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EFFECTS OF DIETARY OXIDATION STATUS AND VITAMIN E LEVEL ON PERPORMANCE, FILLET QUALITY AND ROBUSTNESS TO ACUTE STRESS IN ATLANTIC SALMON (*SALMO SALAR L.*)

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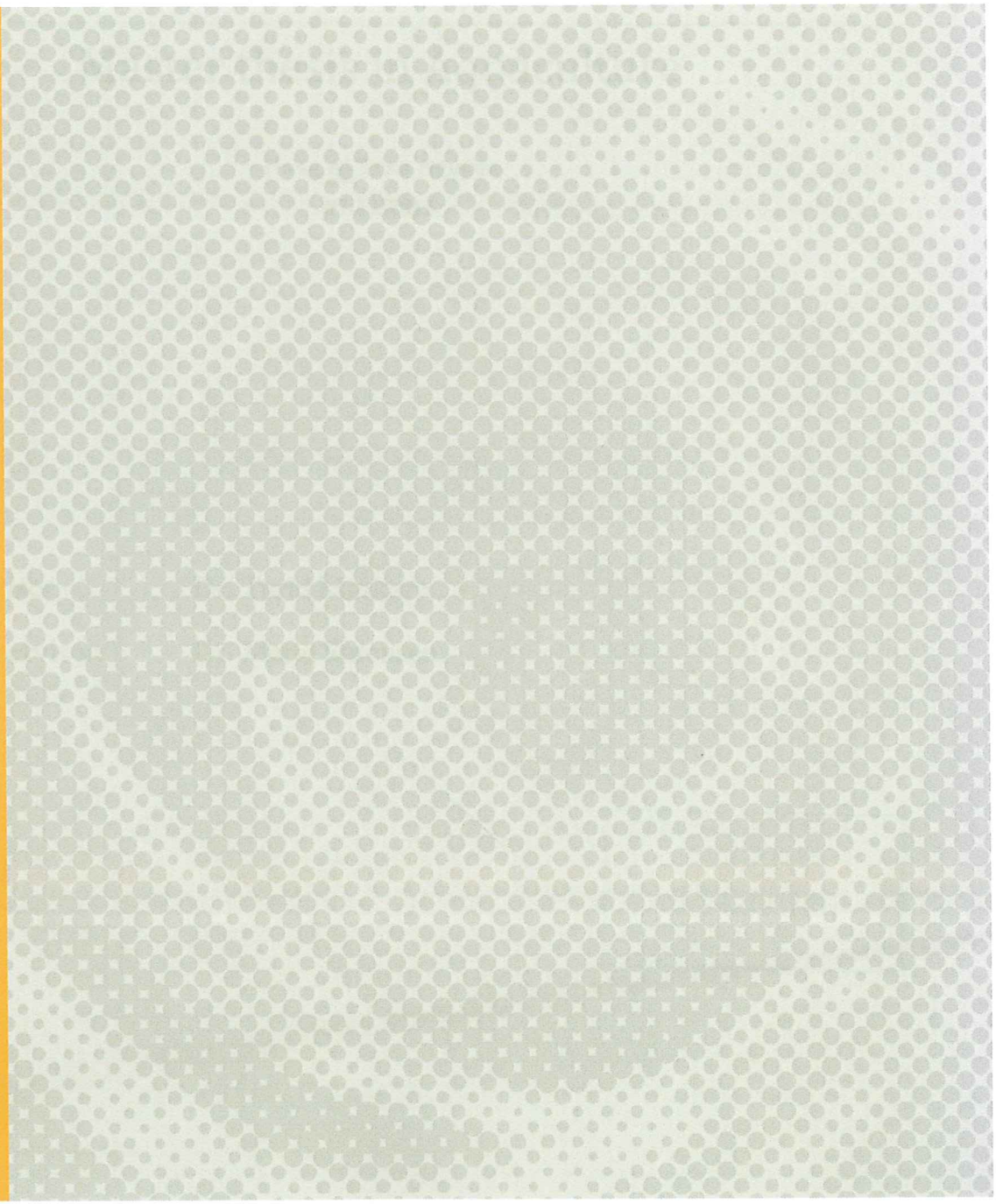

NORWEGIAN UNIVERSITY OF LIFE SCIENCES (UMB)

Effects of dietary oxidation status and vitamin E level on performance, fillet quality and robustness to acute stress in Atlantic salmon (*Salmo salar* L.)

Master thesis in Aquaculture

Hang Ai Tran Nguyen

07/11/2011

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- Co-supervisor: Dr. Mette Sørensen

Dedication

I would feel pride to dedicate this thesis to my philanthropic parents, my lovey-dovey husband and last but not least, my cute boy.

Acknowledgments

The practical part of the study presented in the thesis was carried out at Nofima Marine as a part of my Master of Science degree at the Department of Animal and Aquaculture Sciences, Norwegian University of Life Science, Ås.

I would like to express my heartiest gratitude to my supervisors, Dr. Turid Mørkøre and Dr. Mette Sørensen for their guidance, valuable advices and constructive suggestions throughout the accomplishment of this thesis.

Thanks to all my dear friends at UMB for their positivism, inspiration and most of all their precious friendship.

Furthermore, I would like to thank my closest family, my husband and his family for their love and support during my study time at Norwegian University of Life Sciences.

Ås, 07/11/2011

Hang Ai Tran Nguyen

Abstract

The effects of oxidized dietary lipid and the role of vitamin E on the growth performance, biometric traits, blood parameters, muscle pH and fillet quality of Atlantic salmon (*Salmo salar* L.) were evaluated after a 79-day feeding period. Five hundred sixty fish with mean initial bodyweight of 2.12 kg were distributed into eight cages and fed four different experimental diets. The salmon were fed a commercial diet added fresh or oxidized rapeseed oil with standard (200mg/kg) or elevated (1200mg/kg) vitamin E. The fresh and oxidized rapeseed oil used had the anisidin value of 2.3 and 33 (meq peroxide/kg) and peroxide value of 3.3 and 24 (meq peroxide/kg), respectively.

Growth performance showed no significant dietary effects, but the feed conversion rate (FCR) of the fish fed fresh oil in combination with elevated vitamin E was lowest compared with other diets. No significant differences in organ adhesions (Speilberg score), spleen somatic index, slaughter yield, fillet yield or fillet fat content were observed due to dietary treatment. However, oxidized oil tended to increase heart somatic index and significantly reduced the fillet thickness. Livers of salmon fed high vitamin E levels were larger and paler than those of salmon fed the diets without vitamin E supplementation, irrespective of oil quality.

The fish were slaughtered immediately after netting (non-stressed) or they were kept in seawater with decreasing dissolved oxygen (DO), from 9 to 2mg/L (stressed) before slaughtering. For the non-stressed group, supplementation with vitamin E resulted in higher levels of Na^+ and K^+ in the blood, while oxidized lipid had tended to increase the hemoglobin level although these effects were not significant. The diet added fresh oil without vitamin E supplementation had significantly highest pH after 3h storage, but no significant dietary effects were observed on muscle pH after 24h, pH in blood, liquid loss or gaping., Vitamin E supplementation had a positive effect on the fillet firmness, independent of dietary oil quality.

Acute stress due to exposure to low DO level had a negative effect on the osmoregulation, muscle pH after 3h and liquid loss whereas no significant effects on firmness or gaping were observed. However, vitamin E supplementation appeared to lower Na^+ and K^+ increase and drop in muscle pH due to acute stress. Statistical analyses revealed significant interactions

between stress and oxidized oil. Indeed, hemoglobin level was higher and pH in blood was lower of the fish fed oxidized oil than in those fed.

These results demonstrated that feed containing oxidized oil increased the hemoglobin concentration, accelerated glycolytic activity post-mortem and also negatively affected the fillet thickness. Salmon fed oxidized oil had less ability to cope with acute stress, in particular impaired osmoregulation ability. High vitamin E levels improved the feed utilization, fillet firmness and the ability of the fish to cope with acute stress before slaughtering. However, paler and larger livers, increased Na^+ and K^+ levels in the blood and faster pH drop in the muscle post-mortem might be considered as negative. Hence the level and period of vitamin E supplementation need to be ascertained to achieve optimal production efficiency, physiological status and fillet quality of farmed salmon.

Keywords: *Salmon feed, Oxidized oil, Vitamin E, Slaughter stress, Growth, Blood parameters, Organs, pH, Fillet quality.*

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List of abbreviation

AA	Arachidonic acid
ATP	Adenosine triphosphate
AV	Anisidin value
C	Fresh oil
CE	Fresh oil + vitamin E supplementation
CF	Condition factor
DHA	Docosahexaenoic acid
EPA	Eicosapentaenic acid
EFA	Essential fatty acid
FAO	Food and Agriculture Organization
FCR	Feed conversion rate
IFFO	International Fish Meal and Fish Oil Organiztion
Ox	Oxidized oil
OxE	Oxidized oil + vitamin E supplementation
PUFAs	Polyunsaturated fatty acids
PV	Peroxide value
HUFAs	Highly unsaturated fatty acids
HSI	Hepato-somatic index
SEM	Standard error of mean
TGC	Thermal growth coefficient
WG	Weight gain

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1. Introduction

The world population is increasing dramatically, and is estimated to reach 10 billion people in 2050 (Fig 1). Consequently, production and supply of food are major issues to satisfy global demand, in particular in the developing countries.

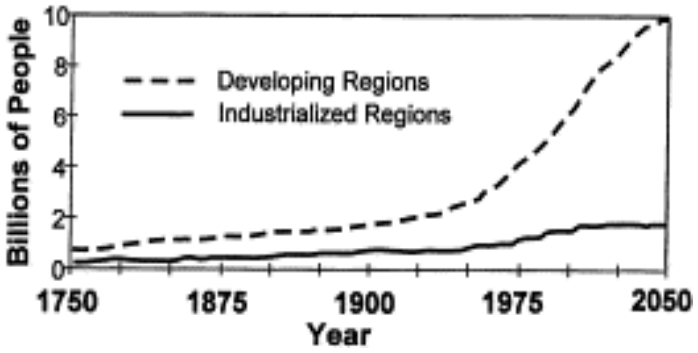

Fig 1. Trends in global human population growth from 1750 to 2050 (data from World Resources Institute, Washington, D.C.) (Welch & Graham 1999)

According to Worldatlats, 70.9% of earth's surface is covered by ocean. Thus, fish is projected to contribute to a significant part of the food supply in the future. As reported by FAO (2010), global total catch and aquaculture production of fish, crustaceans and molluscs reached 142 million tonnes in 2008. China is the largest fish-production country so far, with a production of 47.5 million tonnes in 2008, (32.7 and 14.8 million tonnes from aquaculture and capture fisheries, respectively) (Fig 2). However, most capture fisheries resources are fully exploited, and the volume has stabilized around 90 million tonnes since 2001. During the last decade, aquaculture production has had an average annual growth rate of 6.2 percent from 38.9 million tonnes in 2003 to 52.5 million tonnes in 2008 (FAO, 2010). Of this, 115 million tonnes were used as human food, providing an estimated apparent per capita supply of about 17 kg (live weight equivalents) (Fig 3). Thus, the increased seafood production from aquaculture has to meet the future shortfall in fish supplies.

Norway is the world's largest producers and exporters of Atlantic salmon. Norway's long coastline and cold, fresh seawater provides excellent conditions for aquaculture activities of salmonids. According to The Norwegian Ministry of Fisheries and Coastal Affairs, in 2009, Norway produced approximately 850 000 tonnes of Atlantic Salmon (*Salmo salar*) and the total value of exports reached 23.7 billion NOK, which is an measure of 32 % compared to 2008.

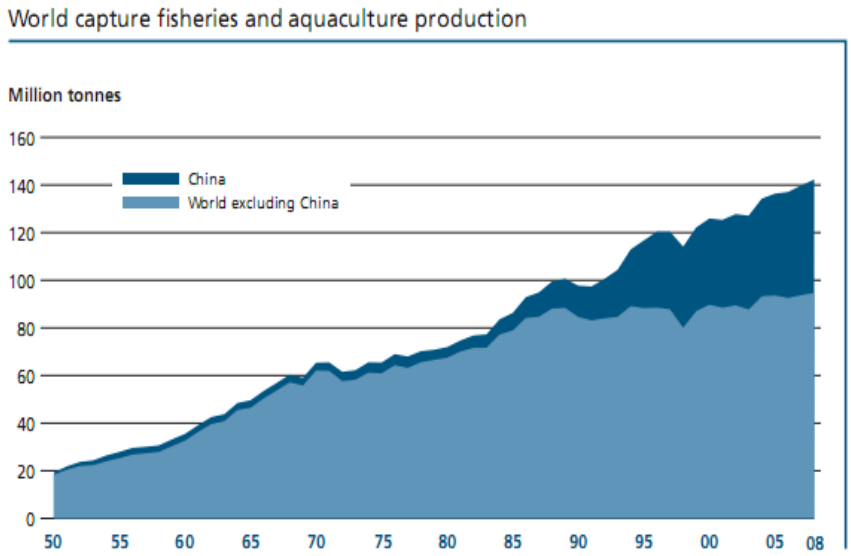

Fig 2. World capture fisheries and aquaculture production (Source: FAO, 2010)

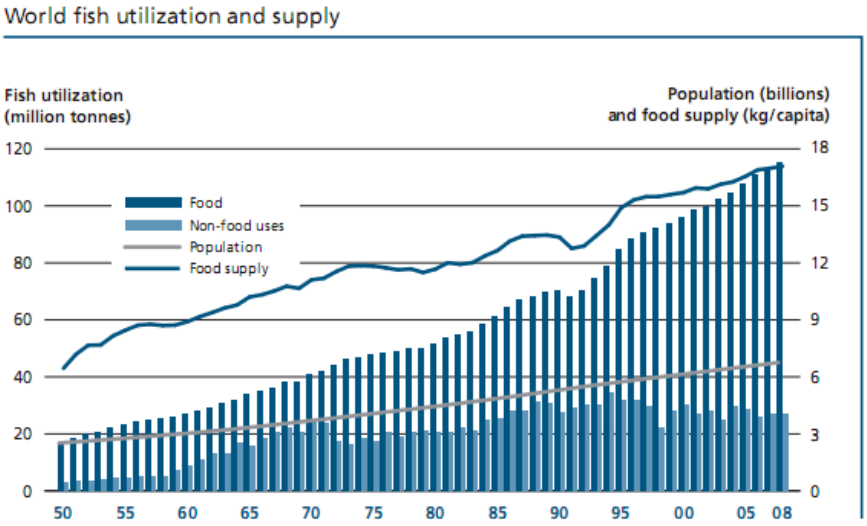

Fig 3. World fish utilization and supply, excluding China. (Source: FAO, 2010)

Generally, it is well known that Atlantic salmon is healthy food for human, mainly due to its content of the long-chained fatty acids EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid), as well as the content of zinc, selenium and iodide. In addition, several important fat-soluble vitamins such as vitamin E, A, D present at high amounts together with these fatty acids in flesh fish (Nortvedt *et al*. 2007). However, flesh quality in fish is affected by various different factors, such as husbandry practices, feed, slaughter method, stress before slaughter and handling post mortem (Ruff *et al*. 2002). Thus, to improve flesh quality pre-slaughter, modifying the fish feed, avoiding stress before slaughter and proper post-mortem handling should be considered.

1. Introduction

The proximate composition of the diet for Atlantic salmon has changed over the past 30 years. The lipid content has increased and the protein content has decreased in order to promote a cost efficient feed to support fast growth, as means to reduce the time needed for a suitable market size of the fish (Nortvedt *et al*. 2007). However, higher lipid content in the feed have also resulted in greater fat deposition in the fish (Einen *et al*. 2007). Also the lipid composition of the flesh reflects the lipid composition of the diet offered to the fish. Thus, feed for Atlantic salmon should contain high lipid level, especially polyunsaturated fatty acids (PUFAs) which are necessary to ensure high growth rate and high content of PUFAs in the flesh fish.

High levels of PUFAs in the feed and consequently also in the flesh may cause challenges because these oils are more prone to oxidation, which may cause problems both for the feed quality as well as fillet quality post slaughtering (Ruff *et al*. 2002). Lipid oxidation in the feed during storage will reduce the content of PUFAs and is also causing rancid odor that may negatively interfere with feed intake. Moreover, for flesh fish, not only does lipid oxidation result in the degradation and loss of highly nutritious PUFAs, but also in the deterioration of color, texture and flavor of the product (Waagbø *et al*. 1993). Vitamin E is a natural antioxidant that can protect HUFAs from oxidation. Improved flesh quality by feeding elevated levels of α-tocopheryl acetate before slaughter has been achieved in sea bass (Gatta *et al*. 2000), trout (Frigg *et al*. 1990) and channel catfish (Gatlin 1992). It is also well established that stress before slaughter has a major impact on the flesh quality of fish. Stress may be caused by crowding, handling and low levels of oxygen or poor water quality due to inadequate water exchange that causes accumulation of excreted carbon dioxide and ammonia during transport from the sea cage to the factory (Erikson *et al*. 1997). Thus, minimal stress for fish before slaughter should be achieved to avoid negative effect on fillet quality.

Not much information is available investigating effects of rancid oil in combination with vitamin E supplementation of the feed on growth performance and product quality. More information is also needed about the interaction between feed quality and pre-slaughter stress on fillet quality of Atlantic salmon. Thus, the aim of the present study was to investigate effects of oxidized dietary lipid and vitamin E supplementation on growth performance, biometric traits, blood parameters and fillet quality. A second aim was to investigate effects of stress associated with low water oxygen prior to slaughtering on product quality.

2. Theoretical background

2.1 Lipid used in feed for Atlantic salmon

Lipids are important constituents in blood lipids, in fat deposits and in biological membranes of the body (Ratnayake & Galli 2009). In fish, the well-know role of fatty acids is to generate metabolic energy in the form of ATP via mitochondrial β-oxidation (Sargent *et al*. 1989). They are not only the prime source of metabolic energy in fish for growth from the egg to the adult fish, but also are the prime source of metabolic energy in fish for reproduction (Sargent *et al*. 1999).

Fish oil used to be the main lipid source in fish diets due to the rich essential highly unsaturated fatty acid (HUFA) content. As reported by Jackson (2007), 43% of the global fish oil production was used for salmon feed (Fig 4).

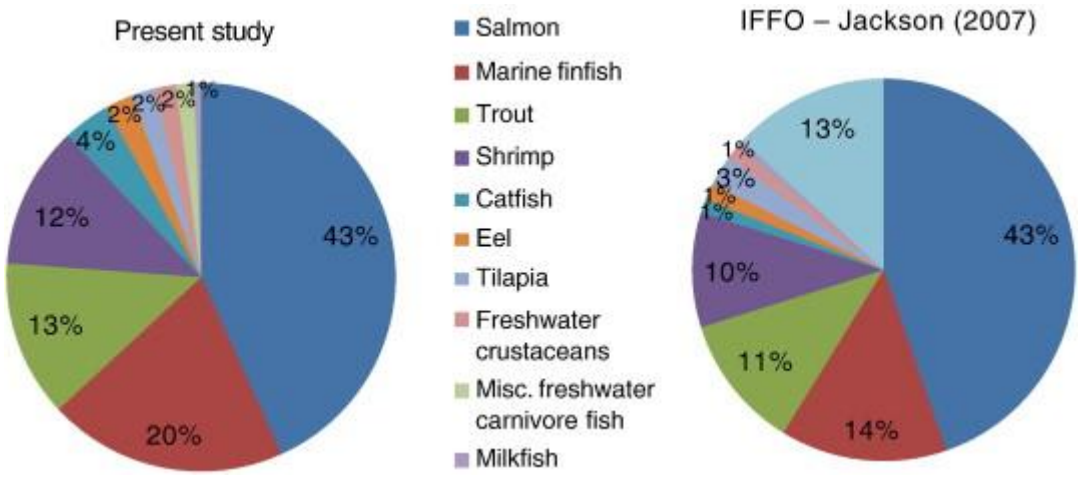

Fig 4. Estimated global use of fish oil within compound aquafeeds in 2006 from Jackson (2007) and IFFO (International Fish Meal and Fish Oil Organization) estimation (values given in percent total aquafeeds:(Jackson 2007))

The global production of fish oil is mainly based on e.g., capelin, herring, sand eel, mackerel, anchovy, and sardine fisheries. However, the limit of sustainability has been reached by overfishing, climate alternations and demand of other users e.g., poultry producers (Opsahl-Ferstad *et al*. 2003; Sargent & Tacon 1999; Tacon & Metian 2008). The supply of fish oil has reached the limit and is not expected to increase in coming years. Consequently, use of plant oils, such as linseed, rapeseed or soybean oils, have become the potential alternative lipid sources in salmonids and freshwater fish feeds without affecting growth performance and feed conversion (Montero *et al*, 2005). Turchini *et al*. (2009) reported that 60 – 75%

replacement of fish oil with alternative lipid sources in almost all finfish do not affect fish performance (growth, feed efficiency, feed intake) and met the demand for essential fatty acid (EFA) requirements by the fish (Turchini *et al*. 2009). Sales & Glencross (2010) used meta-analysis to evaluate the effects of the replacement of marine oils (MO) with canola oil (CO), linseed oil (LO) and soybean oil (SO) on growth, feed conversion and major muscle fatty acid (FA) classes. They concluded that 100% MO replacement with plant oils has a negative effect on growth, but a medium mean effect size (95% confidence intervals) for growth was obtained when replacing by CO (Sales & Glencross 2010).

Essential fatty acids for marine fish such as Atlantic salmon, are docosahexanenoic acid (DHA, 22:6*n*-3), eicosapentaenoic acid (EPA, 20:5*n*-3), and arachidonic acid (AA, 20:4*n*-6) (Sargent *et al*. 1999). They are long chain polyunsaturated fatty acids (PUFA), presented in the cell membranes, being necessary for the normal membrane structure and function (Kjær *et al*. 2008), and play a specific role as precursors of hormones known as eicosanoids (Sargent *et al*. 1999). The essential fatty acid requirements and lipid metabolism are affected by several factors such as development and physiological stage within species, and trophic order and environmental characteristics (temperature, salinity) among species (Glencross 2009). During the fresh water period, salmon parr can convert 18:3(n-3) to 20:5(n-3) and then to 22:6(n-3) and 18:2(n-6) to 20:4(n-6) by the enzymatic pathways of desaturation and elongation. Thus, salmon parr can consume plant oil, especially rapeseed and linseed oil, for normal growth (Tocher *et al*. 2000). In the marine environment, their natural food such as zooplanktonic crustacean and piscine prey contain high level of 20:5(n-3) and 22:6(n-3). Therefore, it might be expected that the genes encoding the desaturase and elongase enzymes responsible for the conversion of 18:3(n-3) to 22:6(n-3) are downregulated (Bell *et al*. 2001). Nevertheless, more recent researches showed that post-smolt salmonids can utilize plant oils if fish fed diets containing enough 18:3(n-3) to satisfy essential fatty acid requirement (Bell *et al*. 2001). In this regard, rapeseed oil is the most potential candidate, due to large content of 18:1(n-9) and levels of 18:2(n-6) and 18:3(n-3) with a ratio of 2:1 which is healthy for human, as well as fish (Bell *et al*. 2001). Moreover, plant oil have received attention as substitute for fish oil by some advantages such as lower cost, lower concentrations of dioxins and other organic pollutants, and sustainable production levels (Turchini *et al*. 2009).

✶ ***Lipid quality and oxidation process***

Marine fish diets contain high levels of polyunsaturated fatty acids which are susceptible to oxidation due to the high number of unsaturated carbon-carbon bonds in the fatty acids (Ackman & Gunnlaugsdottir 1992). Therefore, salmon feed quality may be affected by rancidity from lipid oxidation during storage (Laohabanjong *et al*. 2009).

The mechanism of lipid oxidation has three main stages: initiation, propagation and termination (Fig 5).

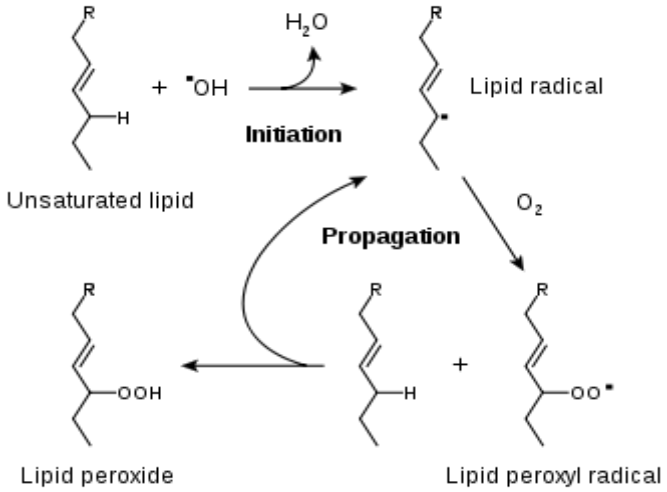

Fig 5. Mechanism of lipid peroxidation. Figure was adapted from the net (http://en.wikipedia.org/wiki/Lipid_peroxidation)

Auto-oxidation of lipid is the first step of this process (initiation). It is initiated when a hydrogen atom of unsaturated fatty acid is abstracted by a free radical (X:OH, O_2^- or others) to form hydroperoxides (PUFA radical). After that, the PUFA radical formed reacts with oxygen to form lipid peroxyl radical. In the propagation stage, lipid peroxyl radical continuously reacts with new unsaturated fatty acid to form lipid peroxide and enters a new turn in the reaction cycle. Termination is achieved when two lipid radicals combine to form a non-radical species (Hamre 2011; Laohabanjong *et al.* 2009). The process of lipid oxidation may go on until PUFAs are useable for rancidity if antioxidants are absent (Hamre 2011).

The conjugated dienes and lipid hydroperoxides are considered as the primary products of lipid auto-oxidation which can damage hormones, vitamins and pigments (Sutton *et al.* 2006). This is followed by decomposing from high molecular weight into low molecular

weight such as aldehydes, alkanes, alkenes, alcohols and acids as secondary oxidation products (Hamre 2011; Sutton *et al*. 2006; Zhong *et al*. 2007). Both primary and secondary oxidation products have effects on the nutritive value of the diet through rancid odor and taste, consequently oxidation status affect growth, feed intake and health of fish (Hamre *et al.* 2001; Laohabanjong *et al*. 2009; Zhong *et al*. 2007). Furthermore, some of them, especially the aldehydes, can bind to the amino groups of protein, thereby reducing the nutritive value of protein (Hamre *et al*. 2001; Laohabanjong *et al*. 2009).

Peroxide value (PV) and anisidin value (AV) are used to evaluate the quality of lipids. The primary reaction products formed during the initial stages of oxidation, called peroxides, give an indication of the progress of lipid oxidation. The peroxide value will vary during oxidation. They reach a maximum value and then followed by a decrease as the peroxides further react and decompose to form the secondary products of lipid oxidation (Fig 6).

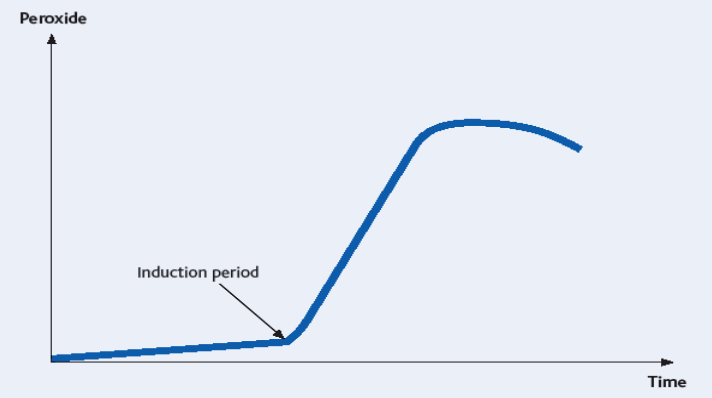

Fig 6. Lipid peroxidation. Figure was adapted from the net (http://www.vitablend.nl/peroxide_value.aspx)

Anisidin value is a measure of the secondary reaction products that occur during lipid oxidation such as aldehydes and ketones (adapted from the net http://www.vitablend.nl/peroxide_value.aspx).

2.2 Vitamin E

Natural vitamin E, including four tocopherols and four tocotrienols, belongs to a group of fat-soluble vitamins (Herrera & Barbas 2001). It is a natural antioxidant which protects organisms against lipid oxidation. The specific biological role is to stabilizes the cell membranes (Hamre 2011; Wang *et al*. 2006), and to impose disease resistance and health through modulation of the immune response in fish (Trichet 2010). Research have showed

that increased dietary PUFA level causes an increased requirement for vitamin E in fish, such as in carp (Schwarz *et al.* 1988), tilapia (Shiau & Shiau 2001), grouper (Lin & Shiau 2005) and Atlantic Salmon (Hamre & Lie 1995).

Among the tocopherol homologues, the antioxidant ability of the α-tocopherol form of vitamin E (Fig 7) is greater than that of other vitamin E homologues.

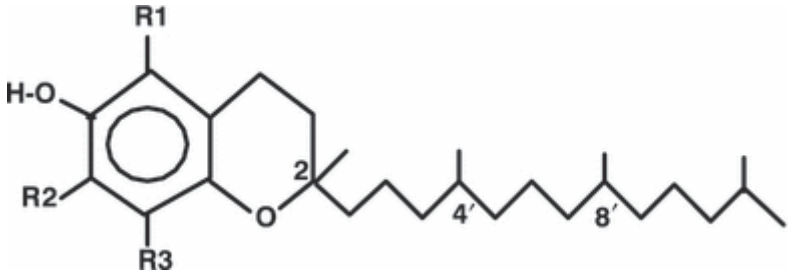

Fig 7. The α-tocopherol (R1–R3 are methyl groups) form of vitamin E (Hamre 2011)

✶ ***The antioxidant function of vitamin E***

The molecul of the α-tocopherol has two different parts which are similar with structure of the membranes. The tail is a phytyl chain with hydrophobic characteristic buried in inner part of the membrane, while the head with the chromanol ring, which carries the reactive and polar OH-group, located at or near the membrane surface (Hamre 2011). When lipid oxidation is initiated, the polar peroxyl radical group is formed. This group floats to the surface of the membrane where it can react with OH-group of the chromanol ring of α-tocopherol (Fig 8). This reaction is terminated by the formation of a lipid hydroperoxyde and the tocopheroxyl radical (Buettner 1993). The lipid peroxyl radical is devoted a hydrogen atom from α-tocopherol instead of HUFA, breaking the chain of reactions involved in lipid auto-oxidation (Hamre 2011).

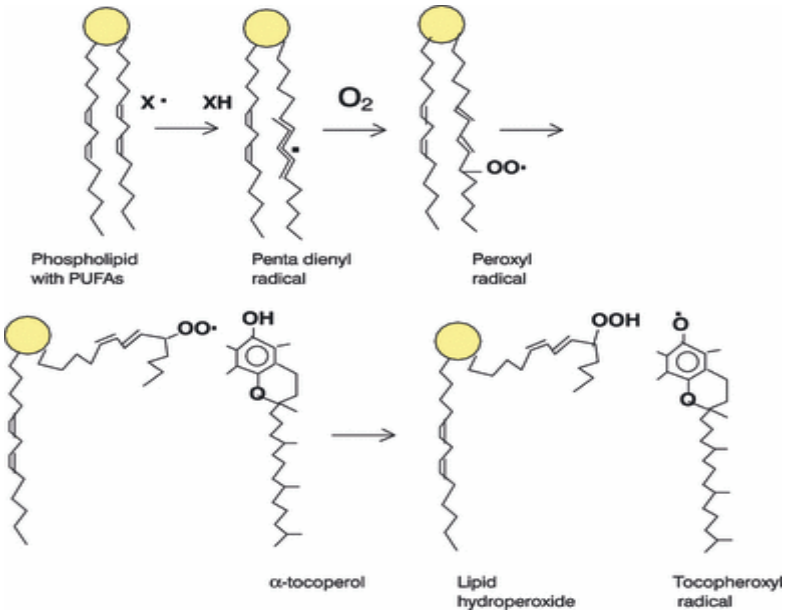

Fig 8. Proposed mechanism for the reaction of α-tocopherol with oxidising lipids (Hamre 2011)

The polar OH-group on the chromarol ring of α-tocopherol has strong polarization. Thus, lipid peroxyl radicals react approximately 10^5 times faster with α-tocopherol than with PUFA in non-polar homogenous solution (Hamre 2011). Moreover, Buettner (1993) calculated that 1000 molecules of PUFA can be protected from oxidation by one molecule of α-tocopherol. This demonstrates that α-tocopherol is the most powerful antioxidant. Vitamin E is recycled by vitamin C (Fig 8) (Tappel 1962). If vitamin E is used in high concentration combined with low concentration of vitamin C, vitamin E may be present mainly as tocopheroxyl radicals, which abstract hydrogen atoms from surrounding molecules (Bowry *et al*. 1992), even hydrogen atoms of PUFA and thereby initiating lipid oxidation. Consequently, vitamin E can act as a proxidant if vitamin C is deficient (Hamre 2011).

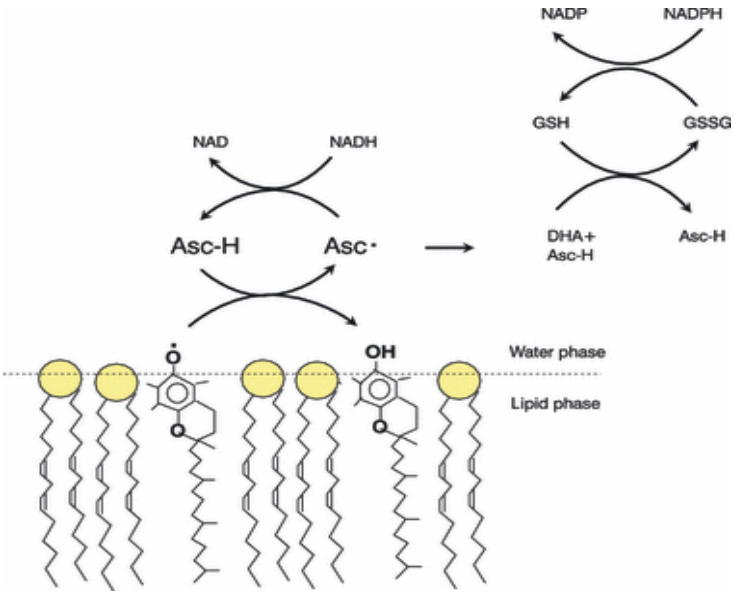

Fig 9. Proposed mechanism for regeneration of α-tocopherol from the tocopheroxyl radical (Asc-H: ascobic acid, NAD: Nicotinamide adenine dinucleotide, NADP: Nicotinamide adenine dinucleotide phosphate, NADPH: nicotinamide adenine dinucleotide phosphate-oxidase, NADH: ubiquinone reductase, GSH: glutathione, GSSG: oxidized glutathione, DHA: docosahexaenoic acid (Hamre 2011)

2.3 Stress

Stress is defined by Hans Selye as "the nonspecific response of the body to any demand made upon it" (Barton 2002). There are several factors inducing stress responses in fish such as poor water conditions, improper feeding or other chronic disturbances (Erikson 1997). Based on these factors, stress may be divided into: (1) chronic and (2) acute stress. Chronic stress may be induced by adverse water condition and improper feeding during the period rearing. Acute stress may occur during capture, anesthesia, grading, hauling, crowding, slaughter, etc (Erikson 1997). Physiological responses of fish to environmental stressors have been groups as primary (e.g., increases in corticosteroid and catecholamine hormones, alterations in neurotransmitter activity), and secondary (e.g., metabolic changes, osmoregulatory disturbance, changes in hematological features and changes in immune function features) (Barton 2002). For instance, Hamre *et al*. (1994) measured hemoglobin levels in Atlantic salmon fry after long term stress (24 weeks) by feeding low dietary vitamin E. The hemoglobin concentration decreased over the decreasing range of experimental vitamin E levels (0, 15, 30, 60 or 120mg vitamin E/kg). Thus, vitamin E deficient fish had low hemoglobin levels (Hamre *et al.* 1994). Skjervold (2002) studied effect of cold water stress on blood parameter: cortisol, glucose, lactate and osmoality in rainbow trout. After 39 hours,

the blood plasma osmoality was increased up to lethal levels. The later author concluded that osmoregulatory imbalance was the main cause of death in rainbow trout suffering from water-filled stomach (Skjervold 2002).

In addition, muscle pH is methods used to evaluate how the stress *pre-slaughter* affect the fish. Muscle pH development is affected by several factors such as feed composition (Mørkøre 2006), starvation (Mørkøre *et al*. 2008), handling or crowding stress before slaughtering (Bahuaud *et al*. 2010; Mørkøre *et al*. 2008; Sigholt *et al.* 1997) and temperature during prolonged storage (Sigholt *et al*. 1997; Stien *et al*. 2005). Lactic acid is formed during anaerobic metabolism in the muscle when glycogen used to product ATP. Lactic acid accumulates concurrent with an acidification of the muscle resulting in a pH drop. After that, the pH increases again due to bacterial production of basic amine (NH_3) (Hansen *et al*. 2007). As the pH falls, the denaturation of protein is started and the proteins may lose their water holding capacity. These processes may affect the physical properties of the fish muscle (Haard 1992). Hansen *et al.* (2007) reported an initial muscle pH around 7 in unstressed salmon immediately after post mortem. When fish are stressed or subjected to exhausting exercise before slaughter, ATP content in the muscle and initial post mortem muscle pH are low. Consequently, the onset of rigor mortis is shortened and could be responsible for the devolved muscle quality (Bagni *et al*. 2007).

2.4 Flesh quality

There are several important attributes of fishery products considered by the consumer such as safety, nutrition, flavor, texture, color and appearance and the suitable of the raw material for processing and preservation (Haard 1992). Moreover, fish farmer has additional ways assessed the impact of product quality. These ways include control of physiological factors, such as biological age and growth rate, control of environmental factors, such as water temperature, pressure, flow and chemistry and control of dietary factors, such as feeding cycle, starvation, overfeeding and the presence or absence of specific dietary components (Haard 1992). Fillet composition, color intensity, texture, gaping and liquid loss are important parameters used to evaluate flesh quality of salmon. Soft texture and fillet gaping are major causes of downgrading of salmon (Michie 2001). Furthermore, liquid loss is economically important as the weight of the fillet decrease and exudations are repulsive (Kiessling *et al*. 2004).

Fillet texture is one of the most important quality parameters in fish that determines the acceptability of the seafood products. Fish texture is usually expressed as flesh firmness, measured either instrumentally or by sensory analysis. This property depends on the connective tissue, consisting of mainly collagen (responsible for tensile strength) and the myofibrils, consisting of myosin and actin (Casas *et al*. 2006). Fish flesh firmness decreases gradually during post mortem storage and can be influenced by several factors such as length of starvation before slaughter (Mørkøre *et al*. 2008), pre-slaughter crowding stress (Bagni *et al*. 2007), dietary lipid level, feeding intensity and feed influencing fat content of the fillet (Einen *et al*. 1999; Mørkøre *et al*. 2001; Mørkøre 2006), method of slaughter, fillet processing, storage temperature (Ruff *et al*. 2002; Sigholt *et al*. 1997) and pH of the flesh (Rasmussen 2001).

Gaping is caused by rupture of the connective tissue, which produces flaking of the fillet. The cause of gaping can crudely be described as the interaction between forces pulling the muscle apart, and the strength of the tissue. Gaping is usually accompanied by tissue softening. However, gaping may also occur in the firm flesh. Salmon fillets with extensive gaping are difficult to process and are not accepted by consumers (Kiessling *et al*. 2004).

Moreover, there are some evidences that in fish a relationship exists between harvest procedures and aspects of quality such as water holding capacity (Rørå *et al*. 2003). Lowered liquid holding capacity of the muscle associates with the changes of muscle structure as fish exposed with stress (Kiessling *et al*. 2004). Exposure to stress, initial muscle pH is low and fall down during the prolonged storage. Since, muscle pH falls, the denaturation of protein is started and the proteins may lose their water holding capacity (Haard 1992).

3. Materials and methods

3.1 Fish and experimental design

The experiment was carried out in seawater at Nofima AS research station (Averøy, Norway) during the period 16th of April to 3rd of July 2011. The fish used were 560 Atlantic salmon (*Salmo salar*, L.) hatched at the Nofima AS research station at Sunndalsøra, Norway, and transferred to seawater autumn 2009 as 0+ smolts. The fish was vaccinated. The body weight at the experimental start was 2.12 kg (range 2.07-2.15 kg). The fish were distributed randomly into eight net pens of 125 m^3 (5m length * 5m width * 5m depth), with 70 fish in each net pen. The fish were assigned to four different diets in duplicates, and fed four times per day by automatic feeders to 10–20% overfeeding. Uneaten feed was collected after each meal and pumped up into wire mesh strainers as described by (Einen *et al*. 1999). Each diet was tested for recovery of dry matter under the environmental conditions present during the experiment as described by (Helland *et al*. 1996). The weight of uneaten feed was corrected for water absorption during feeding and collection. The water temperature at 3 meter depth averaged 9°C during the experiment, with a minimum of 5°C on 16th of April and a maximum of 13.7°C of June (Fig 10).

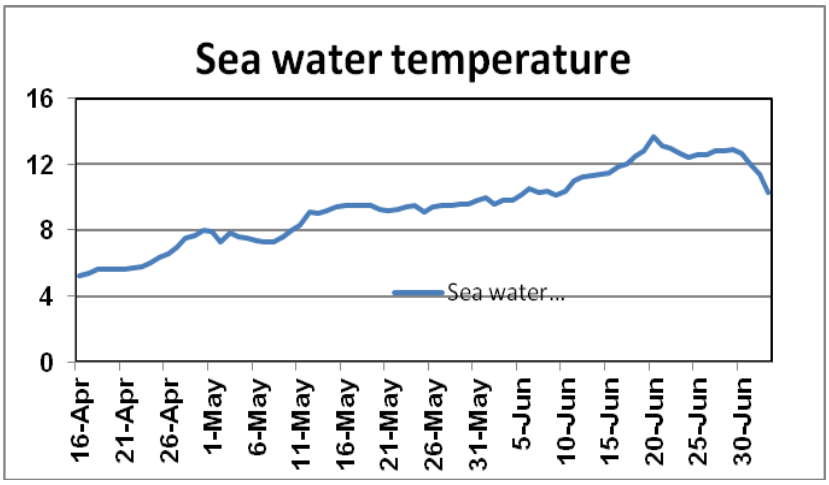

Fig 10. Sea water temperature during the experiment from April 16th to July 3rd

3.2 Experimental diets

The basic feed used was a commercial 7mm extruded diet manufactured by Skretting AS, Stavanger, Norway (Optiline Spirit V 600), containing g kg^{-1} 6.2 water, 32.3 fat, 40.1 protein and 5.3 ash. The astaxanthin, vitamin C and vitamin E content were 41mg/kg, 100mg/kg and

200mg/kg, respectively. The experiment diets were prepared by coating the 25 kg feed with 1250 ml fresh rapeseed oil (Anisidin value, AV 2.3 and peroxide value, PV 3.3 meq peroxide/ kg) or 1250 ml oxidized rapeseed oil (AV 33 and PV 24 meq peroxide/ kg), with or without the addition of 25 grams of vitamin E (dl-α- tocopheryl acetate – DSM Nutritional Products Ltd, Basel, Switzerland). After coating in a blender, the feed was spread on a tray of 1m x 2m for 3 days at approximately 15°C for drying. In order to avoid any differences in taste between the diets, all feeds were coated again with 250 ml of capelin oil and subsequently dried for additional two days before feeding.

Table 1. The four dietary treatments

Dietary treatments		
Oil quality	Vitamin E	Abbreviation
Fresh	Standard (200 mg/kg)	C
Fresh	Elevated (1200 mg/kg)	CE
Oxidized	Standard (200mg/kg)	Ox
Oxidized	Elevated (1200mg/kg)	OxE

3.3 Sampling of the fish

The fish were counted and individually weighted at the beginning of the experiment and at the experimental termination. Fifteen salmon from each net pen were anesthetized by MS 222 (metacaine, 0.1 g L^{-1}; Alpharma Animal Health Ltd), where of blood was immediately collected from 5 fish. These fish and additional five fish were subsequently killed by percussive stunning, whereas five fish were transferred to a tank with seawater (approximately 1.54 m^3) with an initial oxygen level of 9.1mg/L. The fish were kept in the tank for 75 minutes during which time the oxygen level declined to approximately 2mg/L. Thereafter blood was collected from each fish before they were killed by percussive stunning. All 15 fish were gill cut and bled in seawater for 30 minutes. All sampled fish had approximately the same average weight to avoid the effect of weight on the parameters (Table 2).

Table 2. The average weights and standard deviation of fish were sampled in each dietary treatment

Group	C	CE	Ox	OxE
Body weight	3478 (180)	3489 (161)	3500 (273)	3463 (209)

After bleeding, the round body weight and fork length was recorded. Thereafter the fish were examined for internal adhesions, classified by visual examination according to a standardized scoring system for vaccine induced lesions and modified by using a visual analogue scale (VAS) (0–6) (Aunsmo *et al*. 2008). Degree of melanin was classified by macroscopic observation of the abdominal organs (visceral peritoneum) and abdominal wall (parietal peritoneum) and scored on separate (0–3) VAS scales. The weight of liver, heart and spleen was recorded and the liver color was evaluated according to scale from 1 – 3 by four persons where score 1 is pale, 2 is brown and 3 is dark brown.

The gutted fish were weighed and filleted by hand and the weight of the fillets was recorded (left and right). The left fillet side of salmon using normal slaughter procedure (5 fish) and exposing with low dissolved oxygen (DO) stress (5 fish) were photographed for subsequent image analyses for fat determination according to Folkestad *et al.* (2008). Thereafter, muscle pH was measured after 3 and 24 hours storage.

The right fillets of all the fish of each net pen were sent to Nofima laboratory at Ås for analyzing gaping, texture and liquid loss during cold storage.

3.4 Blood sample

Blood was collected from the caudal blood vessel into a vacutainer using heparin as anticoagulants (two tubes per fish) (Fig 11). The heparinised blood samples were analyzed using i-STAT® 300 Portable Clinical Analyzer (I-stat, Abbott, Princeton, NY, USA). The analyzer was used in conjunction with EC8+ and CG4+ disposable cartridges. Blood was automatically heated to 37 °C and analyzed for pH, sodium (Na+), potassium (K+), hemoglobin (Hb).

Fig 11. Blood sampling (Original photo, Nofima)

3.5 Image analyses

Fat analyses were performed on the dorsal fillet between the posterior part of the dorsal fin and the gut (Norwegian Quality Cut, NQC) of the left fillet, using the equipment by the equipment provided by PhotoFish AS (Averøy, Norway). The system consists of closed box with standardized light and color conditions, a digital camera, and a PC for transmitting of the image and software for analyses (Folkestad *et al*. 2008).

Fig 12. The photo box to analysis fat content of the salmon fillets (Original photo, Hang)

3.6 pH measurements

The pH was measured using a pH-meter 330i SET (Wissenschaftlich-Technische-Werkstatten Gmbh & Co.KG, WTW, Weilheim, Germany) with a pH muscle-electrode (Schott pH-electrode, Blueline 21 pH, WTW, Weilheim, Germany) and a temperature probe (TFK325, WTW, Weilheim, Germany) that was directly entered in the fillets (Fig 13).

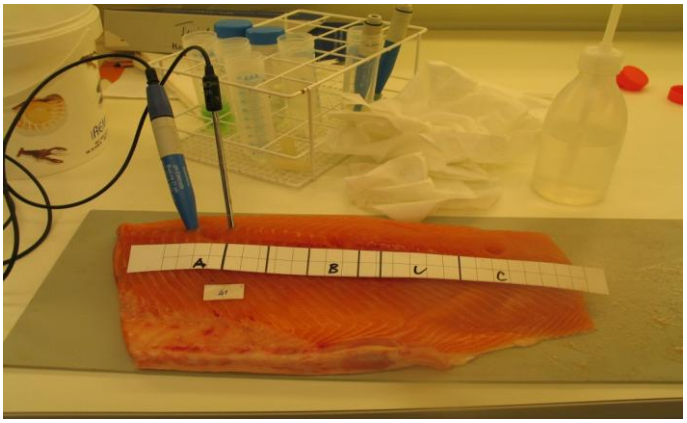

Fig 13. pH measurements (Original photo, Nofima)

3.7 Fillet gaping

The fillet gaping was recorded according to a scale ranging from score 0-5, where score 0 represents no gaping and score five extreme gaping (Andersen *et al*. 1994).

3.8 Liquid loss

Liquid loss during 3 days of storage at 4^{o}C was analyzed based on a method described by Mørkøre *et al*. (2002) and by Mørkøre *et al*. (2007). In brief, a 15 gram slice of muscle was placed on a thin-bedded honeycombed pad (8x12 cm) made of cellulose paper (7 sheets type la-Hochgebleicht, AEP Industries, AA Apeldorn,Holland). The sample and pad were put in a sealed polyethylene bag and stored in a cooling room (3^{o}C) for three days. The liquid loss was calculated as:

$$LL\ (\%) = 100\% \times \text{weight increase of the pad (g)} * \text{initial muscle weight (g)}^{-1}$$

Fig 14. Sample preparation for analyses at Nofima AS laboratory at Ås. (Original Photo. Nofima)

3.9 Texture

A Texture analyser, model TA-XT2 (SMS Stable Micro Systems Ltd., Blackdown Rural Industries, Surrey, UK), equipped with a flat-ended cylinder of 12.5 mm in diameter (type P/0.5) was used for the mechanical analyses. The trigger force was 0.2 N and the test speed 1 mm s^{-1}. Analyses were made longitudinal to the muscle fibres on 2.5 cm thick cutlets (duplicate measurements per fillet). The force-time graphs were recorded by a computer and analysed using the Texture Expert for Windows software (version 4.0.9.0., 2007, Stable Micro Systems Ltd, Surrey, UK). The parameter recorded from the force-time graphs was the area under the graph (Newton*s), frequently termed the total work (denoted as firmness).

3.10 Data analysis

Statistical analysis was performed by the Statistical Analyses System (SAS). ANCOVA with sex as a covariate was used to test for differences in growth, FCR and biometric traits among the dietary treatments (net pen was used as the experimental unit). For the blood parameters and fillet quality characteristics, acute stress (low level of dissolved oxygen (DO)) was also used an explanatory variable. In addition, the interaction between DO level and oil quality and the interaction between DO level and vitamin E level were tested. Furthermore, the interaction between oil quality and vitamin E level was tested, but these results are not reported in the result chapter because no significant interaction was observed. The results are represented as mean (± SEM) and the level of significance was set at P = 0.05.

3.11 Calculation

✯ The following equations were used to analyze treatment effects

Feed conversion ratio	$FCR = (\text{feed intake,g}) \times (\text{wet weight gain, g})^{-1}$
Thermal growth coefficient	$TGC \times 1000 = [(W_1)^{1/3} - (W_0)^{1/3}] \times (\text{days} \times {}^{o}C)^{-1} \times 1000$
Condition factor	$CF = W\ (g) \times (\text{fork length, cm})^{-3} \times 100$
Weight gain	$WG = W_1\ (g) - W_0\ (g)$
Hepato-somatic index	$HSI\ (\%) = 100 \times (\text{live weight, g}) \times W\ (g)^{-1}$
Organ index	Weight of organ/ Body weight (g) x 1000

Where:

W: the body weight of the sampled fish in grams;

W_0: the initial fish mean body weights in grams

W1: the final fish mean body weights in grams

4. Result

4.1 Production parameters

The average body weight of the salmon increased from 2120 grams to 3634 grams during the experimental period of 79 days (Table 3). The growth was not affected by oil quality or vitamin E level in the diet. However fish fed oxidized oil (Ox and OxE groups) had on average 117 g lower weight gain compared to those fed control oil (C and CE groups). Correspondingly, thermal growth coefficient (TGC) was 5% units lower in the salmon fed the oxidized oil. The condition factor (CF) did not change significantly during the experimental period and there was no significant difference between the dietary treatments. The feed conversion ratio of the CE group was significantly lower compared with the C and OxE group.

Table 3. Production parameters (mean ± standard error) of Atlantic salmon (*Salmo salar* L.) fed a commercial diet added fresh or oxidized rapeseed oil with standard (200mg/kg) or elevated (1200mg/kg) vitamin E during a period of 79 days.

Dietary Lipid	Fresh		Oxidized	
Vitamin E	Standard	Elevated	Standard	Elevated
Abbreviation	C	CE	Ox	OxE
Initial body weight, g	2117 ± 3	2136 ± 6	2106 ± 3	2123 ± 2
Final body weight, g	3685 ± 1	3700 ± 1	3640 ± 1	3510 ± 6
Weight gain, g	1568 ± 2	1564 ± 3	1534 ± 2	1387 ± 4
Initial CF[A]	1.34 ± 0.01	1.35 ± 0.00	1.35 ± 0.03	1.36 ± 0.00
Final CF[A]	1.36 ± 0.01	1.37 ± 0.01	1.36 ± 0.02	1.36 ± 0.01
TGC[B]	3.51 ± 0.14	3.48 ± 0.25	3.46 ± 0.09	3.16 ± 0.11
FCR[C]	0.97^{a} ± 0.01	0.92^{b} ± 0.02	0.94^{ab} ± 0.01	0.97^{a} ± 0.00

[A] Condition factor (CF), Body weight (g) x (fork length, cm)$^{-3}$ x 100

[B] Thermal growth coefficient (TGC), $[(\sqrt[3]{Wt} - \sqrt[3]{(Wo)}) / (T \times t)] \times 1000$, where *Wt* is the weight of fish after t days, *Wo* is the initial weight of fish, *T* is temperature in °C, and *t* is time in days

[C] FCR, feed conversion rate, kg feed/ kg weight gain

Different superscripts within the same row denote significant difference ($P<0.05$) between dietary treatment.

4.2 Biometric traits

Fillet thickness of the salmon fed oxidized oil (Ox and OxE groups) was significantly lower compared with that of the salmon fed fresh oil (C and CE groups) (Table 4) and (Table 5). The weight of the heart relative to the body weight tended to be higher in salmon of the Ox and OxE groups than of the C and CE groups (P = 0.0066) (Table 4). Speilberg score was not significantly affected by the oil quality or vitamin E level in the diet, but numerically higher score was observed for the groups fed oxidized oil (Table 4).

Table 4. Organ adhesions, liver color, organ index, yield, fat content and fillet thickness (mean ± standard error) of Atlantic salmon (*Salmo salar* L.) fed a commercial diet added fresh or oxidized rapeseed oil with standard (200mg/kg) or elevated (1200mg/kg) vitamin E during a period of 79 days.

Dietary Lipid	Fresh		Oxidized	
Vitamin E	Standard	Elevated	Standard	Elevated
Abbreviation	C	CE	Ox	OxE
Speilberg score (0-6)	0.8 ± 0.2	0.7 ± 0.1	1.1 ± 0.2	1.1 ± 0.2
Liver score (0-3)	**2.9^{a} ± 0.1**	**2.2^{b} ± 0.1**	**2.9^{a} ± 0.1**	**2.0^{b} ± 0.1**
Liver index, % [A]	**0.88^{b} ± 0.02**	**0.95^{a} ± 0.02**	**0.91^{ab} ± 0.02**	**0.95^{a} ± 0.02**
Heart index, %[B]	0.79 ± 0.02	0.79 ± 0.02	0.83 ± 0.02	0.83 ± 0.02
Spleen index, %[B]	0.11 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.12 ± 0.01
Slaughter yield, %	90.0 ± 0.2	90.2 ± 0.4	89.7 ± 0.2	90.1 ± 0.2
Fillet yield, %	69.5 ± 0.3	69.5 ± 0.4	69.6 ± 0.2	69.9 ± 0.3
Fillet fat content, %	17.0 ± 0.3	16.9 ± 0.2	16.6 ± 0.3	16.5 ± 0.3
Fillet thickness, mm	**27.4^{a}± 0.3**	**27.6^{a}± 0.4**	**26.2^{b}± 0.5**	**26.3^{b}± 0.4**

[A] **Liver index (HSI)**, Liver weight (g)/ Body weight (g) x 100
[B] **Organ index**, Weight of organ/ Body weight (g) x 1000
Different superscripts within the same row denote significant difference (*P<0.05*) between dietary treatments.

High level of vitamin E supplementation resulted in significantly paler (lower color score) (Fig 15) and larger livers but otherwise no significant effect of dietary vitamin E supplementation was observed (Table 5).

Table 5. Organ adhesions, liver color, organ index, yield, fat content and fillet thickness (mean ± standard error) of Atlantic salmon (*Salmo salar* L.) fed a commercial diet added fresh or oxidized rapeseed oil with standard (200mg/kg) or elevated (1200mg/kg) vitamin E during a period of 79 days.

Parameters	Mean Value [A]		P-value	Mean Value [B]		P-value
	Fresh oil	Oxidized oil		Standard vitamin E	Elevated vitamin E	
Speilberg score (0-6)	0.8 ± 0.13	1.1 ± 0.16	0.1321	1.0 ± 0.16	0.9 ± 0.13	0.7695
Liver score (0-3)	2.5 ± 0.08	2.4 ± 0.09	0.1977	**2.87 ± 0.04**	**2.07 ± 0.09**	**<0.0001**
Liver index, %[C]	0.91 ± 0.02	0.93 ± 0.02	0.4094	**0.90 ± 0.02**	**0.96 ± 0.02**	**0.0127**
Heart index, %[D]	**0.79 ± 0.02**	**0.83 ± 0.02**	**0.0666**	0.81 ± 0.02	0.81 ± 0.01	0.8752
Spleen index, %[D]	0.1 ± 0.01	0.1 ± 0.01	0.6318	0.1 ± 0.00	0.1 ± 0.01	0.4687
Slaughter yield,%	90.1 ± 0.2	89.9 ± 0.1	0.4418	89.9 ± 0.1	90.2 ± 0.2	0.2454
Fillet yield,%	62.7 ± 0.2	62.8 ± 0.2	0.6900	62.5 ± 0.2	62.9 ± 0.2	0.1861
Fillet fat content,%	17.0 ± 0.2	16.6 ± 0.2	0.1887	16.8 ± 0.2	16.7 ± 0.2	0.8932
Fillet thickness, mm	**27.5 ± 0.2**	**26.3 ± 0.3**	**0.0014**	26.8 ± 0.3	26.9 ± 0.3	0.8349

[A] Includes oil with and without the addition of vitamin (n=4 net pens, 10 fish per net pen)
[B] Includes diets with fresh and oxidized oil (=4 net pens, 10 fish per net pen)
[C] **Liver index (HSI)**, Liver weight (g)/ Body weight (g) x 100
[D] **Organ index**, Weight of organ/ Body weight (g) x 1000

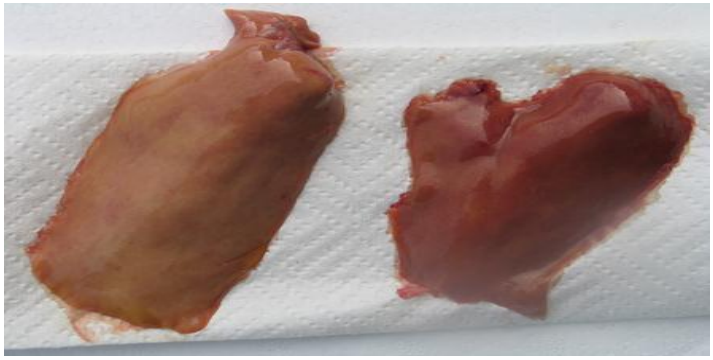

Fig 15. Livers of salmon fed elevated levels of vitamin E (left) and standard levels of vitamin E (right) (Original photo, Nofima)

4.3 Blood parameters

Blood parameters were analyzed in salmon that were harvested according to normal procedures (non-stressed groups) and in fish that were stressed at low dissolved oxygen (DO) content (2mg/L) in the water at the time of harvesting (stressed group).

✶ *Non-stressed groups*

No significant difference due to dietary oil quality was observed for sodium concentration, whereas supplementation of vitamin E gave significantly increased sodium level of the CE compared with the C group (P = 0.04). The same pattern was seen for the salmon fed oxidized oil, but the difference between the Ox and OxE groups was not significant (Fig 16.A). For the potassium level, dietary oxidized oil and addition of vitamin E resulted in a similar trend as for sodium but no significant differences were observed due to dietary treatment (Fig 16.B). Moreover, the dietary oil quality and vitamin E level did not have any significant effect on hemoglobin level or pH in the blood, except for a significantly higher pH of the CE group compared with the Ox group (Fig 17 A and B).

✶ *Stressed groups*

Exposure to low DO in the seawater resulted in significantly increased sodium and potassium level in the blood of all dietary groups (P<0.0001) (Table 6). The non stressed groups had sodium and potassium level ranging from 157.3 to 161.0 mmol/L and from 3.0 to 3.8 mmol/L, respectively. For the stressed groups, these levels increased to 168.9-178.3 mmol/L and 4.5-6.0 mmol/L, respectively (Table 9). The salmon fed oxidized oil had increased level of sodium compared with those fed fresh oil, but addition of vitamin E to the diet diminished the effect of stress. The variation pattern between the dietary treatments was similar for potassium, although less pronounced as compared with sodium (Fig 16). The statistical analyses revealed that a significant interaction between stress and oil quality and stress and vitamin E supplementation for both sodium and potassium (Table 6).

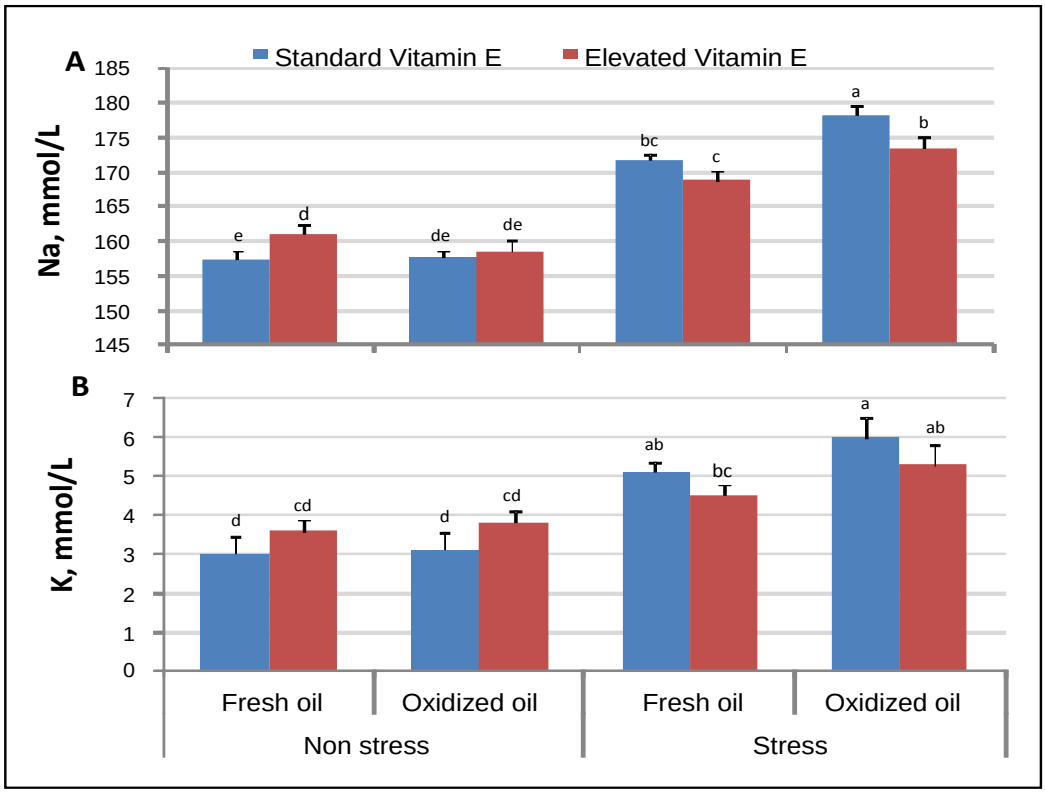

Fig 16. Concentration of sodium (Na, mmol/L) (A) and potassium (K, mmol/L) (B) in the blood of Atlantic salmon (*Salmo salar* L.) exposed high (9mg/L, non-stressed) or low (2mg/L, stressed) dissolved oxygen before slatughtering. The fish were fed a commercial diet added fresh or oxidized rapeseed oil with standard (200mg/kg) or elevated (1200mg/kg) vitamin E during a period of 79 days. Results are given as mean ± SE. Different superscripts denote significant difference between treatments *(P<0.05)*.

Exposure to low DO before slaughtering had no overall effect on the hemoglobin or pH level in the blood (Table 6). However, the hemoglobin level was significantly higher of the Ox groups compared with the C groups (P=0.02) and there tended to be a significant interaction between stress and oil quality (P=0.06) (Table 6). The pH in the blood of the salmon fed fresh rapeseed oil (C and CE group) was significantly lower compared with those fed oxidized rapeseed oil (Ox and OxE group) after stress exposure (Fig 17) and a significant interaction was observed between stress and oil quality (P=0.008) (Table 6). Vitamin E did not significantly affect the hemoglobin level or pH in the blood of the stressed groups, but there tended to an interaction between stress and vitamin E for pH (P=0.085) (Table 6).

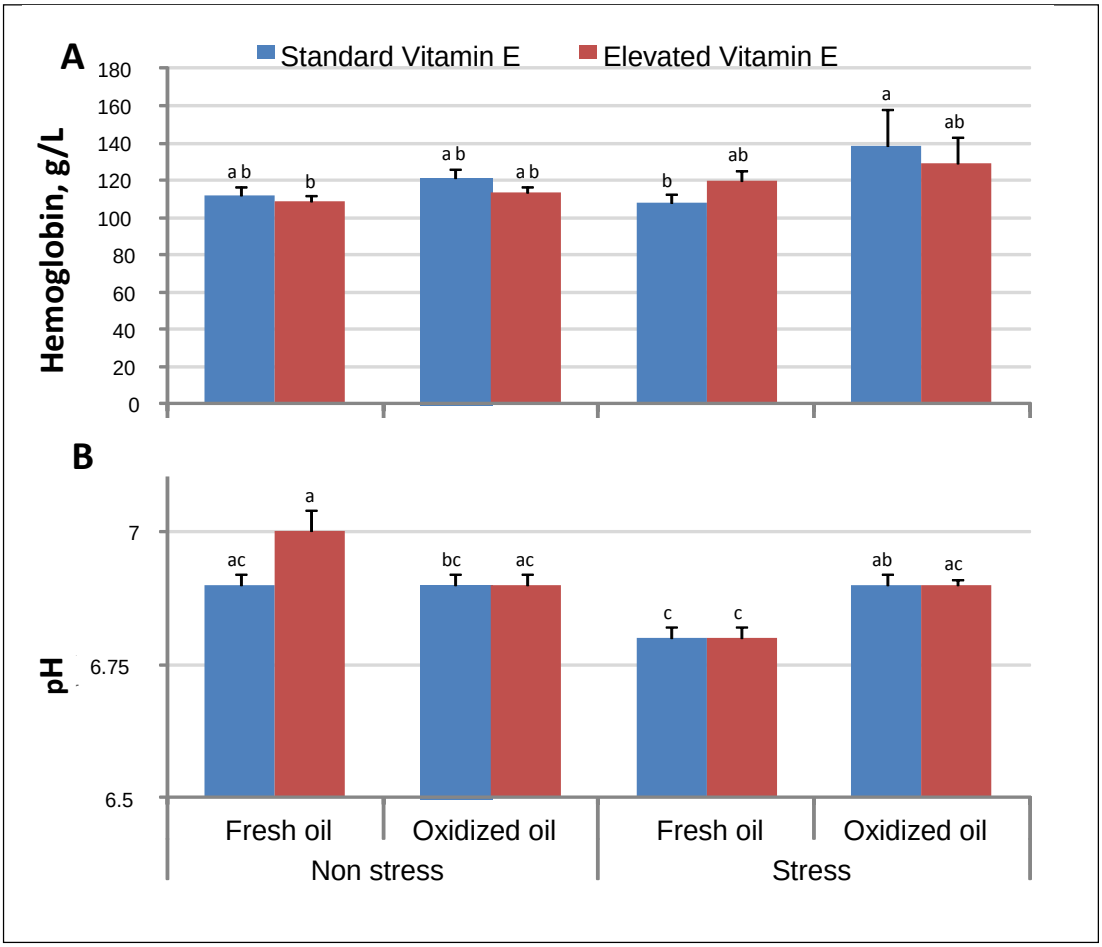

Fig 17. Hemoglobin concentration (g/L) (A) and pH in the blood (B) of Atlantic salmon (*Salmo salar* L.) exposed high (9mg/L, non-stressed) or low (2mg/L, stressed) dissolved oxygen before slatughtering. The fish were fed a commercial diet added fresh or oxidized rapeseed oil with standard (200mg/kg) or elevated (1200mg/kg) vitamin E during a period of 79 days. Results are given as mean ± SE. Different superscripts denote significant difference between treatments *(P<0.05)*.

Table 6. Summary of the statistical analyses for the of blood parameters regarding the impact of stress (low DO level) and the interaction between stress and dietary oil quality and vitamin E level, respectively.

Parameters	P-value		
	Stress	**Oil * Stress**	**Vitamin E *Stress**
Na, mmol/L	**<0.0001**	**<0.0001**	**<0.0001**
K, mmol/L	**<0.0001**	**<0.0001**	**<0.0001**
Hemoglobin, g/L	0.1952	**0.0641**	0.4773
pH-blood	0.1751	**0.0081**	**0.0853**

4.4 Muscle pH

The progress of muscle pH in the non-stressed groups and the stressed groups are presented in Fig 18.

✶ *Non-stressed groups*

The muscle pH declined with time, from an initial pH of 6.7 (3h) to a final pH of 6.4 (24h) on average. Despite the relatively high numerical differences, no significant variations were observed between the oil quality and vitamin E groups, except for a higher muscle pH at 3h for C group. In addition, vitamin E supplementation tended to cause a slight overall reduction of pH.

✶ *Stressed groups*

Exposure to low DO level before slaughter had an overall significant effect on muscle pH. Muscle pH of the stressed groups was 0.2 units lower compared with the non-stressed group after 3h, reaching pH 6.3 after 24h on average. Dietary oxidized oil had no significant effect on pH, but a significant interaction between stress and oil quality was observed on muscle pH both after 3h and 24h. Addition of vitamin E to the diet resulted in a consistent higher pH, although the difference was only significant between the fish fed fresh quality oil (C groups and CE groups) after 24 h. Statistical analyses also revealed that the interaction between stress and vitamin E was significant ($p<0.0001$) (Table 7).

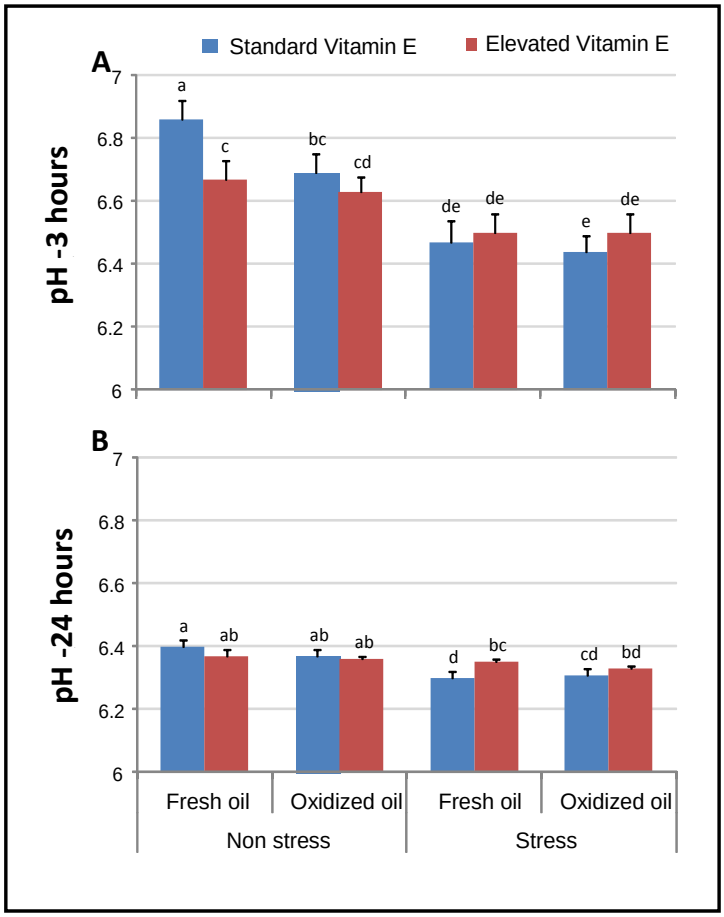

Fig 18. Muscle pH (A,B) of Atlantic salmon (*Salmo salar* L.) exposed high (9mg/L, non-stressed) or low (2mg/L, stressed) dissolved oxygen before slatughtering. The fish were fed a commercial diet added fresh or oxidized rapeseed oil with standard (200mg/kg) or elevated (1200mg/kg) vitamin E during a period of 79 days. Results are given as mean ± SE. Different superscripts denote significant difference between treatments *(P<0.05)*.

Table 7. Summary of the statistical analyses for muscle pH regarding the impact of stress (low DO level) and the interaction between stress and dietary oil quality and vitamin E level, respectively.

Parameters	**P-value Stress**	**P-value Oil * Stress**	**P-value Vitamin E *Stress**
Muscle pH			
3h	**<0.0001**	**<0.0001**	**<0.0001**
24h	**<0.0001**	**0.0004**	**<0.0001**

4.5 Fillet quality

✶ *Non-stressed groups*

Instrumental texture measurements showed the firmness of the non stress groups was not affected by dietary oil quality whereas a significant effect was observed for vitamin E supplementation. Indeed, firmness of fish fed the diets with addition of vitamin E was 11 units harder on average compared with those without vitamin E (Fig 19) (Table 9). Liquid loss and gaping were not affected by dietary oil quality or vitamin E supplementation (Fig 20).

✶ *Stressed groups*

No significant variation was observed between the stressed dietary groups, although firmness of the CE group was numerically highest. The statistical analyses revealed that there was a significant interaction between stress and vitamin E level (Table 8). There was an overall increased liquid loss by stress, but no differences were observed due to dietary treatment. Neither dietary treatment nor oxygen level shows significant effect on gaping.

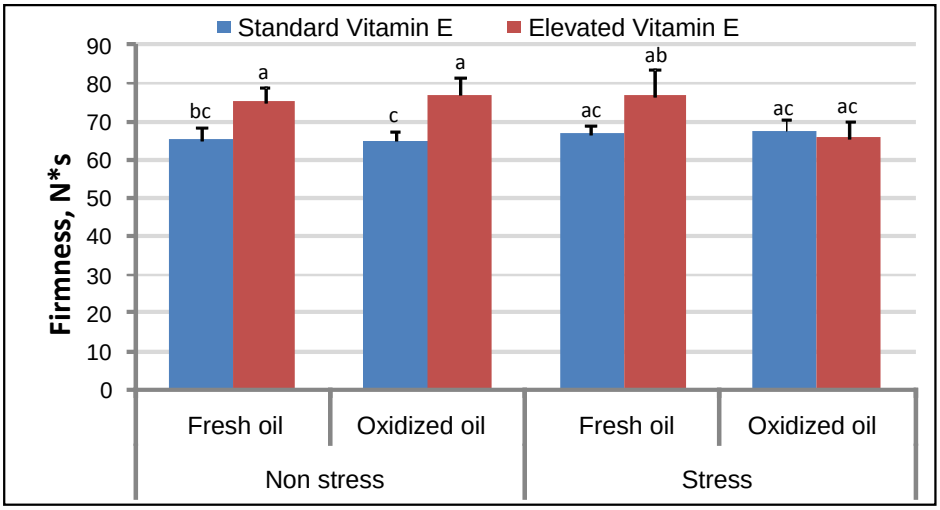

Fig 19. Firmness of Atlantic salmon (*Salmo salar* L.) exposed high (9mg/L, non-stressed) or low (2mg/L, stressed) dissolved oxygen before slatughtering. The fish were fed a commercial diet added fresh or oxidized rapeseed oil with standard (200mg/kg) or elevated (1200mg/kg) vitamin E during a period of 79 days. Results are given as mean ± SE. Different superscripts denote significant difference between treatments *(P<0.05)*.

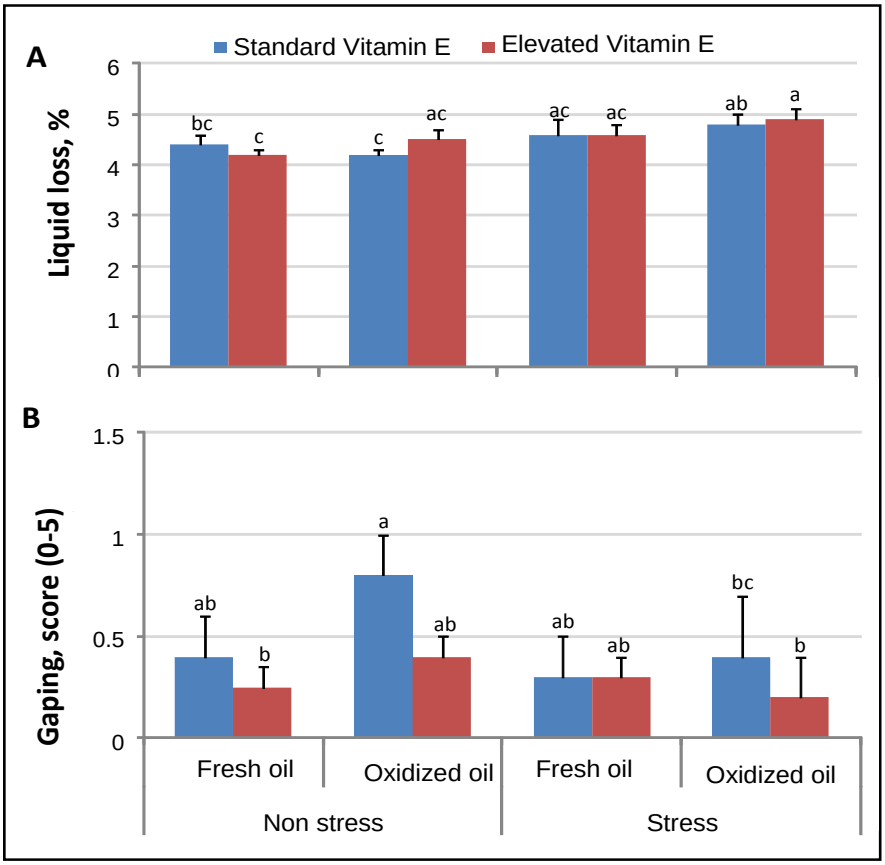

Fig 20. Liquid loss (%) (A) and Gaping score (B) of Atlantic salmon (*Salmo salar* L.) exposed high (9mg/L, non-stressed) or low (2mg/L, stressed) dissolved oxygen before slatughtering. The fish were fed a commercial diet added fresh or oxidized rapeseed oil with standard (200mg/kg) or elevated (1200mg/kg) vitamin E during a period of 79 days. Results are given as mean ± SE. Different superscripts denote significant difference between treatments *(P<0.05)*.

Table 8. Summary of the statistical analyses for firmness, gaping and liquid loss regarding the impact of stress (low DO level) and the interaction between stress and dietary oil quality and vitamin E level, respectively.

Parameters	**P-value Stress**	**P-value Oil * Stress**	**P-value Vitamin E *Stress**
Firmness, N*s	0.3854	0.7489	**0.0100**
Gaping, score (0-5)	0.2494	0.2296	0.2113
Liquid loss, %	**0.0003**	0.4920	0.9242

Table 9. Summary of the mean values (± SE) of blood parameters, muscle pH, firmness, liquid loss, gaping of Atlantic salmon (*Salmon salar* L.) fed a commercial diet added fresh or oxidized rapeseed oil with standard (200mg/kg) or elevated (1200mg/kg) vitamin E during a period of 79 days.

Dietary oil		Fresh		Oxidized	
Vitamin E		Standard	Elevated	Standard	Elevated
Na, mmol/L	Non stress	157.3 ^{e} ± 0.99	161.0 ^{d} ± 1.37	157.6 ^{de} ± 0.87	158.5 ^{de} ± 1.65
	Stress	171.7 ^{bc} ± 0.88	168.9 ^{c} ± 1.33	178.3 ^{a} ± 1.25	173.6 ^{b} ±1.49
K, mmol/L	Non stress	3.0^{d} ± 0.43	3.6 ^{cd} ± 0.25	3.1 ^{d} ± 0.47	3.8 ^{cd} ± 0.33
	Stress	5.1 ^{ab} ± 0.28	4.5 ^{bc} ± 0.30	6.0 ^{a} ± 0.50	5.3 ^{ab} ± 0.51
pH-blood	Non stress	6.9 ^{ac} ± 0.02	7.0 ^{a} ± 0.04	6. 9 ^{bc} ± 0.02	6.9 ^{ac} ± 0.02
	Stress	6.8 ^{c} ± 0.02	6.8 ^{c} ± 0.02	6.9 ^{ab} ± 0.02	6.9 ^{ac} ± 0.02
Hemoglobine, g/L	Non stress	112.2 ^{ab} ± 4.27	108.8 ^{b} ± 2.90	121.2 ^{ab} ± 19.70	113.8 ^{ab} ± 3.02
	Stress	107.8 ^{b} ± 4.84	120.0 ^{ab} ± 5.45	138.7 ^{a} ± 9.4	129.2 ^{ab} ± 14.32
pH-3 hours	Non stress	6.86^{a} ± 0.06	6.67^{c} ± 0.06	6.69^{bc} ± 0.07	6.63^{cd} ± 0.05
	Stress	6.47^{de} ± 0.07	6.50^{de} ± 0.06	6.44^{e} ± 0.05	6.50^{de} ± 0.06
pH-24 hours	Non stress	6.40^{a} ± 0.02	6.37^{ab} ± 0.02	6.37^{ab} ± 0.02	6.36^{ab} ± 0.01
	Stress	6.30^{d} ± 0.02	6.35^{bc} ± 0.01	6.31^{cd} ± 0.02	6.33^{bd}± 0.01
Gaping, score (0-5)	Non stress	0.40^{ab} ± 0.17	0.25^{b} ± 0.10	0.80^{a} ± 0.21	0.40^{ab} ± 0.13
	Stress	0.30^{ab} ± 0.21	0.30^{ab} ± 0.15	0.40^{ab} ± 0.31	0.20^{b} ± 0.20
Firmness, N*s	Non stress	65.41^{bc} ± 3.10	75.26^{a} ± 3.68	64.86^{c} ± 2.87	77.06^{a} ± 4.06
	Stress	66.82^{ac} ± 1.92	76.69^{ab} ± 7.14	67.77^{ac} ± 2.71	65.93^{ac} ± 4.07
Liquid loss, %	Non stress	4.4^{bc} ± 0.15	4.2^{c} ± 0.12	4.2^{c} ± 0.12	4.5^{ac} ± 0.15
	Stress	4.6^{ac} ± 0.26	4.5^{ac} ± 0.21	4.7^{ab} ± 0.25	4.9^{a} ± 0.18

Table 10. Summary of the statistical analyses.

Parameters	P-value				
	Oil	Vitamin E	Stress	Sex	Model
Condition factor	-	-	-	-	-
Fat content	-	-	-	-	-
Slaughter Yield	-	-	-	-	-
Fillet Yield	-	-	-	0.0130	0.0800
Fillet thickness, mm	0.0014	-	-	0.0225	0.0049
Speilberg score	-	-	-	0.0019	0.0137
Liver score (0-3)	-	<0.0001	-	-	<0.0001
Liver index	-	0.0127	0.0544	-	0.0335
Heart index	0.0634	-	-	-	0.0654
Spleen index	-	-	-	-	-
Na	-	-	<0.0001	-	<0.0001
K	-	-	<0.0001	0.0214	<0.0001
Hemoglobin	0.0451	-	-	-	0.0964
pH-blood	-	-	-	-	-
Firmness	-	0.0051	-	0.0060	0.0014
Gaping (6days)	-	-	-	-	-
Liquid loss	-	-	0.0003	-	0.0043

5. Discussion

5.1 Effects of oil quality and vitamin E supplementation on production parameters

Earlier studies with juvenile cod (Zhong *et al.* 2008) and Chinese longsnout catfish (Dong *et al*. 2011) showed no reduction in growth performance for fish fed oxidized oil in the diet. Similarly, no significant effects of dietary oil quality were observed in the present study, although the salmon fed diets added oxidized oil in combination with high vitamin E levels had on average 11% lower weight increase. In contradiction, an adverse effect on growth was found in black sea bream (Peng *et al*. 2009), African catfish (Baker & Davies 1996b; Baker 1997), turbot, halibut (Tocher *et al*. 2003), and Atlantic salmon (Koshio *et al*. 1994) fed oxidized oils. These negative effects might be explained by toxicity and off-flavour of the feed. Oxidized oils are toxic substances with characteristic rancid odour and taste that may reduce the feed intake and subsequently the growth rate (Hamre *et al*. 2001; Laohabanjong *et al.* 2009; Zhong *et al.* 2007). In the present study all feeds were top-coated with fresh marine fish oil to avoid reduced feed intake due to poor palatability. Moreover, if oxidized oils are taken up into the body, they may initiate *in vivo* lipid oxidation, cause damage to proteins and nucleic acids, as well as negatively interfere with the health of fish (Peng *et al*. 2009). The contradicting findings among studies may be explained by species specific sensitivity to oxidized lipid. Another plausible explanation may be differences in feed intake as well as oxidation status of the oils used in the feeding experiment (Tocher *et al*. 2003). The origin of the oil source may be another factor that may influence the response. In the present study, oxidized rapeseed oil was supplemented to the diets, whereas most studies have elucidated the impact of feeding fish diets added oxidized marine fish oils.

Vitamin E supplementation did not show any significant effect on growth performance when fish fed with either fresh oil or oxidized oil in the present study. Similar results were reported in previous studies on several fish species, including turbot, halibut (Tocher *et al*. 2003) and juvenile Atlantic cod (Zhong *et al*. 2008), where addition of dietary vitamin E had no significant effect on growth performance. In contradiction, improvement in growth by dietary vitamin E supplementation was observed in some other species such as black sea bream (Peng *et al*. 2009), African catfish (Baker & Davies 1996b), juvenile hydrid tilapia (Huang & Huang 2004) and sea bream (Tocher *et al*. 2003). The contradictive findings

among studies could be due to the level of lipid oxidization, the level of vitamin E supplementation in the diet and differences among fish species (Peng *et al.* 2009).

Addition of vitamin E to the diet containing fresh oil significantly lowered the FCR, demonstrating that vitamin E supplementation improved the utilization of the feed energy for building muscles. The lack of positive effects by vitamin E supplementation to diets added oxidized oil, indicate that vitamin E played a role as an antioxidant protecting the body from oxidation (e.g. oxidized fatty acids) only when the oxidation level of the dietary oil was below a certain limit. In vivo evidence of the effects of vitamin C and vitamin E on lipid peroxidation is sparse, but unbalanced content of antioxidants (vitamin C and vitamin E) might have contributed to the lack of positive effect in salmon fed oxidized. Vitamin E is not recycled when the vitamin C level is too low and unbalanced ratio between vitamin E and vitamin C leads to accumulation of vitamin E radicals which may act as pro-oxidants (Hamre 2011; Peng *et al*. 2009). Vitamin E, as dl-alpha-tocopherol acetate, is moderately stable in dry multivitamin premixes if stored below room temperature. However, the vitamin is prone to oxidation on storage in the presence of oxidation products such as rancid oils or at high ambient temperatures. In the present study the oxidized oil was added to the feed 5-7 days prior to feeding and the feed was stored at approximately 16°C after preparing. The vitamin level was not analyzed in the present study, but according to the feed producer the content of vitamin C was 100 mg/kg which above the normal requirement of salmon (Sandnes *et al*. 1992; Storebakken 2002).

5.2 Effects of oil quality and vitamin E supplementation on biometric traits

Previous studies have reported that black sea bream fed a diet with high level of lipid oxidation had enlarged liver (Peng *et al*. 2009), probably due to lipid infiltration of the liver cells (Baker & Davies 1996b). In the present study, the livers of the salmon fed oxidized oil were not significantly affected although the livers were numerically larger compared with those of salmon fed fresh oil. Earlier studies have shown that the HSI of African catfish fed high levels of tocopherol acetate, irrespective of dietary oil quality, was significantly smaller than in fish receiving low tocopherol levels (Baker & Davies 1996b). In line with this, Mourente *et al*. (2002) also reported that gilthead sea bream fed diets with addition of vitamin E tended to have smaller livers. In contrast, Zong *et al.* (2008) reported no effect of vitamin E supplementation or feeding of oxidized oil on HSI in Atlantic cod. In the current study, the largest livers were observed in fish fed diets with high vitamin E levels, irrespective of oil

quality. One explanation for the increased liver size with high vitamin E in the feed could be oxidative stress, as metabolic load associated with oxidation status of the cell. As suggested earlier, vitamin E radicals may act as pro-oxidants when vitamin C is deficient.

The heart weight of salmon fed oxidized oil tended to be larger than for those fed fresh oil, irrespective of vitamin E supplementation. This indicates that oxidized oil might have a negative effect on the heart health causing increased cardiac lipidprobably due to an impaired metabolism of these lipids, as suggested by (Gabriel *et al.* 1977). Furthermore, organ adhesions (Speilberg score) also tended to be more severe of salmon fed oxidized oils. It is well documented that certain vaccines/vaccination regimes may promote organ adhesions (Berg *et al*. 2006), but there is no previous study reporting the impact of dietary oil quality on the severity of organ adhesions in fish.

Fish fed fresh oil had a greater fillet thickness but the fillet yield was not significantly affected. These contradictive findings may be explained by the accuracy of the methods used to measure. Fillet thickness was measured by an automatic texture analyzer with a unit scale in mm. Hence, fillet thickness was measure more accurately than fillet weight that was measured in grams. Furthermore the weight of the fillets will depend on how the fish is filleted and degree of trimming.

5.3 Effects of oil quality, vitamin E supplementation and low dissolved oxygen stress on blood parameters

Feeding oxidized oil over a time period of 79 days did not significantly affect the blood parameters measured as sodium, potassium and pH in salmon slaughtered immediately after netting. The lack of response in the present study might be explained by the fish size. Larger fish, like the ones used in the present experiment (2.12kg), are maybe more developed and able to cope with chronic stress caused by the diets (Alves Martins *et al*. 2007). However, hemoglobin was slightly increased in groups fed oxidized oil. The result demonstrated that oxidative stress affected hemoglobin level as well as a tendency to higher heart index. The results are in line with findings reported by Zhong *el al*. (2008), who observed that Atlantic cod fed oxidized oil had a higher haemolysis than those fed fresh oil. Supplementation of vitamin E to the diet tended to increase the sodium and potassium concentrations. This trend was irrespective of oil quality. This effect on unstressed fish is not easy to explain from existing literature, but further studies may reveal whether a dietary mega

dose of vitamin E causes unbalanced ratio between vitamin E and C that results in a pro-oxidant action of vitamin E.

The increased sodium and potassium level in the blood of salmon kept in seawater with an oxygen concentration decreasing from 9 to 2 mg O_2/L before slaughter demonstrate that low dissolved oxygen induced osmoregulatory stress. According to Morales *et al.* (2005), changes in secondary stress responses, such as altered hemoglobin concentration. Normally are not displayed in acute handling stress, although it could be more responsive in chronic stress situations. In the present study, an overall significant effect of dietary oil quality was observed, whereas the hemoglobin level was not significantly affected by low DO before slaughtering. However, a tendency to increased hemoglobin in salmon fed oxidized oil suggests that poor oil quality may decrease the fish susceptibility to adapt to acute environmental stressors. The results also showed that the pH in the blood was significantly affected by hypoxic stress in combination with dietary oxidized oil, though the numerical variation was relatively low (from 6.8 to 6.9). The pH in the blood of hypoxic stressed salmon fed fresh oil was lower than the pH in those fish fed oxidized oil. The most likely explanation is that chronic stress associated with feeding diets containing oxidized oil reduced the response sensitivity to acute stress (i.e. lactate efflux to the blood at low DO stress). Randall *et al.* (1992) reported that vitamin E enriched diets caused smaller increase in plasma lactate level in sturgeon at progressive hypoxic exposure. In the present study no effect of vitamin E enrichment was observed for the hypoxic stressed salmon, but fresh oil in combination with high vitamin E levels gave the highest pH of salmon slaughtered immediately after netting.

5.4 Effects of oil quality, vitamin E supplementation and low dissolved oxygen stress on muscle pH

For the rested fish, the highest initial muscle pH (around 6.86) was observed for fish fed fresh oil quality without addition of vitamin E while no differences were observed among the other groups after 3h. The more rapid pH decline in salmon fed oxidized oils reflects accelerated glycolysis and coincides with results observed in broilers fed oxidized oil (Jensen *et al*. 1997). However, the former authors reported that addition of antioxidant (vitamin E and BHT) arrested the pH decline, whereas in the present study vitamin E addition had the opposite effect. There were no differences in muscle pH among dietary treatments after 24

hours, indicating that the glycogen level in the living salmon was similar for all dietary fish groups.

Muscle pH of the stressed groups dramatically dropped after 3 hours (6.47 on average for all groups), demonstrating that exposure to the low DO significantly accelerated glycolysis and subsequent lactic acid production. Erikson *et al*. (1997) reported that a DO level of 3.0 mg O_2/ L in the anesthesia tank seemed to be sufficient to immobilize the salmon quickly without causing severe anaerobic metabolism in the white muscle or suffocating the fish. The current study suggests that a DO level of 2 mg/L might be a critical limit where anaerobic metabolism in white muscle occurs, or that a progressive declining of DO is causing a more pronounced effect on the glycolysis than a fixed low DO level that may immobilize the fish. At the final sampling (after 24h) the difference between rested and stressed fish became less apparent. However, the muscle pH was significantly higher of the salmon fed the diets containing fresh oil with addition of vitamin E in the stressed groups. Hence, the overall results from the pH analyses indicate that both oxidized oils and vitamin E affect the glycolysis of salmon fillets. Initially a high vitamin E level accelerated the pH decline in un-stressed salmon, whereas high dietary vitamin E content arrested the glycolysis at an earlier point in stressed salmon, causing a higher terminal pH in the muscle. The underlying causes for these observations need to be elucidated in future studies.

5.5 Effects of oil quality, vitamin E supplementation and low dissolved oxygen stress on firmness, gaping and liquid loss

Oxidized oil did not affect firmness, gaping or liquid loss. It is possible that the ability of fish to counteract lipid peroxidation in tissues, induced by feeding oxidized diets, is species specific and size dependent (Mourente *et al.* 2002; Tocher *et al*. 2003). Therefore, larger fish, like the ones used in the present experiment (2.12kg), are probably more developed and able to cope with the stress imposed by the diets. The texture analysis showed that firmness was affected by vitamin E supplementation after a period 79 days, irrespective of oil quality. Fish fed the diets with highest level of vitamin E had firmer fillets, except those fed oxidized oil in combination with stress. The most likely explanation might that vitamin E can protect unsaturated fatty acids in bio-membranes against attack from oxygen free radicals during storage, resulting in cell damage (Peng *et al*. 2009). Although no significant effect of dietary treatment was seen on fillet gaping, there was a consistent pattern showing lower

values for fillets of salmon fed diets supplemented with vitamin E, hence coinciding with a firmer texture.

Acute stress did not have any effect on firmness or gaping but there was a significant effect on liquid losses. Einen *et al*. (2002) suggested that a lower water binding capacity in fish fillets could be due to cell damages, lower protein solubility and protein denaturation and aggregation. Furthermore, it is well documented that a rapid pH decline and a low end pH may promote liquid losses in muscle food (Fennema 1990). In the present study, the higher liquid losses coincide with the rapid pH decline.

Conclusion

Oxidized oil

- No negative effects on growth performance or feed conversion rate
- Increased hemoglobin level and a tendency to larger heart
- Decreased fillet thickness and declined muscle pH after 3h

Vitamin E supplementation

- No significant effect on growth performance but improved feed conversion rate (FCR) of the fish fed fresh oil
- Increased hepatosomatic index and paler liver
- Increased Na^+ and K^+ level in the blood
- Declined muscle pH 3h post mortem
- Increased fillet firmness

Low dissolved oxygen stress

- Increased Na^+ and K^+ level
- Declined muscle pH 3h post mortem
- Increased liquid loss

These results demonstrated that feed containing oxidized oil might have negative effects for Atlantic salmon and reduce their ability to cope with acute stress; in particular causing impaired osmoregulation ability. High vitamin E levels, on the other hand, improved the ability of the fish to cope with acute stress before slaughtering. However, paler and larger livers, increased Na^+ and K^+ levels in the blood and faster pH drop in the muscle post-mortem might be considered as a negative consequence of high dietary vitamin E supplementation. Hence the level and period of feeding high vitamin E levels need to be ascertained to achieve optimal production efficiency, physiological status and fillet quality of farmed salmon.

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