), and inosine and hypoxanthine (Hx) of farmed Atlantic salmon fed a commercial diet (Con) or the same diet supplemented with arginine (Arg) or glutamate (Glu). The fish were transferred from the cages in seawater (8 ℃) to tanks with 2 ℃, 8 ℃ or 16 ℃ seawater for 3.5 hours before they were slaughtered. Results are given (mean±SE) for samples frozen at -80 ℃ at 3 hours (A) and one week post-mortem (B). Different letters denote significant differences ($p<0.05$) between dietary treatments within acclimation temperature.

Table 4.7

Development in adenosine-5'-triphosphate (ATP), adenosine-5'-diphos-phate (ADP), adenosine-5'-monophosphate (AMP), inosine mono-phosphate (IMP), and inosine and hypoxanthine (Hx) three hours post-mortem of farmed Atlantic salmon fed a commercial diet (Con) or the same diet supplemented with arginine (Arg) or glutamate (Glu). The fish were transferred from cages in seawater (8 ℃) to tanks with either 2 ℃, 8 ℃ or 16 ℃ seawater for 3.5 hours before harvesting. Results from ANOVA (model Y=diet+temperature+ε) are presented as P-values for each parameter and for the total model. SEM referres to Standard Error of Mean.

						ANVOA			
ATP 3h		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	20.33^{A}	18.99^{A}	17.87^{A}	24.13^{A}	1.76	0.503	0.002	0.007
	8℃	18.59^{A}	18.68^{A}	20.04^{A}	17.05^{AB}				
	16℃	10.70^{B}	7.95^{B}	10.94^{B}	13.22^{B}				
	Average		15.21	16.28	18.13				
ADP 3h		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	5.13^{A}	5.21	4.27	5.93	0.35	0.753	0.153	0.352
	8℃	4.88^{A}	5.15	4.90	4.58				
	16℃	4.16^{B}	3.67	4.52	4.29				
	Average		4.67	4.56	4.93				
AMP 3h		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	0.29	0.20	0.45	0.22	0.05	0.083	0.311	0.128
	8℃	0.18	0.14	0.22	0.19				
	16℃	0.23	0.15	0.31	0.23				
	Average		0.16^{b}	0.33^{a}	0.21^{ab}				
IMP 3h		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	5.39^{B}	7.24^{B}	6.27^{B}	2.66^{B}	1.90	0.391	0.0001	0.0006
	8℃	6.32^{B}	7.46^{B}	4.56^{B}	6.93^{AB}				
	16℃	18.02^{A}	19.86^{A}	20.25^{A}	13.95^{A}				
	Average		11.52	10.36	7.85				
Ino 3h		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	0.71^{B}	0.72	0.95	0.45	0.14	0.289	0.016	0.034
	8℃	0.59^{B}	0.59	0.77	0.42				
	16℃	1.19^{A}	1.17	1.27	1.14				
	Average		0.83	1.00	0.67				
Hx 3h		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	0.14	0.04^{b}	0.39^{a}	0^{b}	0.06	0.078	0.278	0.113
	8℃	0.11	0.07	0.12	0.15				
	16℃	0.25	0.19	0.36	0.20				
	Average		0.10^{b}	0.29^{a}	0.11^{ab}				

Significant differences ($p<0.05$) between dietary treatments within acclimation temperature are given by different small letters (horisontal), and significant differences ($p<0.05$) between temperature treatments within dietary treatments are given by different capital letters (vertical).

Table 4.8

Development in adenosine-5'-triphosphate (ATP), adenosine-5'-diphos-phate (ADP), adenosine-5'-monophosphate (AMP), inosine mono-phosphate (IMP), and inosine and hypoxanthine (Hx) one week post-mortem of farmed Atlantic salmon fed a commercial diet (Con) or the same diet supplemented with arginine (Arg) or glutamate (Glu). The fish were transferred from cages in seawater (8 ℃) to tanks with either 2 ℃, 8 ℃ or 16 ℃ seawater for 3.5 hours before harvesting. Results from ANOVA (model Y=diet+temperature+ε) are presented as P-values for each parameter and for the total model. SEM referres to Standard Error of Mean.

						ANVOA			
ATP 1w		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	0.12	0.10	0.15	0.11	0.01	0.404	0.579	0.567
	8℃	0.11	0.10	0.11	0.11				
	16℃	0.10	0.09	0.11	0.08				
	Average		0.10	0.12	0.10				
ADP 1w		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	1.56	1.64	1.48	1.54	0.10	0.826	0.495	0.765
	8℃	1.70	1.72	1.68	1.70				
	16℃	1.56	1.45	1.79	1.46				
	Average		1.60	1.65	1.57				
AMP 1w		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	0.11	0	0.05	0.28	0.05	0.339	0.173	0.231
	8℃	0	0	0	0				
	16℃	0	0	0	0				
	Average		0	0.02	0.09				
IMP 1w		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	3.72	4.29	2.87	4.01	1.02	0.087	0.128	0.072
	8℃	6.35	8.80	3.58	6.68				
	16℃	3.65	4.97	1.69	0				
	Average		6.02	2.71	4.99				
Ino 1w		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	14.45	14.94	13.58	14.82	0.76	0.976	0.096	0.295
	8℃	13.30	14.15	13.22	12.54				
	16℃	15.74	13.99	16.91	14.30				
	Average		14.36	14.57	14.56				
Hx 1w		Average	Control	Arginine	Glutamate	SEM	P-diet	P-temp	P-model
	2℃	14.83^{A}	14.02	16.10	14.37^{A}	0.54	0.0005	0.051	0.0007
	8℃	14.19^{AB}	12.75^{b}	16.99^{a}	12.83^{Bb}				
	16℃	12.86^{A}	12.78	14.99	16.33^{A}				
	Average		13.19^{b}	16.03^{a}	14.51^{b}				

Significant differences ($p<0.05$) between dietary treatments within acclimation temperature are given by different small letters (horizontal), and significant differences ($p<0.05$) between temperature treatments within dietary treatments are given by different capital letters (vertical).

5. DISCUSSION

5.1 Growth performance

The present study showed that feeding a diet supplemented with arginine or glutamate did not significantly improve the overall growth of Atlantic salmon during the seawater rearing phase (May 2009-May 2010). In a previous experiment (Oehme et al., 2010), a combination of arginine and glutamate, with supplementation levels of 1.1% and 0.75% respectively, resulted in a significant increased growth from July to September 2007 for salmon transferred to seawater (May 2007).

The results therefore suggest that there might be a synergy between arginine and glutamate, which means they are more successful when they work together than when they are added separately. The feed formulation and fish size may be other factors affecting the final results.

5.2 Composition of fillets

The fat content of the salmon in the present study showed no significant differences between the dietary treatments. However, the liver of the control group was larger of the salmon fed the control diet, indicating larger lipid accumulation of this organ. Fatty livers are frequently associated with metabolic disturbances in mammals. On the other hand, a previous study showed that salmon fed a diet supplemented with a combination of glutamate and arginine for approximately four months after they were transferred to seawater had significantly higher fillet fat content than fish fed a control diet (Oehme et al., 2010).

As expected, the fish that got supplementation of arginine had a higher concentration of free arginine in the skeletal muscle. None of the other selected amino acids or metabolites except urea was significantly different between feeding groups. Arginine can be metabolised to urea by the cytosolic enzyme arginase in the Urea Cycle (Wu and Morris, 1998). However, the content of free glutamate in the muscle was not significantly higher in the salmon that were fed the glutamate added diet. Hence, the supplemented glutamate may have been metabolized in the gut for energy use since glutamate is known to be a suitable energy substrate (Tapiero et al., 2002).

5.3 Fillet contraction

It is well known that handling stress prior to slaughter can result in poor quality of fish fillets and lower fillet yield (Basrur et al., 2010; Erikson et al., 1997a; Matos et al., 2010; Merkin et al., 2010; Sigholt et al., 1997; Skjervold et al., 2001a) . Stress can also cause considerable physiological responses in fish, for example, the release of several hormones such as catecholamines and cortisol from the chromaffin and interrenal cells, respectively (Einarsdóttir and Nilssen, 1996). Elevated plasma levels of cortisol, glucose, lactate and osmolality are other stress response parameters (Einarsdóttir and Nilssen, 1996; Rotllant and Tort, 1997; Skjervold et al., 1999), but in the present study plasma parameters were not determined.

The current study showed that the acclimation temperatures before slaughter had a significant effect on the fillet contraction from 3-12 hours storage, whereas the dietary treatment had a significant effect on the fillet contraction at 24 hours storage. It was shown that the onset of rigor mortis (contraction of fillets) for fish kept at higher temperature (16℃) was faster than the onset for fish kept at lower temperature (2℃). Since stress during harvest processing may accelerate onset of rigor mortis (Sigholt et al., 1997), a common procedure is to keep farmed salmon in refrigerated seawater (approximately 2℃) for 40-60 minutes before slaughtering in order to calm the fish and hence delay time to onset of rigor mortis (Skjervold et al., 1999). The present study showed that salmon reared at 8℃ and thereafter acclimated to 2℃ during 3.5 hours before slaughtering had a delayed rigor development. Reasons may be the lower temperature and activities of muscle at death and after the live-chilling process. However, no previous studies have reported the effect on rigor development and subsequent fillet quality of salmon kept at temperature above their rearing temperature. Keeping the salmon at high acclimation temperature (16℃) may be considered to be a thermal stressor that causes faster onset of rigor mortis.

In addition to seawater temperature before slaughter, dietary effects were also observed. The arginine group that was kept at 8℃ before slaughter reached a significant lower fillet contraction compared to the control group after 24 hours storage; indicating that arginine or metabolites from arginine influences post-mortem muscle contraction.

5.4 Fillet gaping

Gaping is considered to be a post-mortem phenomenon (Robb et al., 2000). The cause of gaping can be described as the interaction between forces pulling the muscle apart, and the strength of the connective tissue, which produces flaking of the fillet (Kiessling et al., 2004). Commercially gaping is a problem because it makes the fillets much more difficult to process. However, neither acclimation temperatures nor the dietary treatment had any significant effect on the gaping in this experiment. The gaping score of all fish was indicated a strong muscle structure of the salmon in general. Gaping is known to vary significantly between seasons. Hence, it is possible that the effect of acclimate temperature or diet would have been more pronounced if the fish were slaughtered when their structure was weaker in general.

5.5 Fillet colour and pigmentation

Colour is an important characteristic used to establish the acceptance of various food products (Barbut, 2004). Consumers purchasing fish in supermarkets have few means of evaluating the flavor, tenderness, and juiciness of fillets. Therefore, the red muscle colour of salmonid fishes is an important quality criterion for consumer acceptance, as they commonly base their selection on visual appearance when purchasing various meats (Barbut, 2001). Muscle colour in salmonids results from deposition of dietary carotenoids (Bjerkeng, 2000; Buttle et al., 2001).

Improved fillet colour of salmon kept at 1℃ for 45 minutes before slaughtering was found by a previous study (Skjervold et al., 2001b). However, the present study showed that acclimation temperatures and diet had no overall effect on the colour

score or content of pigmentation after 5 days or 12 days of storage. These results are not consistent with previous studies that showed that fillets of fish exposed to pre-slaughter handling stress became paler compared to fish with normal handling (Kiessling et al., 2004; Robb et al., 2000). However, the deviating results may be due to different stressors or the size of fish used in these experiments. Because colouration is so important for consumer preferences, the effect of thermal stressor before slaughtering on salmon flesh colour should be further studied.

In line with the present study, (Bjerkeng et al., 1997), found no significant impact of dietary protein source on flesh colouration of salmon fed a diet using fish meal compared to full-fat soybean meal.

5.6 Texture analysis

Food texture covers several related physical properties, including resistance to chewing, tenderness, and juiciness. Soft flesh can cause quality downgrading in the salmon processing industry and reduced acceptability by consumers. Soft flesh in fish is believed to be associated with reduced content and reduced strength of the connective tissue (Hatae et al., 1986; K. Sato and Yoshinaka, 1986; Mørkøre and Austreng, 2004). Neither acclimation temperatures nor dietary treatment had an overall significant effect on fillet firmness measured as breaking force. However, supplementation with glutamate resulted in firmer texture in salmon acclimated to 2℃ or 16℃ at both 5 and 12 days of storage. These results might relate to the effect of glutamate on muscle fibres synthesis as glutamate is a precursor of proline and arginine that are involved in the synthesis of collagen and connective tissue (Reeds et al., 2000). There are a number of various factors responsible for the fillet texture; for example a low pH level and/or a fast pH reduction rate post-mortem may be associated with soft texture in salmon (Mørkøre et al., 2008). The muscle pH of the glutamate group acclimated to 2℃ was higher as compared with the other dietary groups at 3 and 24 hours storage.

5.7 Muscle pH

Lactate is accumulated during anaerobic glycogen breakdown post mortem and the reduction of pH is caused by formation of lactate from breakdown of muscle glycogen; hence lactate and pH in muscle are correlated. The muscle pH decreased during the storage period for all dietary groups, reaching a final level of approximately 6.2. However, there was a small increase of pH for the Arg group at 2℃ and 8℃ acclimation temperature after 12 days of storage, indicating initiation of spoilage of these groups. The reduction rate of the muscle pH affects rigor-related enzyme activity and therefore not only the delay and onset of rigor but also the proteolytic activity causing the resolution of rigor (Skjervold et al., 2002). The final pH in muscle has an important influence on the textural quality of meat, water holding capacity and colour (Mørkøre, 2006). Soft flesh has been associated with rapid pH decline and lower final pH through a weakening of the connective tissue, denaturation of proteins and an increased amount of proteolysis (Mørkøre et al., 2008). Acclimation temperatures had a significant effect on the pH at early storage time. Results showed a lower muscle pH post mortem or a higher lactate level in fish kept at higher temperature before slaughter than fish kept at lower seawater temperatures. These results are in accordance with some previous studies showing lower ultimate pH in

stressed salmon (Mørkøre et al., 2008; Skjervold et al., 2001a). Hence, the results of pH also indicate that high acclimation temperature caused stress responses in the fish. No overall dietary effect was observed, but a higher muscle pH during the first 24 hours of storage of the Glu group indicates that added levels of glutamate improved the ability of the salmon to cope with stress related to slaughter handling or improved buffer capacity of the muscle.

5.8 Cathepsins activity

Cathepsins belong to a large family of lysosomal cysteine proteases which have been associated with post mortem degradation of mammalian and fish muscle (Bahuaud et al., 2008). An increased cathepsin activity indicates breakage of the lysosomal membrane, releasing degrading enzymes, and i.e. the start of fish muscle proteolysis (Bahuaud et al., 2009). In salmon, cathepsins B and L are considered to be the enzymes playing the most important role in post-mortem muscle softening. The level of cathepsin L activity was dominant when compared to cathepsin B, since the activity of cathepsin L was almost two times higher than cathepsin B in all groups. These results were in accordance with previous results reported by Bahuaud et al. (2009).

There was a significant acclimation temperatures effect on the total activity of cathepsin B+L immediately after slaughter. In salmonids, cathepsin B and cathepsin L activity have been associated with the pH level in the muscle. Results from a previous study indicated that a lower pH in stressed fish could explain a higher activity of cathepsin B+L in salmon muscle after slaughtering (Rahnama, 2010). However, the present study was not in accordance with that suggestion. The lowest pH was found in the muscle of fish kept at 16℃ while the highest activity of cathepsin B+L was found in fish kept at 8℃. The results indicate that acclimation temperature before slaughtering (higher, 16℃ or lower, 2℃) than harvest seawater temperature (8℃) influences the activity of cathepsin B+L.

5.9 ATP breakdown products

Nucleotide degradation in fish muscle commences at early post mortem and proceeds throughout storage. Hypoxanthine (Hx) is formed as a by-product from the degradation of ATP. The accumulation of hypoxanthine has shown potential as an index of freshness (Beuchat, 1973). Freshness is thought to be the most important quality parameter. Hx is considered to be a good biomarker for unpleasant flavours in stored salmon, while IMP contributes to the pleasant, fresh flavors (Mørkøre et al., 2010b). There was a significant effect of acclimation temperatures and dietary treatment on the content of Hx after one week storage. The fish kept at 16℃ had a significantly lower content of Hx after one week storage as compared with the fish kept at lower temperatures. According to previous results (Mørkøre et al., 2010a), a significant inverse relationship was found between the rearing temperature in seawater and the accumulation of Hx in the salmon muscle during storage. Several experiments have shown that crowding as a stressor can accelerate breakdown of ATP (Erikson et al., 1997a; Erikson et al., 1997b; Mørkøre et al., 2008), but they are not consistent with the results from the present study as higher acclimation temperature as a thermal stressor before slaughtering resulted in lower accumulation of Hx. High acclimation temperature before slaughter may alter the enzyme activity and possibly

cause some temperature adaptation. When placing the fillets on ice post-mortem, the chilled storage temperature may be more efficient for salmon acclimated to high temperatures than for fillets of salmon acclimated to lower temperature (△℃ higher for salmon acclimated to high temperature).

6 CONCLUSION

Dietary supplementation of arginine or glutamate had no overall effect on slaughter parameters in Atlantic salmon, except from the liver size that was smaller as compared with salmon fed the control diet. A firmer texture of salmon fed the diet supplemented with glutamate and acclimated to 2℃ or 16℃ is considered as a very positive result. The results further indicate that the glutamate level in commercial salmon diets might be too low to obtain a premium texture quality, although the level might be sufficient for adequate production efficiency. Salmon fed diets supplemented with arginine had a slower fillet contraction rate compared with the other groups when they were kept at 8℃ before slaughter. However, the spoilage rate of this group seemed to be faster, as the hypoxanthine level and pH were highest after one week storage. Hence, the present results are questioning the established measure of rigor development as a marker for prolonged freshness of salmon, since high levels of hypoxanthine and increased pH during prolonged storage indicate reduced sensory quality and bacterial growth, respectively.

The present study is the first to report the fillet quality of Atlantic salmon acclimated to pre-slaughter seawater temperature above the rearing temperature. It was shown that the onset of rigor mortis for fish kept at higher temperature (16℃) was faster than the onset for fish kept at lower temperature (2℃). Results also showed a lower muscle pH at early storage time in fish kept at higher seawater temperature before slaughter. High acclimation temperature accelerated activity of cathepsin B at 24 hours storage. On the other hand, salmon kept in 16℃ seawater before slaughter had a lower content of Hx after one week storage, indicating prolonged sensory freshness. Acclimation temperature and dietary treatment had no significant effect on fillet gaping or colour score.

Results from the present thesis have contributed with novel knowledge regarding the impact of dietary treatment of salmon and acclimation temperature before slaughter. However, further studies are required to obtain a greater depth of understanding of the underlying mechanisms and the complex basis of factors affecting the salmon quality, and interaction among the various factors.

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