Influence of dietary tetradecylthioacetic acid (TTA) on lipid content and fatty acid composition in liver of 1+ Atlantic salmon (*Salmo salar* L.) and sex-specific differences in muscle fat provoked by TTA.

Påvirkningen av tetradesyltioeddiksyre (TTA) på fettinnhold og fettsyresammensetning i lever hos 1+ atlantisk laks (*Salmo salar* L.) og kjønns-spesifikke forskjeller i muskelfett fremprovosert av TTA.

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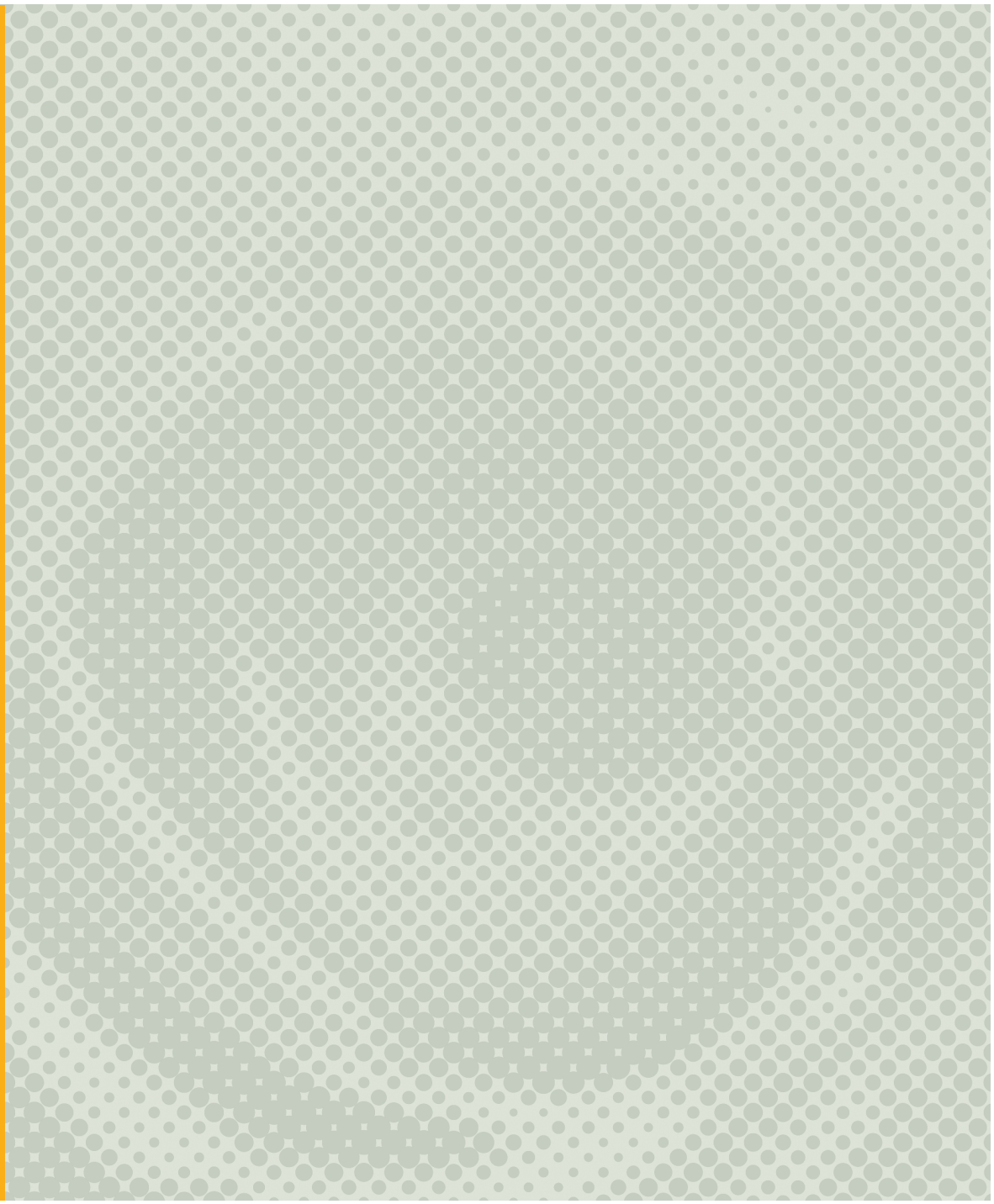

Forord

En femårig studieperiode er nå slutt og masteroppgaven i akvakulturvitenskap skal leveres. Studieløpet på UMB har vært en utrolig fin tid med faglig berikelse og mye sosialt påfyll. Da jeg skulle velge studie var jeg usikker hva jeg ville, men etter at jeg begynte på Ås har jeg aldri vært i tvil. Utdannelsen innen akvakultur og det gode studiemiljøet har vært utrolig givende og noe jeg ikke ville vært foruten. Gjennom utdannelsesforløpet har fiskeernæring alltid vekket min interesse, og da emne for masteroppgaven skulle velges viste jeg at jeg ville skrive innenfor ernæringsfeltet. Jeg kontaktet professor Kjell-Arne Rørvik og etter kort tid var alt i boks, jeg skulle skrive en oppgave om den bioaktive fettsyren TTA. Spennende!

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Ås, juni 2011

Sammendrag

Tetradesyltioeddiksyre (TTA) er en modifisert fettsyre som har vist seg å ha en rekke biologiske og fysiologiske effekter hos pattedyr og fisk. I forsøk med Atlantisk laks (*Salmo salar* L.) har tilsetting av TTA i fôret vist seg å redusere fettreserver i kroppen og øke kapasiteten for fettoksidasjon i muskel og lever. Målet med dette forsøket var evaluere påvirkningen av TTA på fettinnhold og fettsyresammensetning i lever hos laks. Det ble også undersøkt hva salgs effekter TTA kan ha på fettreserver og om dette kan variere mellom kjønn.

Forsøket ble utført med atlantisk laks (1^+ smolt) satt ut i sjøen 14. april 2009 (snittvekt 105 g) og denne fisken ble fulgt inntil avslutningen av forsøket i begynnelsen av mai året etter. Fisken ble fordelt i 12 merder med tre gjentak per fôrbehandling. Under forsøket ble det fôret fire ulike fôrtyper; kontrollfôr (CONTR), kontrollfôr tilsatt 1.5 % glutamat (GLU), kontrollfôr tilsatt 1.5 % arginin (ARG) og kontrollfôr tilsatt 0.25 % TTA (TTA-SD). Innvirkningen av TTA tilsetning på vekst, fôrinntak, kondisjonsfaktor, fôrutnyttelse, fettsyresammensetning i lever, samt fettinnhold i både muskel og lever ble fastslått under forsøksperioden. Det ble også undersøkt om det var kjønns-spesifikke forskjeller mellom fisk gitt de ulike fôrtypene. Fôr tilsatt TTA ble gitt i to perioder under forsøket. I den første perioden ble TTA gitt fra og med sjøutsett og inntil fisken hadde spist den mengden av fôr tilsatt TTA som tilsvarer 0.2 prosent av den opprinnelige kroppsvekten (varighet 10 uker). I den andre perioden ble TTA gitt i 6 uker sent på vinteren. Etter de to periodene ble fôr tilsatt TTA erstattet med kontrollfôr (CONTR).

Det ble ikke funnet negative signifikante effekter av TTA på gjennomsnittlig vekst eller fôrinntak. Det ble allikevel registrert at fisk gitt fôr tilsatt TTA hadde en signifikant lavere vekstrate og fôrinntak de første seks ukene etter sjøutsett, sammenlignet med fisk tildelt de andre fôrtypene (N-TTA-SD). Den hepatiske stomatic indeks (HSI) sammen med fettinnholdet i leveren til fisk gitt TTA var signifikant høyere ved slutten av første TTA fôring, 10 uker etter sjøutsett. Ved sluttuttaket i mai, etter den andre TTA fôringen, ble det derimot observert at fisk gitt TTA-SD, ARG og GLU hadde en signifikant lavere HSI sammenlignet med fisk gitt CONTR. Etter både den første og andre TTA fôringen hadde fisk gitt TTA-SD en tendens til økt andelen av n-3 flerumettede og mettede fettsyrer i leveren, mens nivået av oljesyre var redusert. TTA tilsetning førte til en signifikant reduksjon av fett i muskel og kondisjonsfaktor

sammenliknet med N-TTA-SD. Kjønns-spesifikke forskjeller i muskelfett og kondisjonsfaktor ble også påvist i fisk gitt TTA etter de to fôringsperiodene.

Siden det ikke ble påvist kjønns-spesifikke forskjeller i noen av de andre fôringsgruppene ble forskjellen trolig fremprovosert av TTA. En hypotese er at denne forskjellen mellom kjønn kan være nært knyttet opp mot energi og fett status, samt ønsket om å opprettholde fettreserver for å kunne initiere en kjønnsmodnings-prosess. Mer forskning er imidlertid nødvendig for å forstå underliggende og viktige faktorer som kan påvirke de observerte kjønns-spesifikke forskjellene.

Abstract

Tetradecylthioacetic acid (TTA) is a modified fatty acid that has been shown to have several biological and physiological effects in mammals and fish. In studies with Atlantic salmon (*Salmo salar* L.) dietary TTA supplementation was shown to reduce fat reserves in the body and increase the capacity for fat oxidation in muscle and liver. The aim of this study was to evaluate the influence of TTA on fat content and fatty acid composition in liver of Atlantic salmon. The influence of TTA on fat reserves and if this may vary between the sexes was also investigated.

The present study was conducted with Atlantic salmon (1^+ smolt) transferred to sea at the 14^{th} of April 2009, and until termination of the study in the beginning of May the following year. The fish were distributed in 12 net pens with an initial mean body weight of 105 g. During the experimental period four different diets were used in randomized block design of triplicate net pens. The four different diets were: control feed (CONTR), control feed added 1.5 % glutamate (GLU), control feed added 1.5 % arginine (ARG) and control feed added 0.25 % TTA (TTA-SD). The influence of TTA supplementation on growth rate, feed rate, condition factor, feed efficiency, fatty acid composition in the liver, and lipid content in muscle and liver was determined. We also investigated if there were sex-specific differences among the fish fed the different diets. TTA was fed in two periods. The first period, TTA was fed from sea transfer and until the fish had reached a final consumption of the TTA-supplemented diet equal to 0.2 percent of the initial body weight (duration of 10 weeks). The second period, TTA was fed for 6 weeks during the late winter. After the two TTA feeding periods, the TTA diet was replaced by the CONTR diet.

Dietary TTA supplementation was shown to have no statistical negative effect on overall mean growth or feed rate, however, the TTA fed fish had significantly lower feed rate and growth rate the first six weeks after sea transfer, compared with the fish from the other dietary groups (N-TTA-SD). The hepatic stomatic index (HSI) together with the lipid content of the liver in the fish fed TTA was significantly higher at the end of the first TTA feeding period. At the final sampling in May, after the second TTA feeding period, the HSI was significantly reduced in fish fed TTA-SD, ARG and GLU diet compared with fish fed the CONTR diet. Dietary TTA supplementation had a tendency to increase the percentages of n-3 polyunsatu-

rated fatty acids and saturated fatty acids in the liver, whereas the level of oleic acid was found to be reduced. After the first and second TTA feeding periods, the fish fed TTA had significantly reduced muscle fat content and condition factor compared with the N-TTA-SD group. Sex-specific differences in muscle fat and condition factor were also detected within the fish fed TTA after these two periods. Interestingly, this difference was provoked by TTA, since no similar sex-specific differences were observed in any of the other dietary groups. A possible hypothesis is that the obtained differences might be closely linked to energy and fat status, and the desire to maintain high fat reserves to be able to initiate a maturation process. More research is however needed to understand the underlying and important factors that may influence the observed sex-specific differences.

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1 Introduction

In the world aquaculture production of diadromous fish, Atlantic Salmon is the dominating fish specie, with a total production of 1.5 million tons in 2008 (FAO 2010). Norway is the world leading producer and exporter of Atlantic salmon, with an export of 711 053 metric tons representing a value of 23 656 million NOK in 2009 (FAO 2010; Norwegian Seafood Export Council 2009). Farmed salmon is therefore an important commodity for Norway and contributes to economic growth and employment, especially along the Norwegian coast line (Pettersen & Alsos 2007). Fish farming has had a rapid growth and development from the early 1980s until today (Asche et al. 2008; FAO 2010). The norwegian salmon industry has developed from small family-based businesses to an intensive, modern and globalised industry with large corporations and stable investors (Pettersen & Alsos 2007). The rapid development has grown concurrently with technical innovations and research, making aquaculture to an intensive and cost efficient industry with specialized research fields (Asche 2008).

Even though the salmon industry today is well organized and economically efficient, it faces many challenges. One of the challenges in salmon farming is to obtain an optimal production throughout the seawater phase. Today this is currently not fully realized due to the severe impacts of seasonal environmental variations and its influence on disease, appetite, lipid mobilization, growth and energy status (Alne 2010, 2011; Mørkøre & Rørvik 2001). If the farming of salmon shall continue to grow and be a sustainable industry it is important that these problems are reduced and that the production is as optimal as possible. Innovative research during the last decade has shown that by composing diets that is adapted for seasonal variations and energy demanding periods, it is possible to reduce the before mentioned challenges (Alne et al. 2009a, b; Oehme et al. 2010; Rørvik et al. 2003, 2007). This is done by the strategic supplements of additives or functional ingredients and is known as dynamic or functional diets. In particular, the administration of tetradecylthioacetic acid (TTA) has been found to have beneficial effects (Alne 2010; Kennedy 2007; Moya-Falcón 2005).

TTA is a saturated fatty acid with 16 carbon atoms and belongs to the group of 3-thia fatty acids where the methylene group in the third position from the carboxylic end is replaced with a sulphur atom (Kennedy 2007; Muna et al. 2000; Skrede et al. 1997). This replacement in the β-position of the carbon chain blocks the ability of TTA to undergo β-oxidation (Skrede et al. 1997). TTA is known as a hypolipidemic drug and peroxisome proliferator (Bremer 2001),

which in experiments with mammals has shown to reduce body fat and enhance the *β*-oxidation capacity in mitochondria and peroxisomes (Aarsland et al. 1989; Asiedu et al. 1996; Berge et al. 1989a, b). TTA is also found to reduce plasma lipid levels and have anti-inflammatory effects (Asiedu et al. 1996; Dyrøy et al. 2005). In Atlantic salmon TTA has shown to increases the *β*-position capacity in the white muscle (Rørvik et al. 2007), reduce muscle fat and increase the expression of immune genes and genes related to lipid metabolism in the cardiac ventricles (Alne et al. 2009b; Grammes et al. in press). A reduction in growth, condition factor and body energy during the first spring in sea, has been observed for both salmon transfer to the sea during the autumn (0^+) and spring (1^+) (Alne et al. 2011). The administration of TTA during these periods with subsequent natural outbreaks of diseases has shown to significant increase the survival rates (Alne et al. 2009b; Rørvik et al. 2007), and it is therefore believed that TTA may re-allocate deposited fat reserves and increase the amount of available energy as a result of increase fatty acid (FA) oxidation. The influence of TTA on the energy status of the fish was also found to reduce the frequency of early sexual mature males (Alne et al. 2009a; Arge et al. in press). These studies show the beneficial biological effects TTA may have on the salmon and how TTA may be used as a functional feed ingredient in diets to Atlantic salmon. It also illustrates how TTA may be used as a tool for understanding the importance of functional feed in modern fish nutrition.

3-thia fatty acids like TTA is efficiently taken up by the liver cells (Skrede et al. 1997), and an up-regulation in liver FAs oxidation activity is observed in Atlantic salmon, cod and rainbow trout fed dietary TTA (Kennedy et al. 2007a, b; Moya-Falcón 2005). In rainbow trout TTA also increased the proportion of n-3 fatty acids and reduced the expression of Δ6 desaturase in the liver (Kennedy et al. 2007a). In a study with Atlantic salmon TTA was shown to significantly increase the hepatosomatic index (HSI) and the levels of monounsaturated FAs (Moya-Falcón et al. 2004). In Alne et al. (2009b) a numeric increase in HSI was observed for the salmon fed TTA-supplemented diets, no further investigation was however done on the liver, and to our knowledge little research has been conducted on the influence of TTA on lipid content and fatty acid composition of the salmon liver during the sea water phase. The main objective of this thesis was to use TTA as a functional ingredient to 1^+ Atlantic salmon and evaluate the influence of dietary TTA on fat content and fatty acid composition in the liver. The influence of TTA on fat reserves and if this may vary between the sexes was also investigated.

2 Theoretical background

2.1 Smoltification and osmoregulation

The seawater phase is looked upon as a vulnerable and difficult stage during the salmon production cycle. One reason for this is the challenges related to the transfer of salmon from freshwater to seawater. The process where the salmon is pre-adapting to a life in seawater is known as smoltification, where the salmon parr develops into smolt. This is a complex process where the salmon has to undergo several physiological, morphological, and biochemical changes to be able to obtain normal osmoregulation and survive in seawater (reviewed by Boeuf 1993; Hoar 1988; McCormick & Saunders 1987). Osmoregulation is known as the mechanism for maintaining a constant internal ion concentration (Wurts 1987) and are abundantly described in the literature by Evans & Caliborne (2005), Salte (2002) and Stefansson (2007). Briefly, in freshwater the salt concentration is lower than in the tissue fluids and blood of the salmon. Due to osmosis the salmon will therefore take in water passively, mainly through the gills epithelium, skin and oral cavity. To maintain a correct internal salt concentration and fluid balance the salmon has to excrete large volumes of diluted urine. To compensate for the loss of salts the salmon absorbs ions from the surrounding water and through their diet. The regulation of fluids and ions in freshwater is known as hyper-osmoregulation (point 1 fig 2.1). In saltwater the environment is opposite that in freshwater. Here the salt concentration is significantly higher than in the tissue fluids and blood of the salmon. The salmon will therefore lose water to the surrounding environment. To compensate for the loss of water and avoid dehydration, the salmon drinks large amounts of salt water. The excess salt in form of monovalent ions (Cl^- and Na^+) from the drinking is removed through excretion across the gill epithelium, whereas the di- and trivalent ions (Mg^{2+}, PO_4^{3-}, SO_4^{2-}) is removed together with small amounts of urine through secretion by the kidneys. Good ability to removal of salts is necessary to obtain fluid balance and normal functions of the cells (Salte 2002). The regulation of fluids and ions in saltwater is known as hypo-osmoregulation (point 2 fig 2.1).

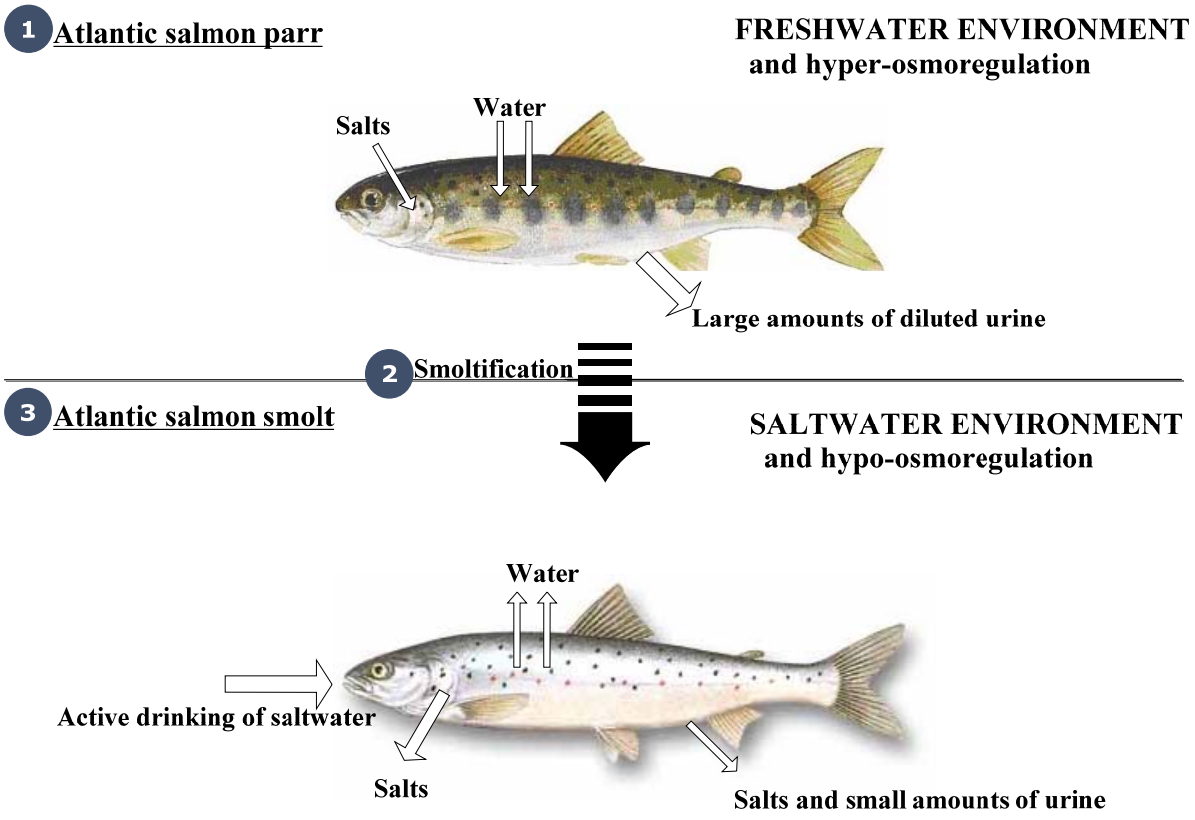


Fig 2.1 Osmoregulation in fresh and seawater environments and the transformation of Atlantic salmon parr (**1**) to smolt (**3**) trough the smoltification process (**2**). The figure is made in SmartDraw and is modified after the figures in Salte (2002) and Stefansson (2007).

The major changes in morphology, physiology and biochemistry during smoltification are under endocrine hormonal control and the secretion of hormones is mainly regulated by endogenous rhythms and environmental factors (Stefansson 2007). To be able to adapt to seawater the salmon develops so called hypo-osmoregulatory mechanism that involves functional changes in main osmoregulatory organs (McCormick & Saunders 1987; Salte 2002). This is illustrated in the fig 2.2 and shows that the number of chloride cells in the gills and the Na^+, K^+- ATPase activity increases (Langdon & Thorpe 1985; McCormick & Saunders 1987). The Na^+, K^+- ATPase is known to be involved in the transport of monovalent ions across membranes (Shuttleworth 1989), and high activity of this enzyme and ion transport capacity is found in mitochondria-rich gill chloride cells (Epstein et al. 1980). The increase of these mechanisms is therefore important for seawater tolerance, together with a decrease in kidney

glomerular filtration rate and reabsorption of ions by the urinary bladder. The dotted line in the figure indicates the period of maximum salinity tolerance and represents the best time for transferring the smolt to sea. This period is often referred to as the "smolt window", and if the smolt is not set out to sea during this period the current changes will reverse (desmoltification). An imbalance in osmoregulation may be seen as accumulation of Cl^- and Na^+ in plasma and these levels are often used as an index of smolt status (Staurnes et al. 2001).

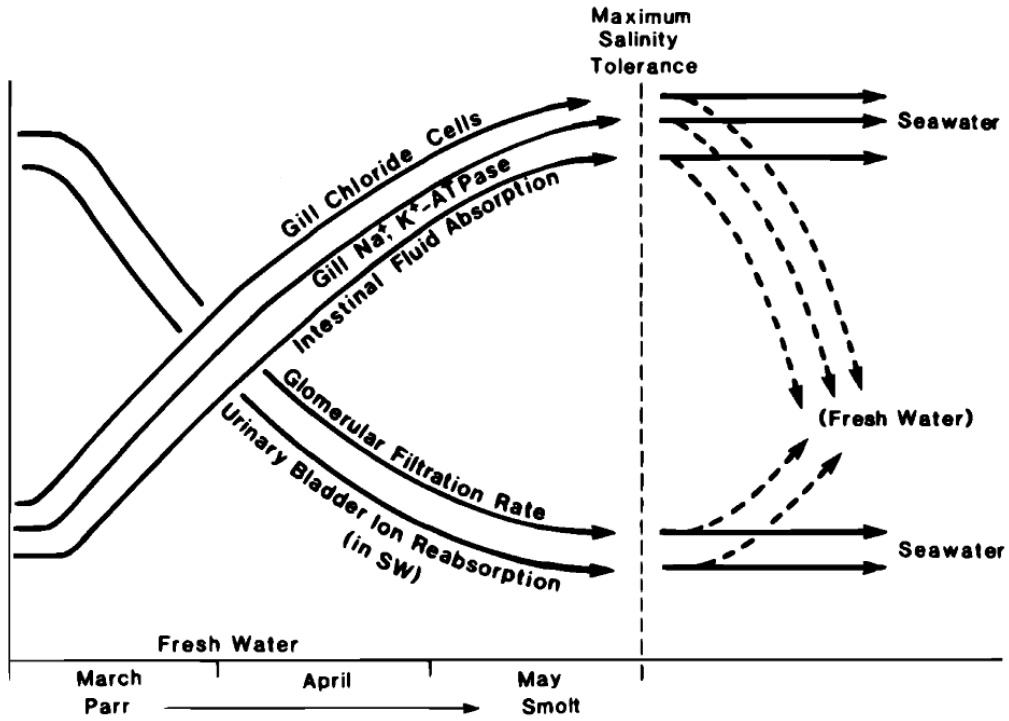


Fig 2.2 Changes in main osmoregulatory organs during the smoltification process (McCormick & Saunders 1987).

2.2 The smolt and post-smolt stage; seasonality and performance

In Norway, the salmon farmers transfer the smolt to sea at two different seasons during the year (Alne et al. 2011; Mørkøre & Rørvik 2001). This is done to be able to produce fresh marketable salmon throughout the annual cycle. From a production point of view, the first set-out is during the autumn, around 8 months after hatching and is denoted as 0^+ smolt. The second set-out is during the spring, around 16 months after hatching and is denoted as 1^+ smolt. The production of 0^+ smolt for transfer in autumn is made possible by the manipulation of photoperiod and water temperatures to alter the time of smoltification (Duston & Saunders 1995; Sigholt et al. 1995; Solbakken et al. 1994), and is often referred to as "out-of-season" smolt. The production of 1^+ smolt is conducted under natural conditions and is known as the traditional way of producing smolt, often referred to as "in-season smolt". Seasonal variations in energy stores, condition factor, growth and feed utilization have been well documented in both 1^+ and 0^+ salmon throughout the sea water phase (Alne et al. 2011; Mørkøre & Rørvik 2001).

The use of deposited fat and glycogen during smoltification and sea water exposure is observed in salmonids (Jobling et al. 2002a; Sheridan 1989). This indicates that these processes are energy demanding and that the salmon is dependent on accessible energy to fulfill their metabolic requirements. Studies have shown that the osmoregulatory adaption may act as a stressor that suppress the growth and feed intake in the first weeks after sea transfer (Handeland et al. 2000; Jobling et al. 2002a; Jørgensen & Jobling 1994; Rørvik et al. 2007; Usher et al. 1991). Results obtained by (Alne et al. 2011), also illustrate that the season and photoperiod are important factors that may influence the performance of the smolt significantly.

The 0^+ smolt maintain a good growth rate and start to accumulate fat after sea transfer in the autumn (Alne et al. 2011; Lysfjord et al. 2004). During the following spring, the growth rate, condition factor and muscle fat has been observed to decrease until June (Alne et al. 2011). This drop in performance has also been experienced by Norwegian commercial salmon farmers (Alne et al. 2011), and a decline in condition factor during the spring for 0^+ has also been observed by Oppedal et al. (2006). During the period from May to June the retention of fat and energy in 0^+ smolt has been found to be significantly lower than the periods March-May and June-September (Alne et al. 2011), indicating that the energy demand is high during

the period from May to June. In contrast to the 0^+ smolt, the 1^+ smolt seems to have a period with low performance in the weeks after sea transfer. During the period after sea transfer in the spring, the condition factor, muscle fat and body energy levels has all been observed to decrease and the period is often burdened with reduced growth and feed intake (Alne et al. 2011; Bendiksen et al. 2003; Jobling et al. 2002a, b; Lysfjord et al. 2004; Måsøval et al. 1994; Usher et al. 1991). This is clearly shown in Alne et al. (2011), were muscle fat, condition factor, energy and fat retention where significantly reduced from sea transfer in May until June/July (fig 2.3). From mid-June and during the autumn the condition factor significantly increased, while muscle fat and the retention of fat and energy significantly increased from mid-July to September. In the same study, but in another experiment with 1^+ smolt the thermal growth coefficient (TGC) showed the same pattern. The increase in fat accumulation during autumn coincides with other studies with 1^+ smolt (Lysfjord et al. 2004; Mørkøre & Rørvik 2001; Måsøval et al. 1994; Roth et al. 2005), and seems to be characteristic for smolt transferred to sea during the spring (1^+) and autumn (0^+). The period of shorter photoperiod during the autumn can therefore be looked upon as a fat accumulating period, whereas during the first spring the fish degrades deposited fat and converts this to accessible energy.

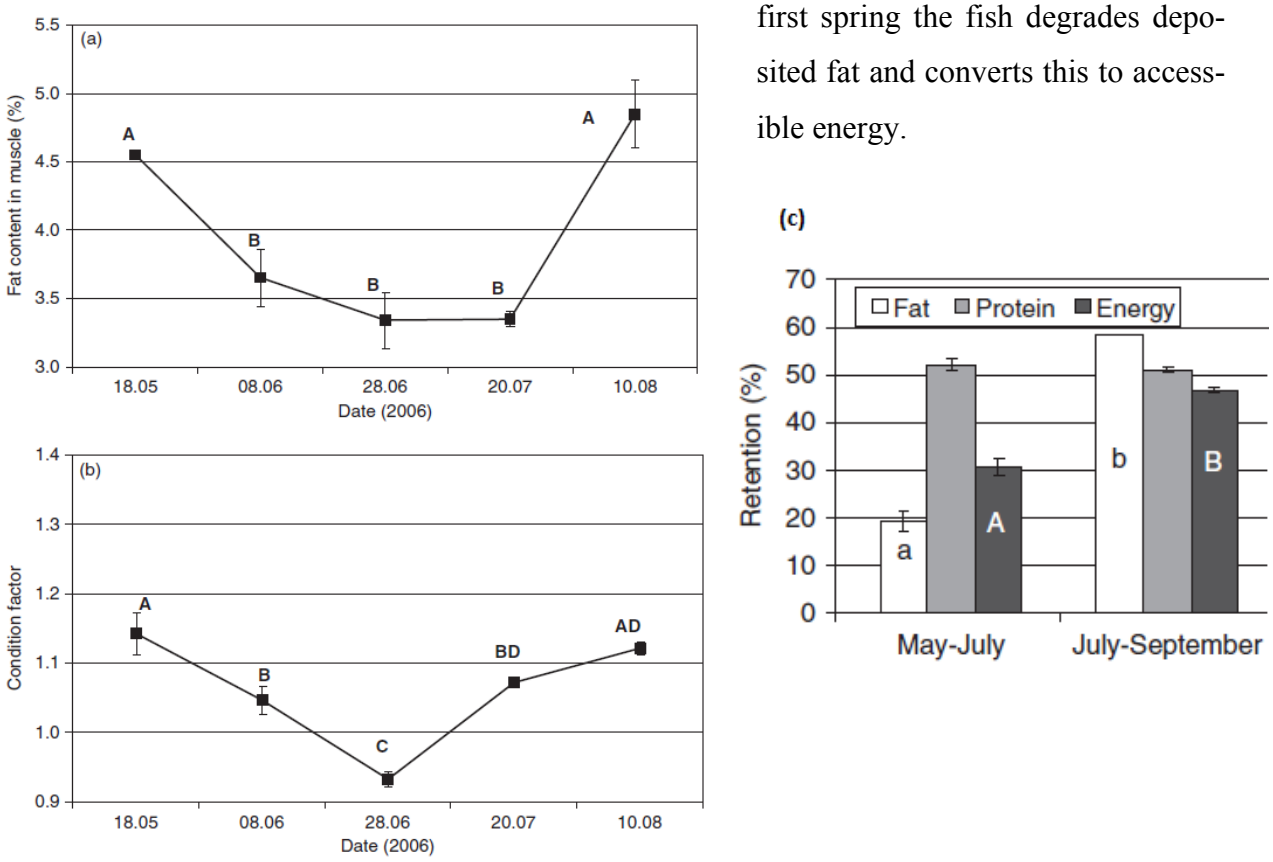


Fig 2.3 Changes in muscle fat content (a), the condition factor (b) and retention of nutrients (c) for 1+ Atlantic salmon after sea transfer from the study of Alne et al. (2011). Significant differences between sampling dates and periods are indicated by different letters on the curves/bars. The variation between net pens within sampling dates/periods is given as the standard error of the mean.

The following presented data shows that both 0^+ and 1^+ smolt have reduced growth, condition factor and body energy levels during their first spring in sea (Alne et al. 2011). This is an important finding since there is normally an increased growth by increasing temperatures (Austreng et al. 1987), and higher growth rate has often been observed with longer day length (reviewed by Boeuf & Le Bail 1999). In the cause of the 1^+ smolt osmoregulatory stress may be part of the reason for reduced growth and feed intake. If osmoregulatory stress may also be a contributing factor for the drop in performance for the 0^+ is unclear, however it has been speculated if hormonal regulation may be a factor to the low performance in 0^+ smolt (Alne 2010). The results obtained by Alne et al. 2011, also show that the muscle fat content of 1^+ and 0^+ salmon with fish weight of 100-1500 grams correlates well ($R^2 = 0.99$) with the whole body energy (Alne 2010). Muscle fat may therefore be a good marker and indicator for reduction of total body energy levels, and illustrate that the first spring and early summer is an energy demanding period for salmon smolt and post-smolt. Natural disease outbreaks are often observed during this period (Alne et al. 2009b; Eggset et al. 1997; Rørvik et al. 2003, 2007), and it has been suggested that the low performance and energy status of both the 0^+ and 1^+ salmon smolt may be a predisposing factor for these outbreaks (Alne 2010; Rørvik et al. 2007). This underlines the importance of seasonal and biological factors and how this may have negative implications during the seawater phase.

Lipids, of which especially fatty acids (FAs) are a major part of the feed to Atlantic salmon and represent the primary source of energy in the diet (Frøyland et al. 1998; Torstensen et al. 2000). High dietary inclusion levels of lipids are often used in commercial salmon farming. The reduction in performance and energy reserves during the spring/summer suggests that energy requirements are higher than the salmon is able to obtain form the diet (Alne et al. 2011; Rørvik et al. 2007). It has therefore been conducted studies on other possible ways for the salmon to obtain higher and more efficient stored energy utilization, i.e. if more of the deposited lipids can be utilized for energy production (Alne et al. 2009b; Moya-Falcón et al. 2004; Rørvik et al. 2007). The outcome of these studies shows that by administrating small amounts of dietary TTA, the fish's FA oxidation capacity increased and the body lipid levels was shown to decrease. Re-allocation and mobilization of deposited lipids to increase the amount of available energy for the fish was found beneficial, especially during the described energy demanding periods where disease outbreaks often occur (Alne et al. 2009b; Rørvik et al. 2007). The influence TTA has on the energy status of the fish was also found to reduce the frequency of early sexual mature males (Alne et al. 2009a; Arge et al. in press).

TTA is known as a so called "functional" or "bioactive" feed ingredient, which may influence the metabolism and thereby affect the status of the fish. The concept with functional or dynamic feed formulations is to exploit different dietary components like functional fatty acids, vitamins, alginate, nucleotides, probiotics etc to boost or improve the performance of the fish during challenging and demanding periods (e.g. sea transfer, energy demanding periods, seasonal variations, diseases outbreaks, before or after handling and treatments etc.) (BioMar 2011; Ewos 2011). This concept is today used by the main Norwegian salmon feed manufactures like Skretting, Ewos and BioMar, that all have different assortments of functional feed lines (e.g. figure 2.4). TTA has shown to influence the lipid metabolism in both mammals and fish. To understand the influence TTA may have in fish nutrition it is important with a basic insight in lipid metabolism, in particularly the catabolism and oxidation of fat.

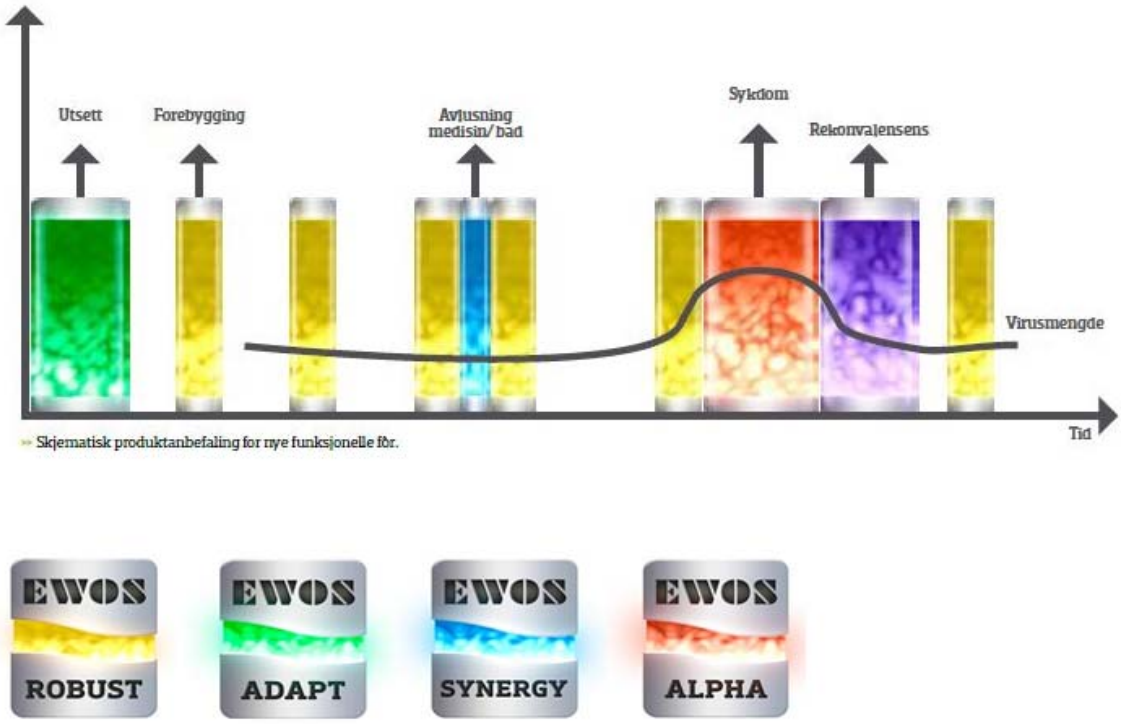


Fig 2.4 The new functional feed line to EWOS, with an example of when it is recommended to feed the different diets (in Norwegian) (Ewos 2011) .

2.3 Lipids and lipid metabolism

Lipids are a diverse group of organic molecules that is insoluble in water but soluble in organic solvents. In general, lipids are the most energy rich nutrient with an energy density of 39 kJ g^{-1}, which is considerably higher than the energy density of both proteins (23.6 kJ g^{-1}) and carbohydrates (17.2 kJ g^{-1}). Besides being high energy nutrients, lipids serve as important structural components in cell membranes and some lipids acts as predecessor to hormones and prostaglandins (Mathews et al. 2000). Lipids are also carriers of lipid-soluble carotenoids, vitamins and minerals (Ruyter et al. 2000; Torstensen et al. 2001a). Dietary lipids also provide essential fatty acids (EFAs) as 18:3n-3, 18:2n-6, 20:5n-3 and 22:6n-3 that is vital for development of tissues and normal growth (Ruyter et al. 2000; Sargent et al. 1995; Sargent et al. 2002). The main function of FAs in all organisms is to undergo β-oxidation and produce metabolic energy in the form of ATP (Sargent et al. 2002).

2.3.1 Digestion, absorption and transportation of lipids

The digestion, absorption and transportation of lipids in Atlantic salmon mainly occurs as shown in figure 2.5, described by Torstensen et al. (2001a) and Sjaastad et al. (2003). The digestion of dietary lipids is done in the blind sacks and intestines with the help of bile salts from the liver and lipolytic enzymes (lipase) from the pancreas. The bile salt emulsifies the lipids and increases the accessibility of the lipases to attack the surface of the lipid molecules and break them down to FAs and glycerol. The degradation products of the lipid molecules (mainly free fatty acids and monoglycerides) and bile salts then form micelles. These objects are small enough to be absorbed by enterocytes. When the micelle comes in contact with the enterocytes, the micelles dissolve and FAs and monoglycerides (MAG) diffuse across the cell membrane. In the enterocytes the long chain FAs are reesterified with MAG to form triacylglycerols (TAG), while medium chain FAs are not esterified. The TAG or FAs are then converted into lipoproteins, so called chylomicrons (CMs). The CMs contain cholesterol and phospholipids (PL) that is absorbed and formed from the intestine and enterocyte. The CMs are circular particles that are covered by the lipoprotein coat, which allows the CMs to be transported in the blood system. In mammals the CMs are transported in the lymph system (Sjaastad et al. 2003) but in fish no such system has been detected. It has therefore been assumed that fish secrete the CMs directly into the blood system and is from here transported to peripheral tissues and the liver (Torstensen et al. 2001a).

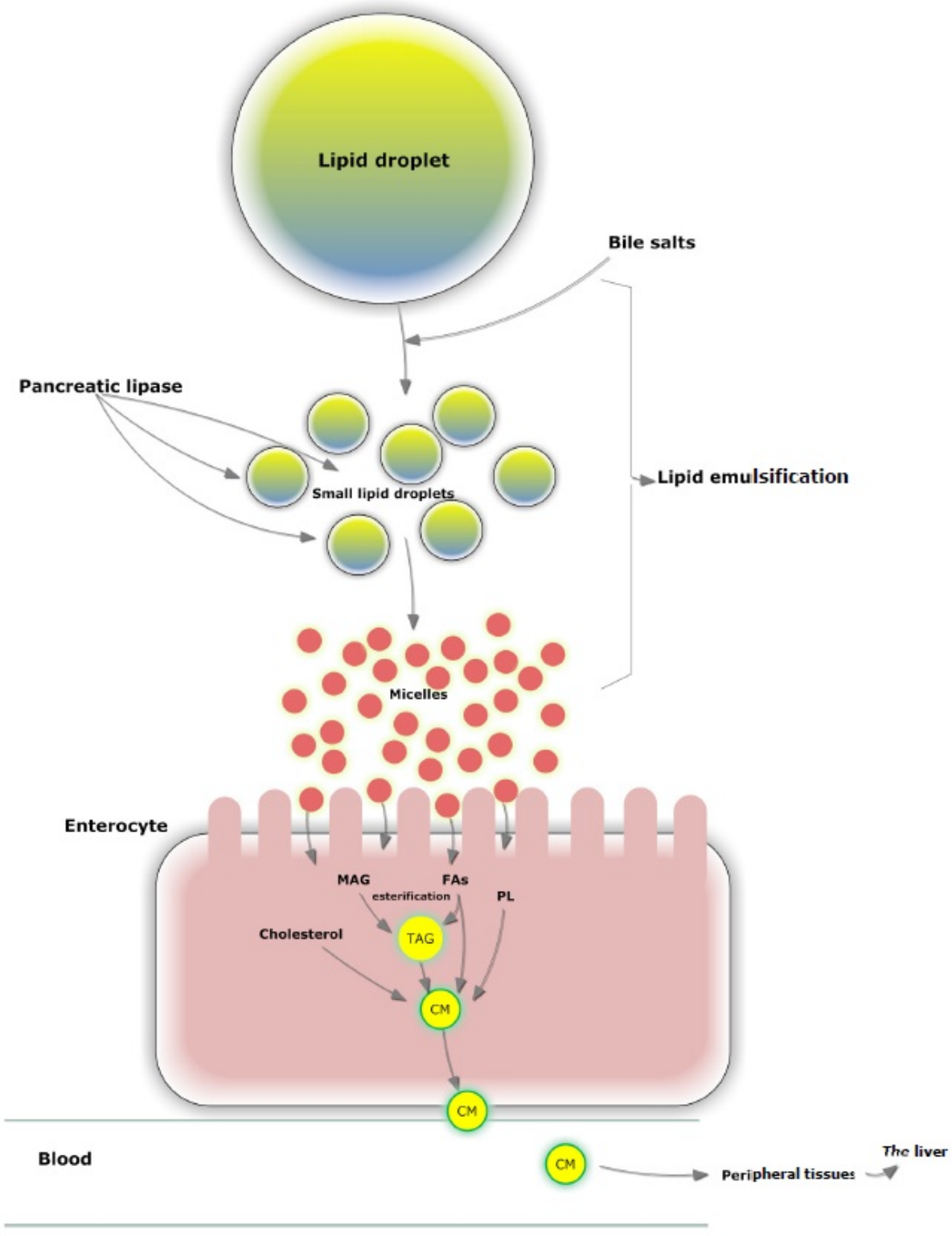


Fig 2.5 Digestion, absorption and transportation of lipids (MAG: monoglycerides FAs: fatty acids, PL: phospholipids CM: chylomicrons. The figure is made in SmartDraw and is modified after Torstensen et al. (2001a) and Sjaastad et al. (2003).

2.3.2 Elongation, desaturation and oxidation of FA

FAs may undergo one or several different metabolic processes in the fish body. They may be synthesized or modified trough desaturation, elongation or chain shortening. Further, the FAs may be esterified into structural and reserve lipids or be a major source of energy by undergoing *β*-oxidation (reviewed by Sargent et al. 2002).The fate of the various lipids will depend on the specie of the fish, physiological status and the FAs composition of the diet (Torstensen et al. 2001a).

The saturated FAs 16:0 and 18:0 are known to be synthesized *de novo* in fish and mammals and these FAs can again be metabolized to 16:1n-7 and 18:1n-9, respectively, with the enzyme Δ^9-desaturase. In fish dietary C_{18}- FAs may be elongated or desaturated to C_{20}- and C_{22}- FAs and the ability to do this varies between species (Ruyter & Thomassen 1999). Salmonids produce 20:5n-3 and 22:6n-3 from 18:3n-3, and 20:4n-6 from 18:2n-6 by Δ^6- and Δ^5-desaturases and elongases (Sargent et al. 2002). Although salmon has the ability to elongate and desaturate EFAs, it is necessary to supplement the diet with 18:3n-3 and 18:2n-6 together with certain amount of 20:5n-3 and 22:6n-3 to meet their optimal EFAs requirement, especially during the fry stage (Ruyter et al. 2000). Cell cultures from Atlantic salmon have been found to have a better ability to elongate and desaturase 18:4n-3 to 20:5n-3 than cell culture from turbot (Ghioni et al. 1999). It has been suggested that anadromous fish has a better ability to elongate and desaturate than marine fish, due to the FAs composition of the natural diet of the marine fish (Sargent et al. 2002). For example may 18:3n-3 in the diet for juvenile rainbow trout in freshwater meet the requirement for n-3 polyunsaturated FAs (Castell et al. 1972).

The catabolism of fatty acids for the release of energy is known as *β*-oxidation. When the CMs reach its target tissue, the FAs are released by lipoprotein lipase and take up by the cells to generate energy trough *β*-oxidation, which occurs in two cell organelles, mitochondria and peroxisomes (Frøyland et al. 1998). The mitochondria consist of an outer and inner membrane, and the *β*-oxidation process take place in the matrix within the inner mitochondrial membrane (Mathews et al. 2000). Before the FAs can undergo *β*-oxidation the FAs are activated to fatty acyl-CoA and then further to fatty acyl-carnitine to be able transported from the cytosol and into the matrix. This is done with the help of the carnitine acyltransferase I (CTP 1) that is located in the outer mitochondrial membrane (Reddy & Hashimoto 2001). When the fatty acyl-carnitine has entered the matrix, carnitine acyltransferase II (CTP II) exchanges the

acyl-carnitine for free carnitine and produces fatty acyl-CoA. Further the fatty acyl-CoA undergoes four main steps: (1) dehydration, (2) hydration, (3) dehydrogenation and (4) thiolytic cleavage (Mathews et al. 2000). These steps make up the *β*-oxidation process, which results in $FADH_2$, NADH and acetyl-CoA. Acetyl-CoA will further be processed in the tricarboxylic acid cycle (TCA cycle) (Mathews et al. 2000; Torstensen et al. 2001a). Mitochondrial *β*-oxidation is known to oxidize short (< C_8), medium (C_8-C_{12}) and long (C_{14}-C_{20}) fatty acid chains (Reddy & Hashimoto 2001).

The peroxisomes only have one membrane and the *β*-oxidation here is different from mitochondrial *β*-oxidation. Peroxisomes lack a link to the TCA cycle and are therefore incapable of producing ATP. Furthermore, the peroxisomes can only chain-shorten fatty acids and is not able to fully degrade the fatty acids into acetyl-CoA units (Wanders et al. 2001). The peroxisome works therefore primarily as chain-shortener for long and very long (> C_{20}) fatty acids (Reddy & Hashimoto 2001; Torstensen et al. 2001a). After the FAs are degraded in the peroxisomes, they can be completely oxidized in the mitochondria. CTP I and II has no role in the uptake of fatty acids in the peroxisomes (Reddy & Hashimoto 2001), but may have a role in exporting chain-shortened products to the mitochondria in mammals (Wanders et al. 2001). The peroxisomes *β*-oxidation process produces H_2O_2 which is decomposed to O_2 and water (Wanders et al. 2001).

Mitochondrial *β*-oxidation takes places in all cells, but to a large extent in organs such as liver, heart, muscle and kidneys (Frøyland et al. 2000; Torstensen et al. 2001a). Frøyland et al. (2000), found that the red muscle in Atlantic salmon has the highest fatty acids oxidation capacity. Here the mitochondrial *β*-oxidation accounted for 80 % of the oxidation capacity, while peroxisomal *β*-oxidation account for 20 %. In the white muscle these values where 60 % and 40 %, respectively. Since the white muscle accounts for a large part of the body mass (60 % of body weight in that study), it is of considerable importance for the total oxidation of fatty acids in the salmon body. The liver was found to have a mitochondrial *β*-oxidation capacity of 70 % and a peroxisomal *β*-oxidation capacity of 30 %. In the liver of juvenile salmon however, the peroxisomal *β*-oxidation dominated and the overall oxidation capacity was higher in the juvenile salmons compared with adult salmons (Frøyland et al. 2000).

2.3.3 Lipid mobilization and deposition

Various fish species have different ways of storing excess fat, for example do lean marine fish as cod store large amounts of lipids in the liver (Torstensen et al. 2001a). Salmonids are known to store large amounts of fat in the muscle and especially in the myosepta tissue around the muscle fibers (Jobling et al. 1998; Nanton et al. 2007). Fat is also stored in visceral adipose tissues and the excess FAs is most commonly stored as triacylglycerols (TAG) (Jobling et al. 1998). High dietary fat levels may therefore lead to an increase in the estrification of FAs to TAG. Lipids-rich diets are known to increase the deposition of lipids in Atlantic salmon, and high levels of excess fat may have a negative effect on both fish health and quality (Sargent et al. 2002).

2.4 Tetradecylthioacetic acid

TTA is a saturated fatty acid with 16 carbon atoms and belongs to the group of 3-thia fatty acids where the methylene group in the third position from the carboxylic end is replaced with a sulphur atom (fig 2.6) (Kennedy 2007; Muna et al. 2000). The chemical properties of TTA are very similar to other FAs and it is assumed that TTA is digested, absorbed and transported (as described in 2.3.1) as other FAs. The replacement with the sulphur atom in the *β*-position of the carbon chain, however, blocks the ability of TTA to undergo normal *β*-oxidation pathways as described in 2.3.2 (Moya-Falcón 2005; Skrede et al. 1997). TTA is therefore activated into TTA-CoA and then incorporated into PL and acylglycerols (Aarsland & Berge 1991; Hvattum et al. 1993). TTA can however be metabolized to dicarboxylic acid trough ω-oxidation in endoplasmatic reticulum and peroxisomes (Muna et al. 2000). After this the dicarboxylic acids can be oxidized by the mitochondria or peroxisomes, and it may also be excreted in the urine trough the kidneys (Bergseth & Bremer 1990; Moya-Falcón 2005). This is known to occur at low rates and high amounts of TTA metabolites have been observed to accumulate in the kidney of the salmon (Moya-Falcón et al. 2004).

Figur 2.6 The chemical structure of tetradecylthioacetic acid, (Kennedy 2007).

TTA has been substantially studied and has shown to have many beneficial properties when administrated to mammals and fish (Aarsland et al. 1989; Alne et al. 2009a, b; Berge et al. 1989a, b; Kennedy et al. 2007a, b; Moya-Falcón et al. 2004; Rørvik et al. 2007). The most important effects of TTA are the increase in *β*-oxidation and its subsequent fat-reducing effects (hyperlipidemic effects). TTA is known as a peroxisome proliferator (Bremer 2001), which is a term for some substances having a regulatory effect on lipid metabolism. TTA does this by being a ligand to the peroxisome proliferation activation receptors (PPARs) (Bremer 2001), which controls the expression of genes regulating lipid metabolism and transport of FAs (Larsen et al. 2005). When TTA was fed to mammals (mainly rats) the size and number of both peroxisomes and mitochondria were observed to increase, FAs oxidation capacity increased, CPT I and II activity was up-regulated, drops in plasma lipid levels and cholesterol where induced, and a reduction in hepatic TAG levels was observed (Aarsland et al. 1989; Asiedu et al. 1996; Berge et al. 1989a, b; Madsen et al. 2002). A reduction in hepatic TAG secretion in cultured rat hepatocytes has also been shown (Skrede et al. 1994). TTA reduces the total body fat in rats, together with having an influence on the lipid composition in different organs (Asiedu et al. 1996). The supplementation of TTA has also been observed to increase the oxidation of fat in the heart of mice (Hafstad et al. 2009).

In the last decade TTA has also gained attention in aquaculture based research and has shown to have a lot of the same effects on fish as with mammals. In fish however, the effects appear to vary in some extent between the species. In Kennedy et al. (2007a) rainbow trout was fed 0.5 % TTA over a period of 8 weeks. In this study the CTP I and acyl CoA oxidase (ACO) activity in liver and red muscle was increased and an up regulated expression of CTP-I in the white muscle was observed. TTA fed to cod showed the same increase in ACO and CTP I activity in the liver, but in contrast the ACO activity in the white and red muscle was reduced and no effect of TTA was observed on the activity of CTP I in muscle tissues (Kennedy et al. 2007b). In these studies with cod grown from approx. 125 to 310 grams (Kennedy et al. 2007b) and rainbow trout grown from approx. 430 to 800 grams (Kennedy et al. 2007a), TTA had no significant effect of body weight, growth rate, feed conversion ratio or fat content. In a study with juvenile Atlantic salmon fed 0.3 % and 0.6 % of dietary TTA, the final body weight, thermal growth coefficient and relative feed intake was significantly lower compared with the fish not fed TTA (Moya-Falcón et al. 2004). The supplementation of TTA also resulted in a higher mitochondrial *β*-oxidation capacity, and the fish fed 0.6 % TTA showed a reduction in total lipid levels.

Recent studies has also revealed that TTA can be beneficial as a functional feed ingredient for Atlantic salmon during the sea water phase (Alne 2010), especially in earlier described periods (se 2.2) of low performance with subsequent natural outbreaks of diseases (Alne et al. 2009b; Rørvik et al. 2007). In Rørvik et al. (2007) the mortality during a natural outbreak of infectious pancreatic necrosis (IPN) in 1^+ post smolt 8 weeks after sea transfer, was reduced from 7.8 % to 2.3 % by the supplementation of dietary TTA. The fish fed TTA had the lowest plasma chloride levels, which were significantly lower than in the fish fed a high fat diet. High levels of plasma chloride in smolt are often a sign of osmoregulatory imbalance (Staurnes et al. 2001), and this may indicate that TTA fed fish had a better osmoregulatory status than the other fish. Muscle lipid content was also decreased, and mitochondrial β-oxidation was observed to be significantly increased in the white muscle of TTA fed fish. This indicates that the fish fed TTA had a higher capacity for energy utilization and this may partly explain the lower levels of plasma chloride and reduced osmotic stress. It is suggested that these factors are important elements and contributors to an increased ability to survive or resist natural outbreaks of IPN. In Alne et al. (2009b) pre-feeding of dietary TTA was found to increase the survival in 0^+ salmon during a natural outbreak of heart and skeletal muscle inflammation (HSMI) during their first spring in sea. Here TTA reduced the mortality from 4.7 % to 2.5 %, up regulated the expression of genes related to lipid oxidation in the heart (PPAR-α, PPAR-β, CPT 1, ACO, LPL), increased cardiosomatic index (CSI) and growth. TTA has shown to have anti-inflammatory properties (Dyrøy et al. 2005), and it suggested that these properties and an increase in FAs oxidation and degradation may be important factors for increased growth and survival. In Grammes (in press), the cardiac ventricle of the fish fed TTA during this outbreak of HSMI also showed increased expression of immune genes (TNFα, VACM-1, IgM and CD8-α). This study suggested that the elevation in cardiac recruitment of immune cells and increased CSI might lead to a more robust fish, with a better ability to survive or resist natural outbreaks of HSMI.

Increased FAs oxidation and lowering of fat reserves by dietary TTA supplemented diets has also shown to have other beneficial effects. TTA fed to 1^+ salmon during the weeks after sea transfer was shown to significantly reduce the level of fat in the muscle and the frequency of early sexual mature males (jack maturation) during their first autumn in sea (Alne et al. 2009a). A recent study also reveals that TTA fed to 0^+ smolt during their first spring in sea (March-May), significantly reduce the male gonadosomatic index (GSI) and the incidences of sexual mature males the following fall (Arge et al. in press). TTA was here found to signifi-

cantly reduce the muscle fat during the spring. Previous studies have indicated that the levels of stored fat and energy in the fish body in late winter or spring may be of great importance for the onset of sexual maturation (Kadri et al. 1996; Rowe & Thorpe 1990; Thorpe et al. 1990). The TTA studies verify this, and during the two trials TTA was not observed to affect the final body weight or growth.

The mentioned studies reveal the importance of functional diets in modern fish nutrition and how this may be utilized in the farming of Atlantic salmon. It also illustrates how TTA may be used as model for understanding the importance of fat and energy status in fish. Figure 2.7 and 2.8 illustrates and summaries the beneficial properties of TTA in 0^+ and 1^+ salmon respectively, during the sea water phase.

Influence of TTA on 0+ Atlantic salmon

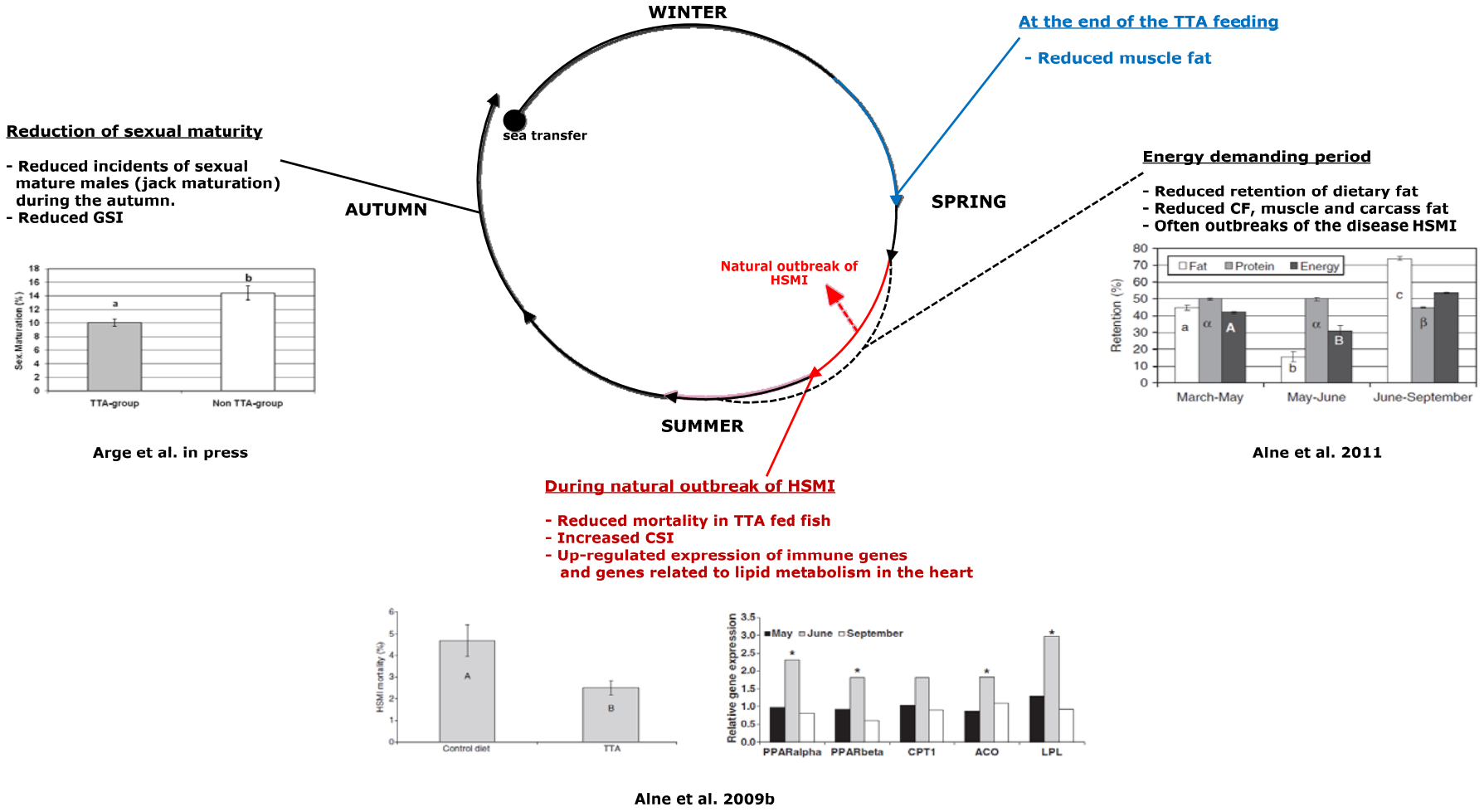


Fig 2.7 The influence of TTA on 0+ Atlantic salmon during the seawater phase.

Influence of TTA on 1+ Atlantic salmon

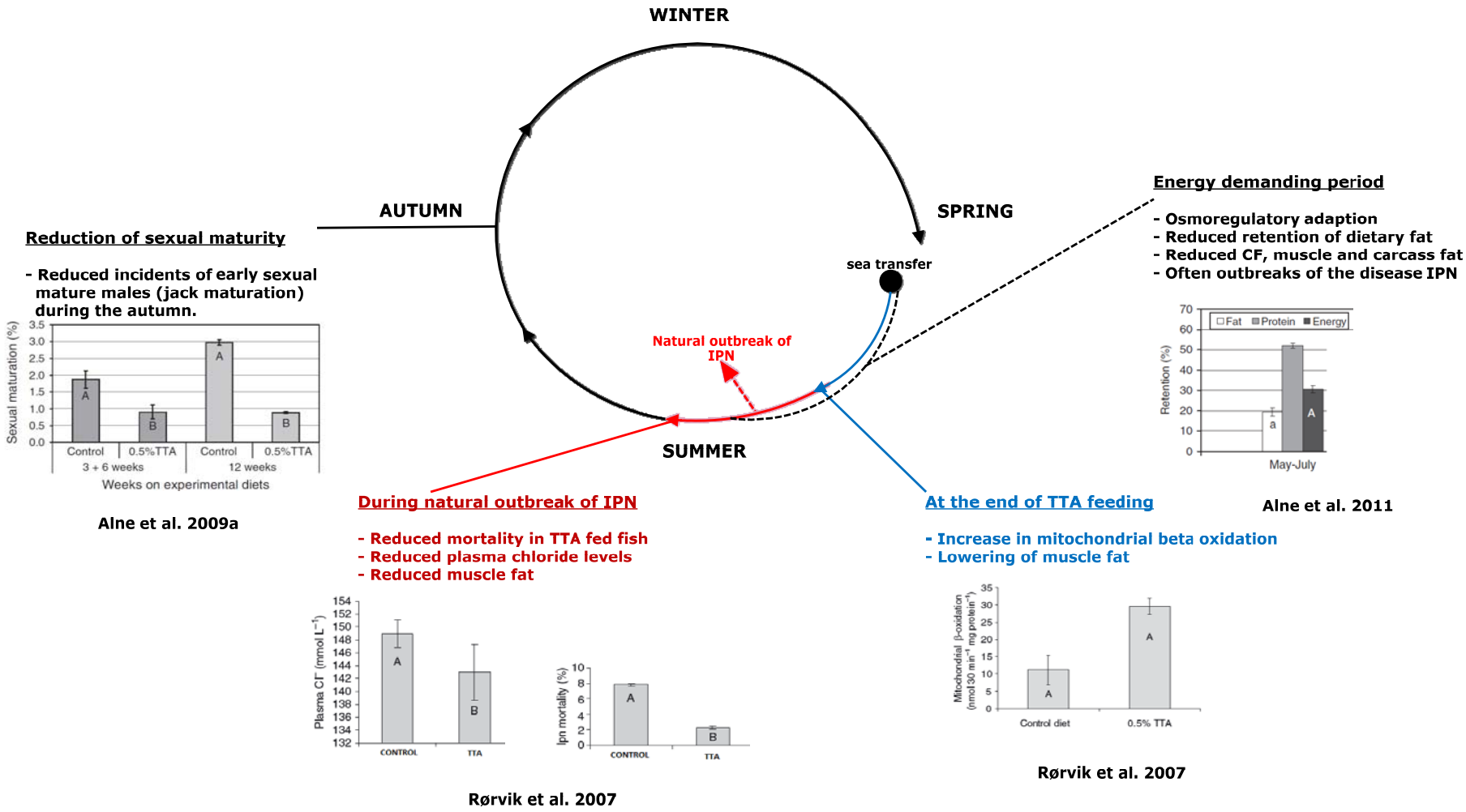


Fig. 2.8 The influence of TTA on 1+ Atlantic salmon during the seawater phase.

The liver is one of the most important organs in lipid metabolism and TTA has shown to influence the liver in mammals and fish. The main influence of dietary TTA in the liver is an increase in FAs oxidation activity, which is observed in Atlantic salmon, cod and rainbow trout (Kennedy et al. 2007a; Kennedy et al. 2007b; Moya-Falcón 2005). In Kennedy et al. (2007a), TTA also increased the proportion of n-3 fatty acids in the liver of rainbow trout and the expression of Δ6 desaturase was observed to be significantly lower in the liver of the TTA fed fish. In cod where lipids are known to be stored in large amounts in the liver, the fish fed TTA had a significantly lower HSI (Kennedy et al. 2007b). In contrast, Atlantic salmon fed TTA showed a significant increase in HSI (Moya-Falcón et al. 2004). In Moya-Falcon et al. (2004), the TTA supplementation also caused changes in the fatty acid composition of the liver, mainly increasing the levels of monounsaturated FAs. In Alne et al. (2009b) a numeric increase in HSI was observed for the salmon fed TTA-supplemented diets. No further investigation was done on the liver, and to our knowledge little research has been conducted on the influence of TTA on fatty acid composition of the salmon liver during the sea water phase.

High dietary inclusion levels of TTA have been observed to have negative effects on fish. In Moya-Falcon et al. (2004), high amounts of accumulated sulphur oxygenated TTA metabolites where found in the kidneys. It was suggested that this accumulation of metabolites may have negative effects on growth, and be a partial explanation for the higher mortality among the fish fed TTA observed in that study. It has been documented that TTA may result in changes of kidney morphology and function (Gjøen et al. 2007). Evaluation of the amount, time of administration and duration of dietary TTA-supplementation is therefore important. The inclusion of 0.25 % TTA for 6 weeks in the diet to salmon lead to no gross changes in the kidneys of the fish (Alne et al. 2009b), and this indicates that this level of TTA and duration may be beneficial. It is important to mention that TTA has not yet received the necessary clearance for human consumption (Grammes 2011), and if TTA shall be administrated to Atlantic salmon, an application has to be filed to the EU (Rørvik pers. comm). It is also required that the level of TTA in the flesh is low and that this is documented. This limits the use of TTA as a functional feed ingredient in diets for Atlantic salmon. A recent study show however, that TTA in the flesh is rapidly diluted and that the amount of TTA is considerable low when TTA is administrated early during the sea water phase (Arge et al. in press), se fig 2.9.

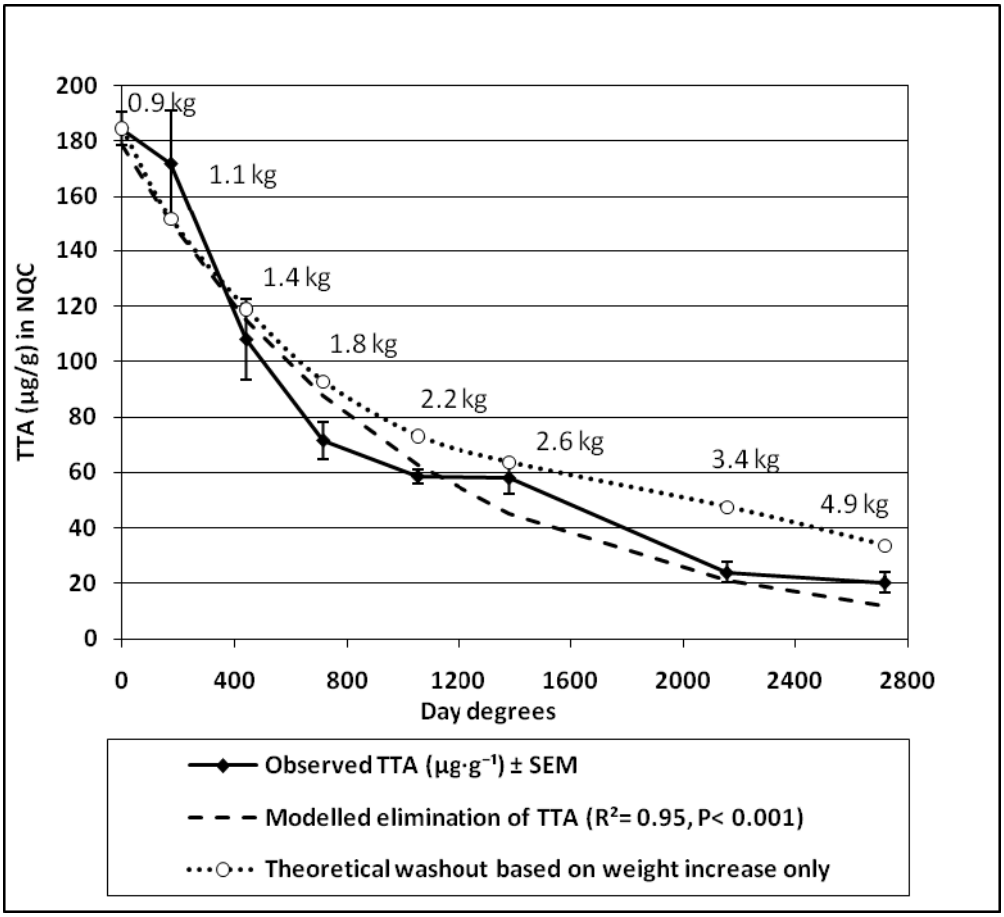


Fig 2.9 The observed and modelled elimination/dilution of TTA (µg·g^{-1}) in the muscle of S0 salmon in Arge et al. (in press). Dotted line with circles represents a theoretical reduction based on weight increase. The bodyweight of the fish is shown above the time points above the line.

2.5 Objectives

The present was performed to evaluate the general effect of TTA as a functional and bioactive feed ingredient in the diet for Atlantic salmon during the seawater phase.

Specific aims:

- Evaluate the influence of dietary TTA on fat content and fatty acid composition in the liver of 1^+ Atlantic salmon.

- Evaluate any sex-specific differences within the fish fed the experimental diets.

- Determine the influence of dietary TTA on growth performance, feeding rate, feed utilization, condition factor and the content of lipids in the muscle and liver.

It was decided to use a dietary inclusion level of 0.25 % as in Alne et al. (2009b) and feed TTA in periods where the fish is in need of energy and the oxidation of own fat reserves in the fish are activated, see section 2.2 and figure 2.3.

3 Materials and methods

3.1 Fish and experimental design

The trial was carried out at Nofima Marins Sea Water Research Station at Averøy, on the west coast of Norway (62 ºN). The experiment was conducted over a period of 13 months, from sea transfer in April 2009 until termination in May 2010. On the 15th of April 2009 a total of 6000 Atlantic salmon (*Salmo salar* L.) 1^{+} smolt were bulk weighted, and divided in 12 different tanks, each of 500 fish, on a vehicle at Straumsnes hatchery at Tingvoll. The smolt were then transported to Averøy and put to sea in 12 different pens (5 x 5 x 5m), the same day. The fish had a mean body weight of 105 g ± 0.05. Three net pens were used for each of four dietary treatments in a randomized block design. All the net pens were located at the same pier and divided into three different blocks depending on the position on the pier (fig 3.1).

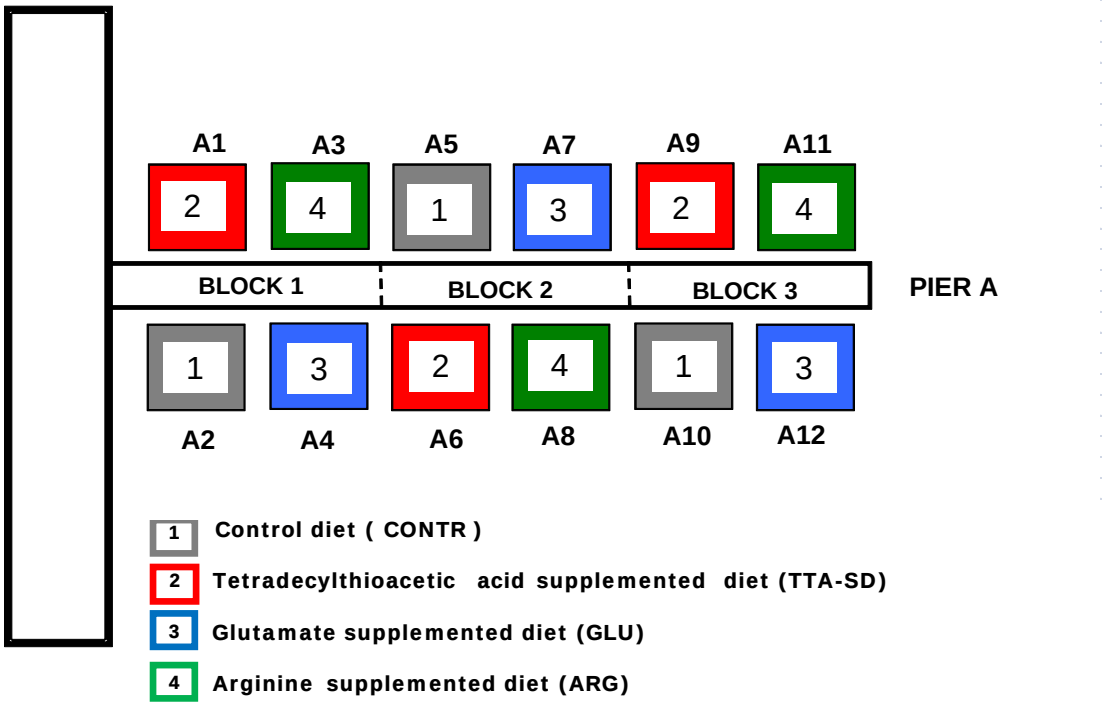


Fig 3.1 The experimental design setup. The boxes represent the net pens (A1-A12) and the different numbers (1-4) and colors represent the different dietary treatments.

3.2 Dietary treatments and feeding

The basis for all diets was commercially extruded fish meal-based pellets (3, 4.5, 7 and 9 mm) manufactured by Skretting AS, Averøy, Norway. The different experimental diets were obtained by coating the basic commercial diet in a blender (Table 3.1). The coating procedure for the control diet (CONTR) was as followed: 3.6 % (0.9 l) of distilled water was coated onto the basic commercial pellets. After this the pellets where dried on a tray for one day, before 2 % (0.5 l) rapeseed oil (heated to 70 °C) were coated over the surface of the pellets. The same coating procedure was used for all the other experimental diets, except for the different supplementation of additives. In the GLU diet L-glutamate was dissolved in the distilled water to an inclusion level of 1.5 % and the same was done in the Arg diet with L-arginine. For the TTA-SD diet the tetradecylthioacetic acid was dissolved in the rapeseed oil, to a final inclusion level of 0.25 % of the diet. The heating of the rapeseed oil was done in order to dissolve the TTA.

Table 3.1 Experimental diets used during the experiment

Diet name	Diets
CONTR	Basic Skretting diet + 3.6 % distilled water and 2 % rapeseed oil
GLU	CONTR + 1.5 % L-glutamate[a]
ARG	CONTR + 1.5 % L-arginine[b]
TTA-SD	CONTR + 0.25 % TTA[c]

TTA, tetradecylthioacetic acid.
[a] L-glutamate, Meihua Holdings Group Co. Ltd, Hebei, China.
[b] L-arginine, Fenchem Biotek Ltd, Najing, China.
[c] TTA, Thia medica AS, Bergen, Norway.

The feeding of the TTA diet was conducted for two periods during the experiment (fig 3.2). The first period TTA was feed for 10 weeks, from sea until transfer 26th of June when the fish had reached a final consumption of TTA-supplemented diet equal to 0.2 percent of the initial biomass in each experimental net-pen. The second period TTA was fed for 6 weeks, from 16th of January to the 26th of February. After the TTA feeding periods the TTA-SD diet was replaced by the control diet. During the trial the pellet size was adjusted to fish size in accordance with the feed manufacturer's guidelines. The fish were fed by automatic feeders in excess of the assumed feed intake, four times per day. Waste feed was collected after each feeding period and pumped up into wire mesh strainers. For more details see Helland et al. (1996) and Einen et al. (1999).

3.3 Sampling and recordings

Sea water temperature at 3 meters depth was measured and recorded every day from the start until the end of the experiment (fig 3.2). The water temperature was 6.9 °C at sea transfer and had an average of 8.7 °C during the experiment, with a minimum of 2.7 °C at the 27-28th of February and a maximum of 16.3 °C at the 11th of August. All fish within the net pens were anesthetized batchwize with MS 222 (Metacaine 0.1 g L^{-1}; Alpharma, Animal Health Ltd, Hampshire, UK) and weighed in bulk at each of the sampling dates presented in table 3.2. This was done in order to determine the growth rates. Before sea transfer 30 (10 x 3) fish were sampled (S0). Thereafter 10 fish from each net pen, representing the average with regard to body weight were randomly sampled for analysis at the sampling points. For each of the sampled fish the body weight, liver weight and fork length (fig 3.3) was recorded, and Norwegian Quality Cut (NQC; fig 3.3) (NS9401 1994) and liver were collected. NQC and liver tissues were stored at -20 °C prior to analyses. The gender was determined by inspection of the gonads and all sampled fish were killed by a sharp cranial blow after they where anesthetized.

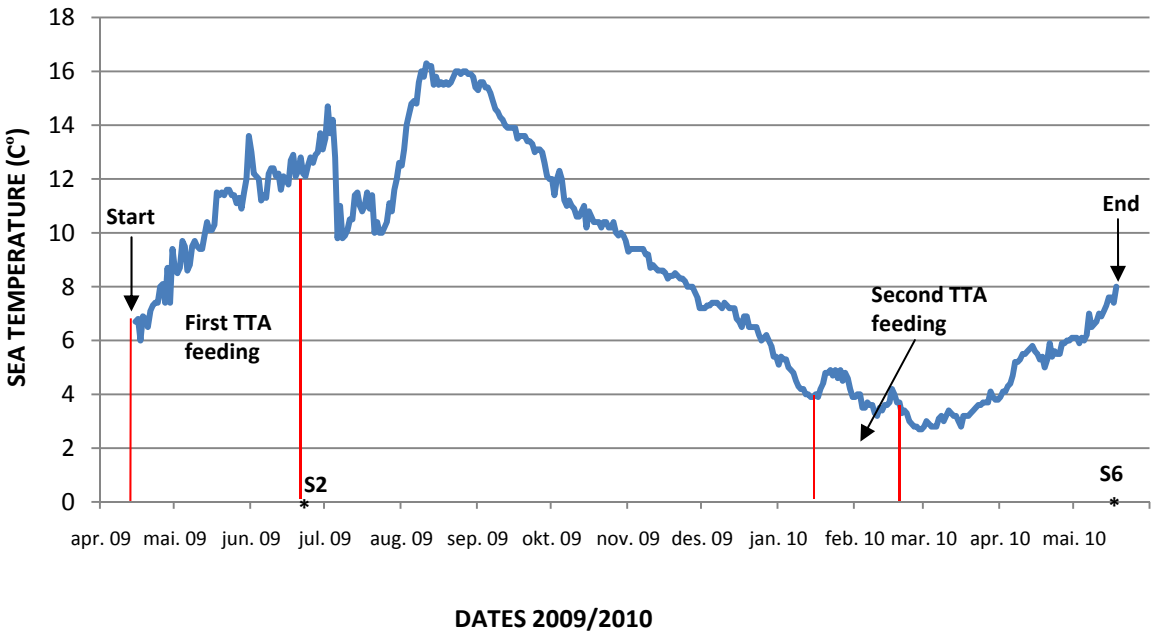


Fig 3.2 Water temperature (ºC) from the start to the end of the experimental period and the two TTA feeding periods. The duration of TTA supplementation are represented as the time between the red lines. The two sampling points (S2 and S6) after the TTA feeding periods is indicated by *.

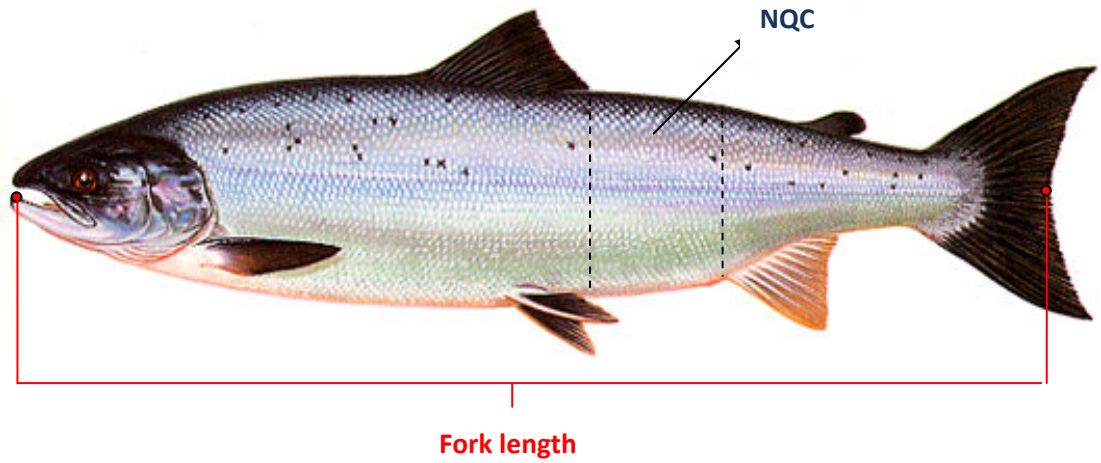


Fig 3.3 NQC is the piece between the end of the dorsal fin and the gut. Fork length is length from the snout to the median of the caudal fin.

Table 3.2 Sampling dates and growth periods with additional codes during the experiment.

Sampling code	Week number	Sampling dates	Growth Periods	Period code
S0	16	15th of April		
S1	22	26-27th of May	S0-S1	P1
S2	26	23-24th of June	S1-S2	P2
S3	31	28th of July	S2-S3	P3
S4	38	15-17th of Sept	S3-S4	P4
S5	47	16-17th of Des	S4-S5	P5
S6	20	18th of May	S5-S6	P6

3.4 Chemical analyses of feed

The control diets were analyzed for dry matter, ash, crude protein, crude lipids, starch and energy (table 3.3). Dry matter was determined gravimetrically by drying at 105 °C to constant weight. Ash was determined by flame combustion and heating to 550 °C until constant weight. The crude protein was analyzed as Nitrogen x 6.25 using the automated Kjeldahl method (Kjeltec Auto System, Tecator, Sweden). Crude lipid was analyzed by HCL acidic-hydrolysis and extraction in petroleum ether using the SOXTEC HT 6 system and SOXTEC1047 Hydrolyzing Unit (Tecator, Sweden). Starch was analyzed as glucose after enzymatic hydrolysis using a Megazyme K-TSTA 05/06 total starch assay kit (Megazyme International Ltd, Wicklow, Ireland). The energy content was determined by using a Parr 1271 Bomb Calorimeter (Parr, Moline, IL, USA).

Table 3.3 Chemical composition of the control diet (CONTR) with the different pellets size.

Pellets size	3 mm	4.5 mm	7 mm	9 mm
Dry matter (g kg^{-1})	912	898	889	927
In DM:				
Crude lipid (g kg^{-1})	275	322	374	378
Crude protein[1] (g kg^{-1})	514	469	435	380
Ash (g kg^{-1})	72	82	56	58
Starch (g kg^{-1})	59	56	82	82
Other carbohydrates* (g kg^{-1})	80	71	53	102
Energy (MJ kg^{-1})	25.2	25.9	27.2	27.1
Astaxanthin (mg kg^{-1})	63	55	45	39

DM, Dry Matter,
*calculated as: 1000 – (crude lipid + crude protein + ash + starch)

3.5 Analysis of muscle fat

Muscle fat content was measured in the left side of the NQC for all sampled fish and determined by image analysis (Photofish AS, Ås, Norway). The image analysis system consists of a light proof box equipped with standardized illumination and color conditions, a digital camera and a calibration card as describe by Folkestad et al. (2008). This current analysis method is developed for measuring pigment and muscle fat of Atlantic salmon. As the prediction not are developed for smolt in the weeks after sea transfer (Rørvik pers. comm.), the analysis of muscle fat at S0 and S1 is not included in the results.

3.6 Total lipid analyses

Liver samples from 10 fish within each net pen were separated into female and male livers, before the livers of each gender were homogenized into pooled samples. The total lipids were extracted from the liver samples according to method of Folch et al. (1957) and the total lipid content was determined gravimetrically. 1.5 g of the liver homogenates were weighted out and placed in a numbered sample flask. Then 6 ml saltwater solution (9 % NaCl) and 50 ml chloroform/methanol (2:1, v/v) were added, before the samples were homogenized for 90 seconds with an Ultra-Turrax knife-homogenizer (IKA Werke GmbH & Co. KG, Germany). After 60 seconds with homogenization 6 ml of saltwater was added and the mixed solutions were separated into two layers. The top layer consisting of mainly salt water and methanol (water soluble phase) and the bottom layer consisting of mainly lipids and chloroform (lipid phase). Afterwards the solutions were filtered into a measuring cylinder and placed in freezer (-20 °C) for further separation over night. The water soluble phases were then discarded and 20 ml of the lipid phase were transferred with a pipette into numbered beakers. The rest of the lipid phase were transferred into test tubes and corked for fatty acid analysis. The chloroform in the breakers was then evaporated, by placing the beakers on a heat plate. After the evaporation the beakers were place in an incubator with a temperature of 100 °C for 30 minutes. Finally, the beakers cooled off in room temperature before end weighing. Parallel samples were conducted when there was sufficient sample material. If a coefficient of variation (CV) over 5 % were detected the samples were reanalyzed. For each series of analysis control samples were carried out, using LT-fishmeal as a reference of the performed analysis. The total lipid content was calculated using equation 6, see calculations 3.8.

3.7 Fatty acid analysis of liver

Fat from the liver samples were extracted using the Folch method as described in section 3.6 and methylated over night with 2m2-dimethoxypropane, methanolic HCL and benzene at room temperature, as described by Mason & Waller (1964) and Hoshi et al. (1973). The next day 2 ml of hexane was added and the samples were neutralized by 4 ml of 6 % $NaHCO_3$ solution. The test tubes were then corked and mixed, so that the mixed solution was separated into two layers. After this the test tubes were placed in the freezer (-40 °C) over night for further separation. The top layer consisting of hexane, benzene and metylated lipids was then transferred to new test tubes, dried at 60 °C with nitrogen flushing and re-dissolved in hexane. Finally, the different fatty acids were separated and determined in a gas chromatograph (Hewlett Packard 6890 GC system) with a split injector SGE capillary column (length 60 m, diameter 0.25 mm and film thickness of 0.25 µm). The carrier gas was helium and the pre-selected oven program had a temperature regime that consisted of a raise from 50 to 180 °C at the rate of 10 °C/min, and then a raise to 240 °C at the rate of 0.7 °C/min. The results were analyzed using Aligent ChemStation software and the relative quantity of each FA is given as the percentage of total FAs. This was found by measuring the area under the chromatograph peak for each FA.

3.8 *Calculations*

Monitoring of data and calculations was carried out using Microsoft® Excel ® 2003 and 2007 (Microsoft, Redmond, WA, USA). The following calculations are used in this thesis.

The growth rate was calculated as the thermal growth coefficient according to Cho (1992). The TGC is multiplied by 1000 to simplify the numbers:

$$TGC = (W_b^{1/3} - W_a^{1/3}) \times (\sum T) \times 1000 \quad \textbf{(1)}$$

W_b = Final weight
W_a = Initial weight
$\sum T$ = sum of day degrees in Celsius

*The overall weighted TGC is corrected for weight difference during the different periods.

Feed conversion ratio was calculated as:

FCR = feed intake during the period (kg) / weight gain during the period (kg) **(2)**

*The overall weighted FCR is corrected for weight difference during the different periods.

Specific feeding rate: was calculated as:

SFR = (weight of feed supplied x $((B_0 + B_1)/2)^{-}1)$ x 100 **(3)**

B_0 = initial biomass
B_1 = final biomass
*The overall weighted SFR is corrected for weight difference during the different periods.

Fulton's condition factor (CF) was used to measure the condition of the fish and was calculated as followed:

$$CF = W/L^3 \quad \textbf{(4)}$$

W = Weight (g)
L = Fork length (cm)

To measure the relative liver weight, hepatic stomatic index (HSI) was used.

$$HSI = \text{liver weight (g)} \times [\text{body weight (g)}]^{-1} \times 100 \quad \textbf{(5)}$$

The total lipid content in liver was calculated after the following equation:

$$\text{Total lipid content (\%)} = (\text{g fat} * 100) / ((I * U)/75) \quad \textbf{(6)}$$

g fat = Evaporated sample in breaker
100 = %
I = Weighted out sample
U = Transferred lipid/chloroform extract in ml
75 = The solvents total volume

The lipid liver index was calculated as described in Kjær et al. (2009). The lipid liver index in this experiment was done with total lipid content of pooled liver samples:

Weight of lipid in the liver = (average liver weight/100) * measured lipid percentage in pooled liver samples **(7)**

Liver lipid index = (weight of lipid in the liver/average fish weight) * 100 (**8**)

3.9 Statistical analysis

Data from the trial were statistically analyzed by analysis of variance (ANOVA) using the general linear model (GLM) statement of the Statistical Analysis Software (SAS) release 9.0 for Windows (SAS Institute Inc. Cary, NC, USA). A total of four dietary treatments were tested in a randomized block design of triplicate net pens. During the statistical model run net-pen was used as experimental unit, where as TGC, body weight, CF, HSI, muscle fat, liver fat, lipid liver index and FAs were used as dependent variables. Gender, sampling date, block and diet were used as class variables. Various combinations of these variables were tested. First difference in gender was tested within each dietary treated group (model 1) and if significant differences were proven, the average values of each experimental unit were estimated as mean of the two sexes (see 3.9.3). After correction for the effect of gender, model 2 was run. If block was found insignificant, diet was the only experimental factor used when evaluating dietary effects. The lsmeans statement (least-square means) was used to detect differences among the predetermined variables, between and within treatments. R^2 expresses the proportion of the variance explained by the model and equals the between-group sum-of-squares divided by the total sum-of-squares (type III). The level of significant was indicated at $P \leq 0.05$ and $P \leq 0.1$ was considered as a trend. The results are presented as lsmeans ± standard error of the mean (SEM), if not otherwise stated.

3.9.1 Model 1: Test of the effect of gender within the dietary treatments:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

Y_{ij} = response variable
μ = general mean
α_i = effect of gender within the dietary group *i*
ε_{ij} = random fault

3.9.2 Model 2: The main GLM model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk}$$

Y_{iijk} = response variable
μ = general mean
α_i = effect of block *i*
β_j = effect of diet *j*
ε_{ijk} = random fault

3.9.3 Correction for the effect of gender:

When significant difference between genders was detected, the average value of each experimental unit was corrected by taking the overall mean of the average male and female parameter. By doing this, the proportion of the male and female means will contribute equally in relation to the average of the net pen. This is referred to as the weighted mean.

If no significant differences between genders were detected, the following equation was used:

$$(X * \% \text{ of female}) + (Y * \% \text{ of male})$$

X = average of pooled male samples

Y = average of pooled female samples

By doing this, all individual sampled fish (irrespective of sex) will contribute equal to an overall average of all the individuals in the net-pen. This is referred to as the un-weighted mean.

This thesis is focusing on the influence of dietary TTA, and if no significant differences were found among the fish groups given non TTA-supplemented diets (CONTR, GLU and ARG diets) these fish groups were pooled into one group of fish not fed TTA supplemented diets (N-TTA-SD). Number of experimental units (net-pens) then becomes nine for N-TTA-SD compared with three for fish fed the TTA-supplemented diet (TTA-SD).

4 Results

4.1 Body weight, growth and feeding rate

Significant differences in body weight were not observed within the fish group fed non TTA supplemented diets (N-TTA-SD) during the experimental period and both fish the fed TTA supplemented diet (TTA-SD) and the N-TTA-SD group had an initial body weight of 105 g ± 0.05. At the first sampling (S1) after sea transfer in May, the body weight was influenced by both block *(P < 0.001)* and diet *(P = 0.001),* together explaining 94 % of the observed variation in the model. Here N-TTA-SD had a significant higher body weight *(169 g ± 2)* than TTA-SD *(162 g ± 4).* As shown in figure 4.2, the same was observed in June (S2). Here both block *(P = 0.01)* and diet *(P = 0.002)* was also found to significantly influence body weight. No significant difference were observed in July (S3), but in September (S4) the TTA-SD group (*828 g ± 14)* had a significantly lower body weight (*P = 0.05*) then N-TTA-SD group (*876 g ± 11)*. At sampling in December (S5) no significant difference in body weight was detected. The development of the body weight during the experimental period is shown in figure 4.1. At the final sampling the mean body weight was 2947 ± 27 g for TTA-SD group and 3073 ± 31 g for N-TTA-SD group, resulting in a slight weight difference of 126 grams. No significant effect of block was found at the end weighing, but test between the dietary groups revealed that the body weight of TTA-SD was significantly (*P = 0.05*) lower than N-TTA-SD (fig 4.2).

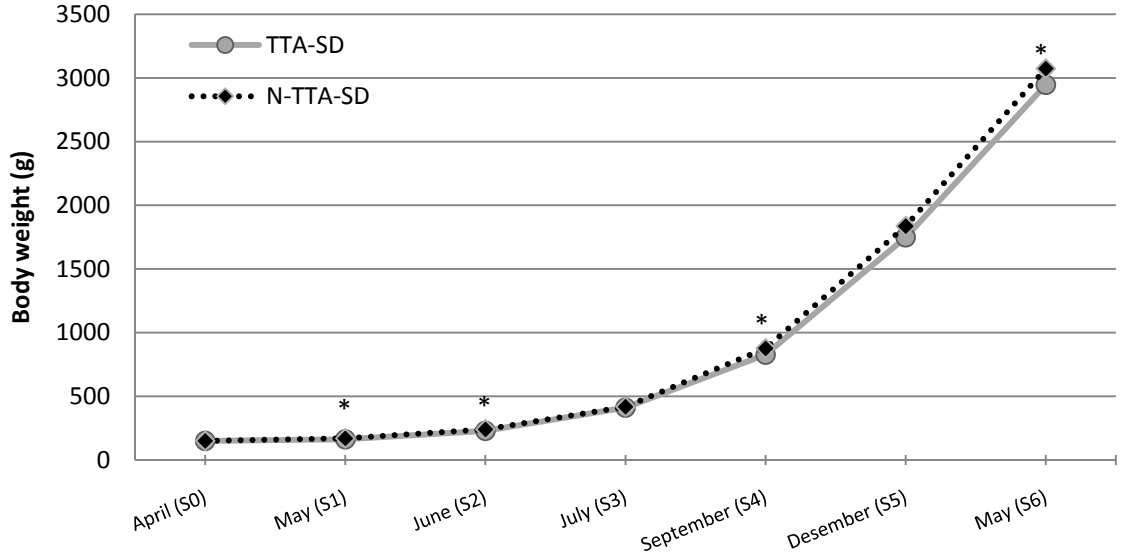


Fig 4.1. Development of body weight for Atlantic salmon fed TTA supplemented diet (TTA-SD) and not TTA supplemented diets (N-TTA-SD) during the experimental period. Sampling periods where significant differences were observed between the groups are indicated by * (P < 0.05).

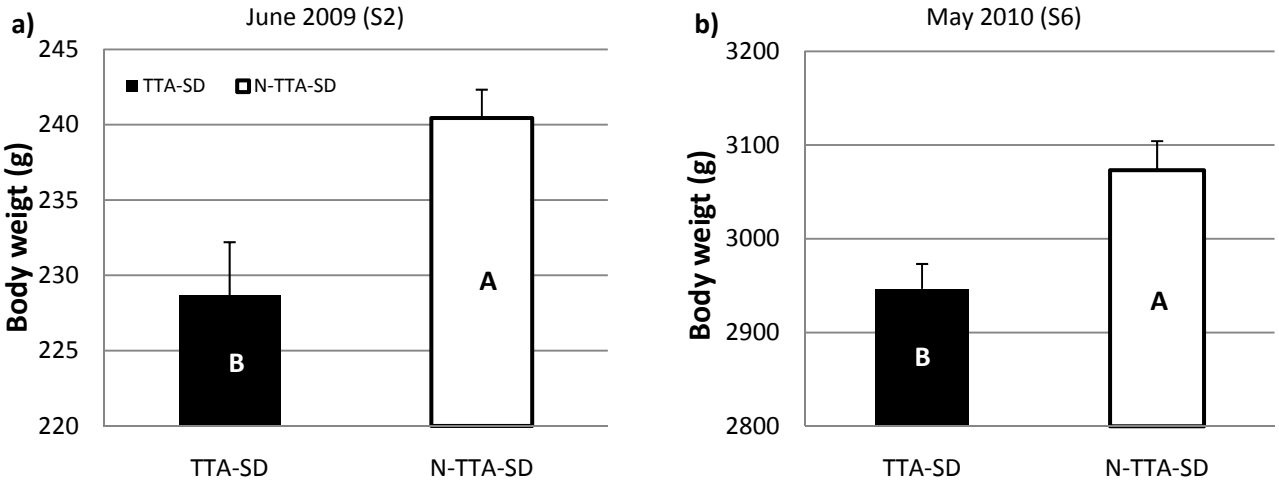


Fig 4.2 Body weight for Atlantic salmon fed TTA supplemented diet (TTA-SD) and not TTA supplemented diets (N-TTA-SD) at S2 (a) and S6 (b). Mean values for the groups are presented over the bars and significant differences between dietary treated groups are indicated by different letters on the bars. The variation between net pens within the TTA-SD and N-TTA-SD group is given as the standard error of the mean (S.E.M TTA-SD: n = 3, N-TTA-SD: n = 9).

No significant differences in TGC or SFR were observed within the N-TTA-SD group during the experimental period. The TGC and SFR between the different sampling points during the experimental period are presented in table 4.1. During the first 6 weeks after sea transfer (P1) the TGC was significantly lower in the TTA-SD group compared with N-TTA-SD group ($P = 0.001$). During this period the TTA-SD group was also observed to have a significantly lower SFR ($P = 0.02$), and both TGC and SFR where strongly influenced by block ($P < 0.0001$). The TGC for TTA fed fish improved as time progressed, and no significant differences were found in TGC or SFR between the fish groups throughout the other periods during the experiment. The overall weighted mean TGC and SFR did not significantly differ between the two groups *($P = 0.5$ and $P = 0.6$, respectively)*.

Both the N-TTA-SD and TTA-SD group showed seasonal variation in TGC and SFR. The TGC was significantly highest during P3, P4 and P6 for both groups. The SFR was significantly highest during P3 and P4. The SFR was significantly lowest during P6, while the TGC was low during P1 and P2 for both groups.

Tabell 4.1 Thermal growth coefficient and specific feeding rate for TTA-SD group and N-TTA-SD group within each sampling period (P1-P6) and the overall TGC for the experimental period. Means in one line sharing a common small superscript letter are not significantly different. Significant differences in TGC and SFR between dietary treatments are indicated by a different capital subscript letters in one column. The variation between net pens within the TTA-SD and N-TTA-SD group is given as the standard error of the mean (S.E.M TTA-SD: n = 3, N-TTA-SD: n = 9).

Parameter	**Dietary treatment**	**Sampling time**						
		P1$^{(S0\text{-}S1)}$	**P2**$^{(S1\text{-}S2)}$	**P3**$^{(S2\text{-}S3)}$	**P4**$^{(S3\text{-}S4)}$	**P5**$^{(S4\text{-}S5)}$	**P6**$^{(S5\text{-}S6)}$	**Weighted mean**
Thermal growth coefficient (TGC)	TTA-SD	2.07 ± 0.1 $^{c, B}$	2.04± 0.1^{c}	3.38± 0.1^{a}	2.68 ± 0.09^{b}	3.29 ± 0.03^{a}	3.10 ± 0.01^{a}	3.07 ± 0.01
	N-TTA-SD	2.29 ± 0.07 $^{d, A}$	2.08 ± 0.06^{e}	3.23 ± 0.07^{ab}	2.87 ± 0.05^{c}	3.33 ± 0.04^{a}	3.09 ± 0.05^{b}	3.10 ± 0.02
Specific feeding rate (SFR)	TTA-SD	0.73 ± 0.05 $^{c, B}$	0.94 ± 0.05^{b}	1.26 ± 0.04^{a}	1.29 ± 0.05^{a}	0.83 ± 0.01^{b}	0.36 ± 0.00^{d}	0.73 ± 0.01
	N-TTA-SD	0.77 ± 0.02 $^{d, A}$	0.96 ± 0.02^{c}	1.21 ± 0.02^{b}	1.34 ± 0.03^{a}	0.82 ± 0.01^{d}	0.36 ± 0.00^{e}	0.73 ± 0.01

4.2 Feed conversion ratio

As mainly non-significant and marginal differences were observed in FCR between the fish groups, the changes in FCR are presented as the mean of all net pens (fig 4.3). The FCR was observed to increase relatively more during P2, P4 and P6 than during P3 and P5. During P1 the fish fed TTA-SD diet (*0.66 ± 0.01*) had a significantly higher FCR (*P = 0.02)* than the fish fed Contr diet (*0.62 ± 0.01*). During P4 the fish fed TTA-SD diet (*0.95 ± 0.01*) had a significantly higher FCR than the fish fed ARG diet (*0.91 ± 0.00, P = 0.02*) and GLU diet (*0.92 ± 0.01, P = 0.05*). When pooling the fish not fed TTA, no significant differences were detected between the N-TTA-SD and TTA-SD during P1, however, at P4 the N-TTA-SD (*0.92 ± 0.00*) had a significantly lower FCR (*P = 0.02*) than TTA-SD group. During P6 the fish fed GLU diet had significantly higher FCR than all the other fish groups.

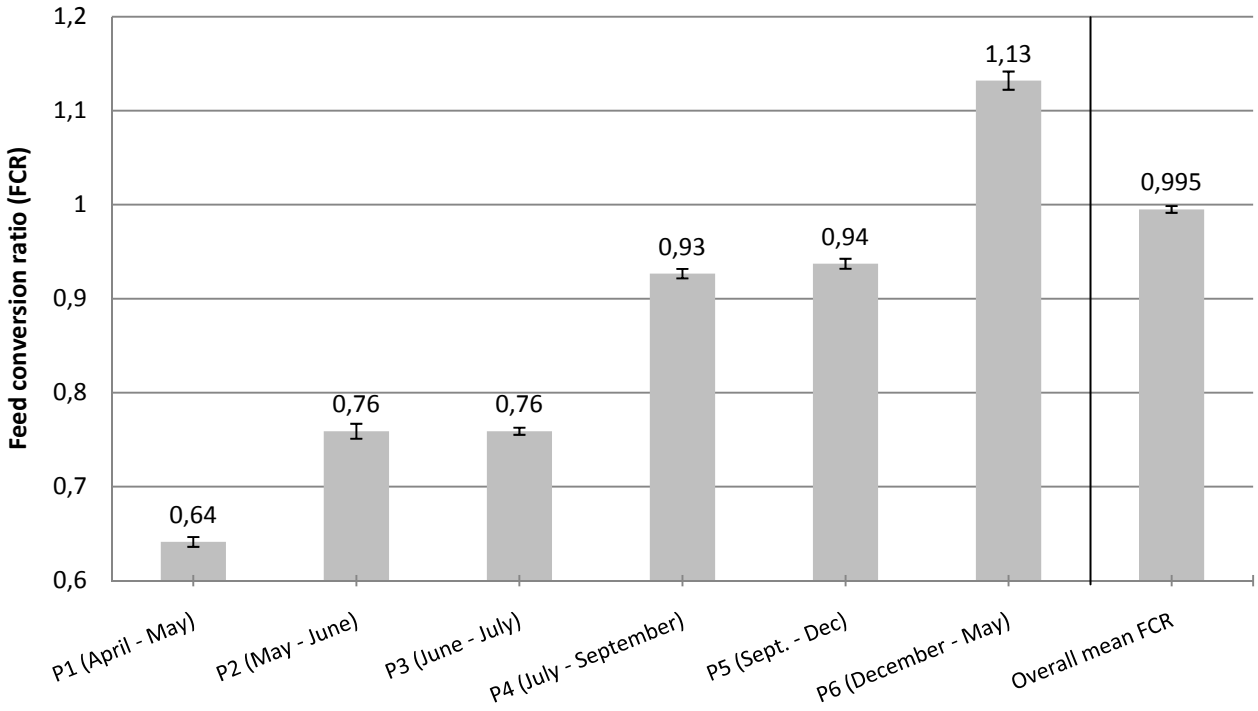


Fig 4.3 Changes in mean feed conversion ratio for all net pens throughout the experimental period. (S.E.M = 12).

4.3 Condition Factor

No effect of block on CF was detected and no significant differences in CF were observed within N-TTA-SD during the experimental period. Both TTA-SD and N-TTA-SD showed seasonal variations in CF. As shown in fig 4.4, CF of N-TTA-SD and the TTA-SD decreased significantly from sea transfer in April until June, before it significantly increases during late summer and autumn. From September to May the TTA-SD had no significant increase (*P = 0.4*), while N-TTA-SD had a significant increase (*P = 0.0003*) from 1.39 in December to 1.45 in May.

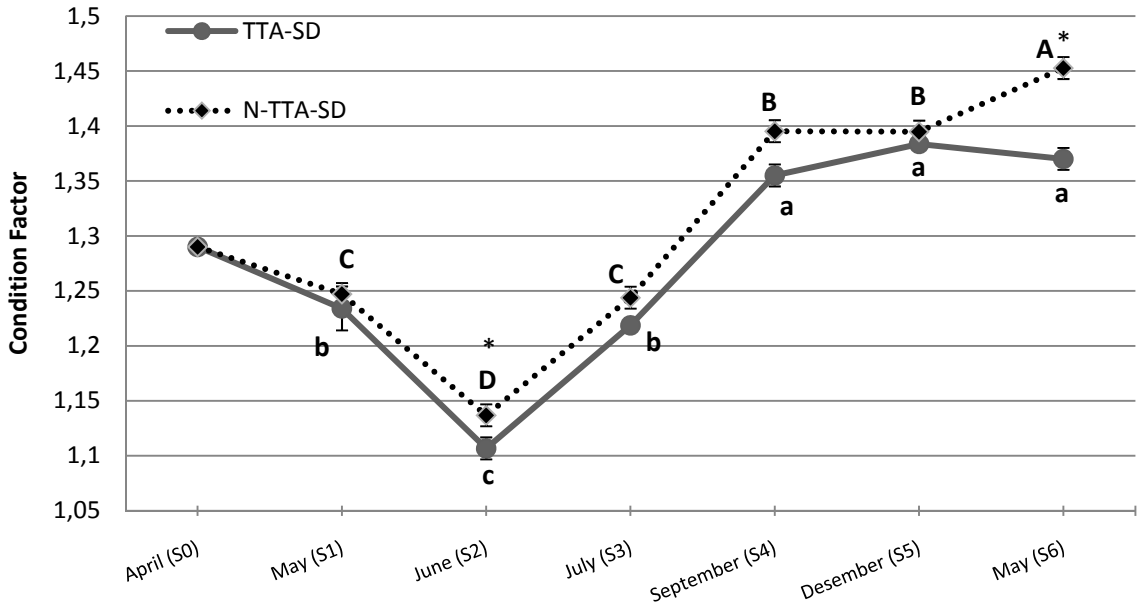


Fig 4.4 Development of condition factor for Atlantic salmon fed TTA supplemented diet (TTA-SD) and not TTA supplemented diets (N-TTA-SD) during the experimental period. Upper case letters show significant differences between sampling points for N-TTA and lower case letters show significant differences between sampling points for TTA. Periods where significant differences were observed between the two dietary groups are indicated by *. The variation between net pens within the TTA-SD and N-TTA-SD groups at the different sampling dates is given as the standard error of the mean.

Diet was shown to have a significant effect on CF at S2 (*P = 0.01*) and S6 *(P = 0.005*). At these two sampling periods TTA-SD had significant lower CF then N-TTA-SD (fig 4.5). At S4 there was detected a trend (*P = 0.07*) towards a lower CF for TTA-SD (*1.36 ± 0.01*) compared to N-TTA-SD (*1.40 ± 0.01*). No significant differences between the TTA-SD and N-TTA-SD groups were detected at the other sampling periods. In S2 and S6 gender was shown to have a significant effect on CF within the TTA-SD group. As shown in fig 4.6, females had a significant lower CF then the males at S2 (*P = 0.03*) and the opposite occurred at S6 (*P = 0.01*). No significant difference between genders was observed within the N-TTA-SD group.

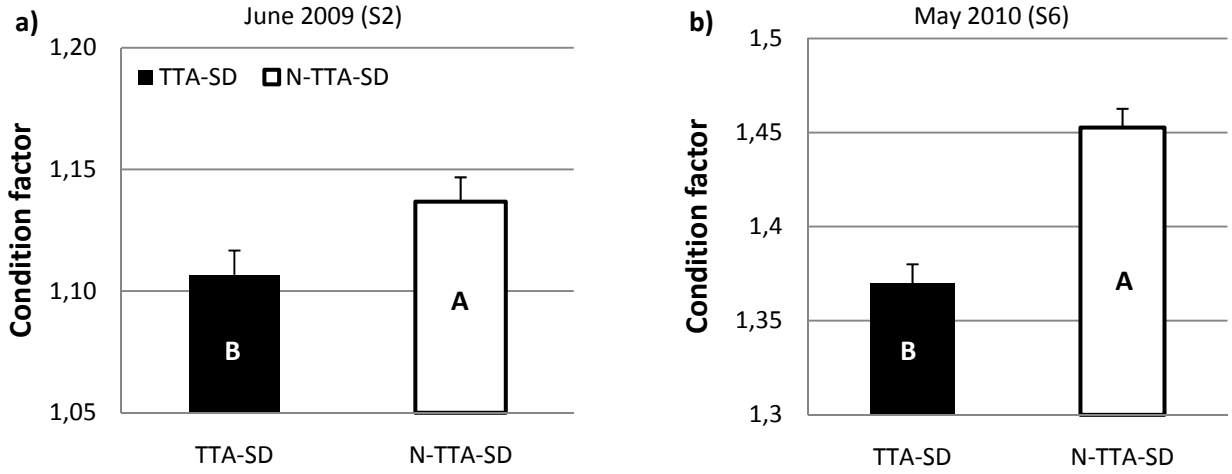


Fig 4.5 The measured condition factor (CF) of Atlantic salmon fed TTA supplemented diet (TTA-SD) and not TTA supplemented diets (N-TTA-SD) sampled from S2 (a) and S6 (b). Significant differences between dietary treated groups are indicated by different letters on the bars. The variation between net pens within the TTA-SD and N-TTA-SD group is given as the standard error of the mean (S.E.M TTA-SD: n = 3, N-TTA-SD: n = 9).

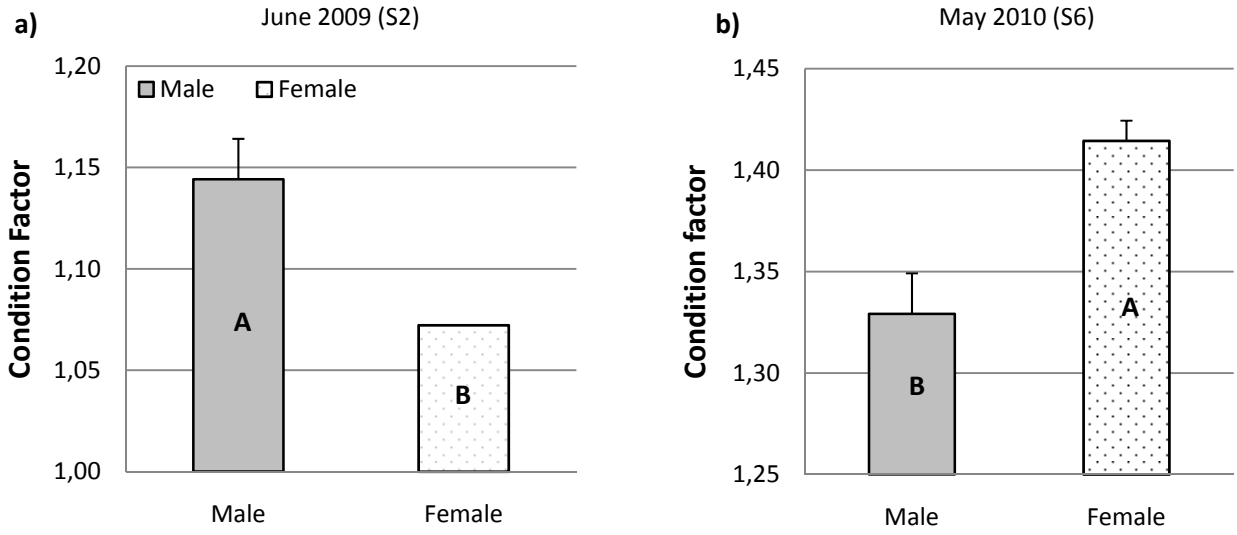


Fig 4.6 The measured condition factor (CF) in female and male Atlantic salmon fed TTA supplemented diet (TTA-SD) sampled from S2 (a) and S6 (b). Significant differences between genders within the net pens fed TTA are indicated by different letters on the bars. The variation between net-pens within the gender is given as the standard error of the mean (S.E.M n = 3).

4.4 Fat content in muscle and liver

The fish fed the different diets had a steady increase in muscle fat from June until December (fig 4.7). After this the TTA-SD showed a significant reduction ($P = 0.03$) in muscle fat from 17.2 % in December to 16.3 % in May. There were found significant differences in muscle fat within the N-TTA-SD group at the sampling points S3 and S5. At S3 block was found to influence muscle fat significantly $(P < 0.001)$ and the fish fed Arg diet (9.0 ± 0.3) had a significant lower muscle fat than fish fed Contr diet $(9.6 \pm 0.4, P = 0.002)$, Glu diet $(9.6 \pm 0.3, P = 0.004)$ and TTA-SD diet $(9.6 \pm 0.4, P = 0.004)$. The muscle fat content did not differ between fish fed Contr, Glu and TTA-SD diet. At S5 the fish fed Contr diet (17.5 ± 0.3) had a significantly lower muscle fat content than fish fed Glu diet $(18 \pm 0.08, P = 0.02)$. The TTA-SD fed fish (17.2 ± 0.1) had a significantly lower muscle fat then fish fed both Glu $(P = 0.002)$ and Arg diet $(17.8 \pm 0.19\ P = 0.01)$, however it was not significantly lower than the fish fed Contr diet ($P = 0.2$) at this sampling point.

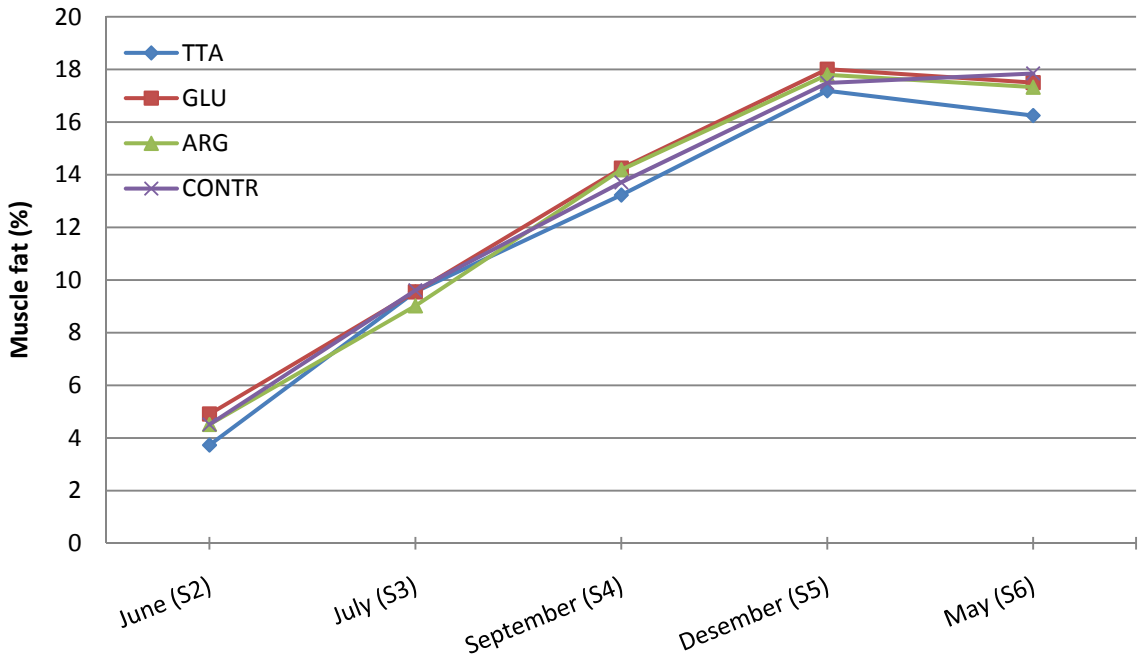


Fig 4.7 Development in muscle fat in the NQC of Atlantic salmon fed TTA-SD, GLU, ARG or CONTR diet during the experimental period.

At the sampling points S2, S4 and S6 no significant differences within the N-TTA-SD group was detected. As with condition factor the muscle fat content was found to be significantly decreased by dietary TTA, compared to N-TTA-SD at S2 (*P = 0.005*) and S6 (*P = 0.0004*), see figure 4.8. The muscle fat content in the TTA-SD also showed the same pattern in relation to difference between genders as with condition factor (fig 4.9). At S2 the muscle fat content of the females had a tendency to be lower than the males *(P = 0.07)*. At S6, however, the females had a significant higher muscle fat content than the males *(P = 0.008)*. No significant difference between genders was observed within N-TTA-SD group.

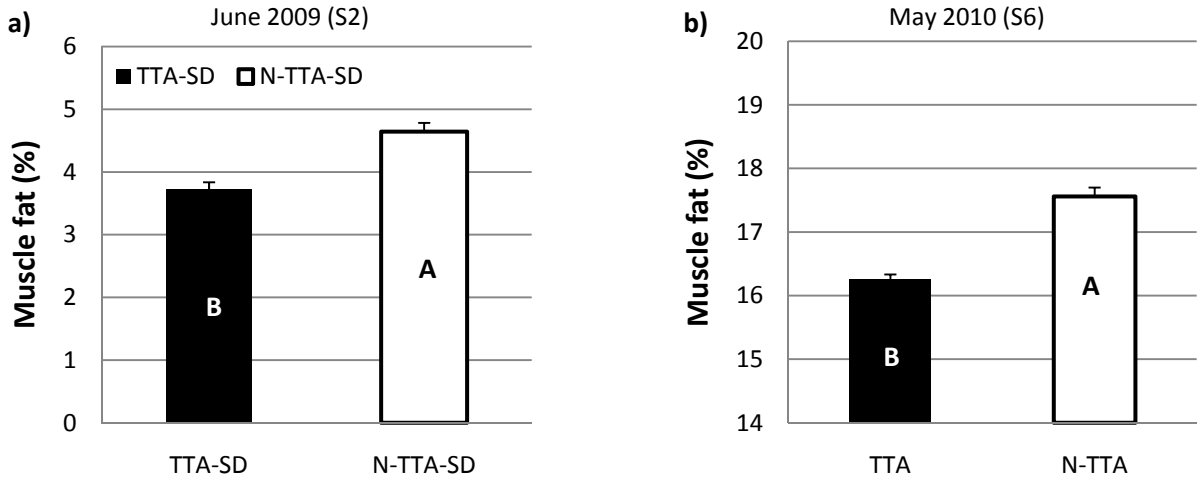


Fig 4.8 The measured muscle fat (%) content in NQC of Atlantic salmon fed TTA supplemented diet (TTA-SD) and not TTA supplemented diets (N-TTA-SD) sampled from S2 (a) and S6 (b). Significant differences between dietary treated groups are indicated by different letters on the bars. The variation between net pens within the TTA-SD and N-TTA-SD group is given as the standard error of the mean (S.E.M TTA-SD: n = 3, N-TTA-SD: n = 9).

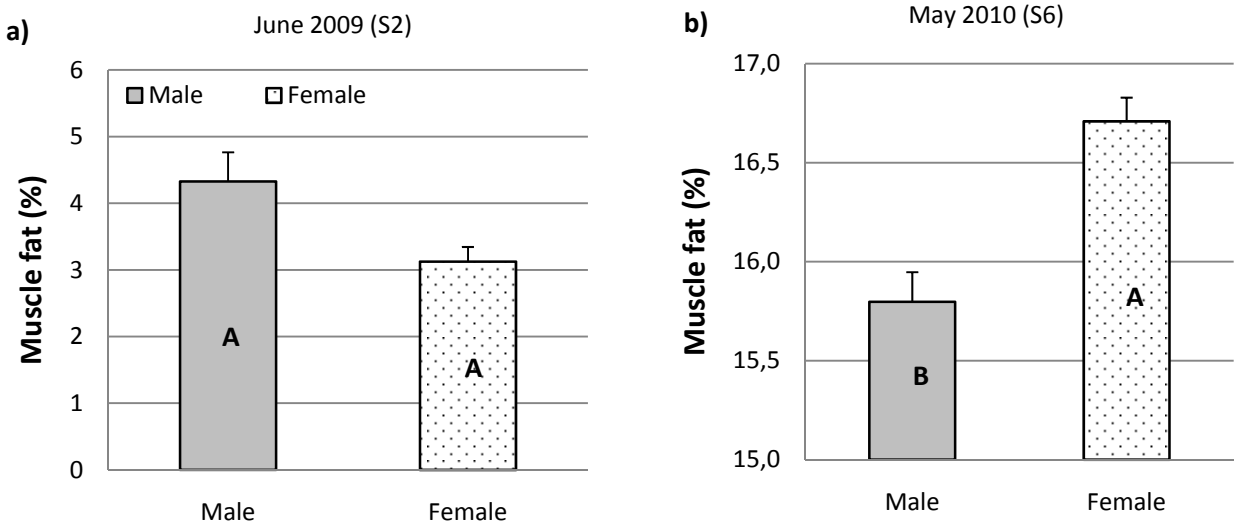


Fig 4.9 The measured muscle fat (%) content in NQC in female and male Atlantic salmon fed TTA supplemented diet (TTA-SD) sampled from S2 (a) and S6 (b). Significant differences between genders within the TTA fed fish are indicated by different letters on the bars. Variation between net pens within the gender is given as the standard error of the mean (S.E.M n = 3).

No significant difference in liver fat within the N-TTA-SD group was detected during the experimental period (S4 missing). Although TTA-SD had a significant lower muscle fat content in S2 compared with N-TTA-SD (fig 4.8), the liver fat content was significantly higher (fig 4.10). A higher liver fat content was also detected at S3 (table 4.2). At S6 the N-TTA-SD group had numerically higher fat content then the TTA-SD group, but the two groups did not significantly differ. No significant difference between genders in liver fat within the two dietary groups was detected.

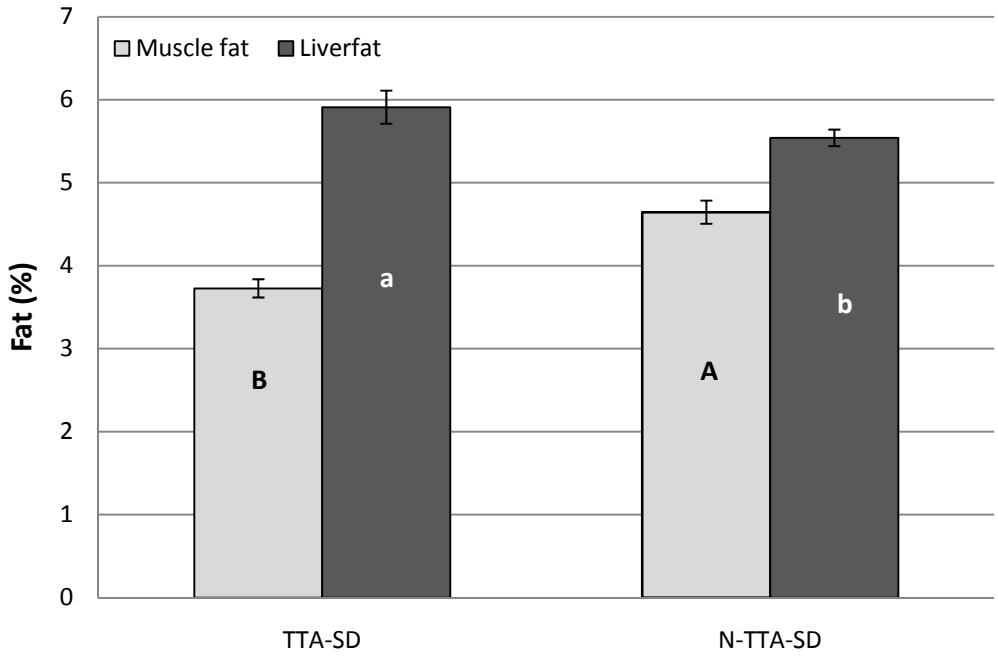


Fig 4.10 Fat content (%) in NQC and liver of Atlantic salmon fed TTA supplemented diet (TTA-SD) and not TTA supplemented diets (N-TTA-SD) sampled from S2 (June 2009). Significant differences between groups in muscle fat are indicated by capital letters on the bars. Significant differences between the groups in liver fat are indicated by small letters on the bars. The variation between net pens within the TTA-SD and N-TTA-SD group is given as the standard error of the mean (S.E.M TTA-SD: n = 3, N-TTA-SD: n = 9).

Table 4.2 Fat content in liver of Atlantic salmon fed TTA supplemented diet (TTA-SD) and not TTA supplemented diets (N-TTA-SD) at each sampling date. (mean ± SEM, TTA-SD: n = 3 N-TTA-SD: n = 9).

	Dietary treatment		**Feed effect**	
Sampling time	N-TTA-SD	TTA-SD	*P*	R^2
18 April (S0)	5.1 ± 0.1	5.1 ± 0.1	-	-
26 May (S1)	7.6 ± 0.2	8.1 ± 0.5	0.24	0.14
23 June (S2)	5.1 ± 0.1	5.8 ± 0.2	0.01	0.49
29 July (S3)	5.5 ± 0.1	5.9 ± 0.1	0.01	0.47
16 September (S4)	*	*	-	-
9 December (S5)	8.5 ± 0.4	8.1 ± 0.6	0.60	0.03
18 May (S6)	7.6 ± 0.3	6.7 ± 0.2	0.16	0.19

*Results on fat content in liver are missing.

4.5 Hepatosomatic index and liver lipid index

At S2 the HSI was strongly affected by both block (*P = 0.002*) and dietary treatment (*P = 0.0003*), together explaining 91 % of the observed variation. The HSI was higher for the TTA-SD than N-TTA-SD group (fig 4.11 a). The liver lipid index was also found to increase in fish fed TTA, being significantly (*P = 0.0004*) higher in TTA-SD (*0.06 ± 0.005*) compared with N-TTA-SD (*0.047 ± 0.001*). At S6 significant differences in HSI were detected between fish within the N-TTA-SD group. Fish fed both the Arg (*0.88 ± 0.02*) and the Glu diet (*0.91 ± 0.02*) had a significantly lower HSI (*P = 0.002 and P = 0.01, respectively*) than the fish fed control diet (*0.99 ± 0.02*). No significant differences were found in HSI within the N-TTA-SD group at the other sampling points during the trial. Because of the significant differences within the N-TTA-SD group at S6, the TTA-SD was tested against the fish fed the control diet (CONTR). When tested it was observed that TTA-SD had a significantly lower HSI (*P = 0.003; fig 4.11 b*). We also detected a significant difference in HSI between genders within the TTA-SD group, the males had significant lower HSI than the females (*P = 0.04; fig 4.12*). No difference in liver lipid index was found between the fish fed TTA-SD and Contr diet or between the genders within the dietary groups at S6.

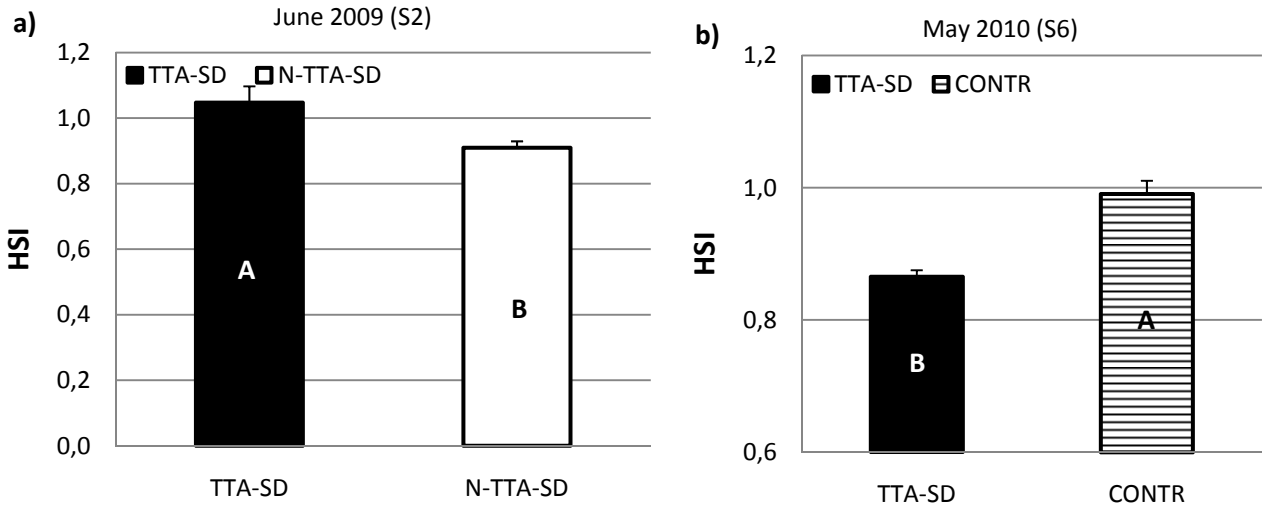


Fig 4.11 Hepatic stomatic index (HSI) of Atlantic salmon fed TTA supplemented diet (TTA-SD) and not TTA supplemented diets (N-TTA-SD) sampled from S2 (a) and HSI of Atlantic salmon fed TTA supplanted diet (TTA-SD) and control diet (CONTR) sampled from S6 (b). Significant differences between dietary treated groups are indicated by different letters on the bars. The variation between net pens within the TTA-SD, N-TTA-SD and CONTR group is given as the standard error of the mean (S.E.M TTA-SD: n = 3, N-TTA-SD: n = 9 CONTR: n = 3).

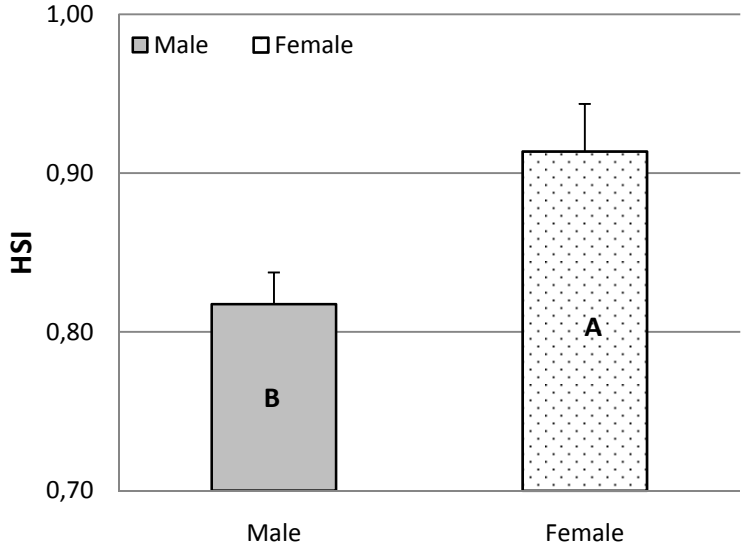


Fig 4.12 Hepatic stomatic index (HSI) in female and male Atlantic salmon fed TTA supplemented diet (TTA-SD) sampled from S6 (May 2010). Significant differences between genders within the TTA fed fish are indicated by different letters on the bars. Variation between net pens within the gender is given as the standard error of the mean (S.E.M n = 3).

4.6 FA composition of the liver after TTA feeding

As shown in table 4.3, the liver of TTA-SD at S2 had a significantly increased percentage of the FAs 24:0 and 20:4n-6, significantly decreased percentages of 18:2n-6 and 18:3n-3 when compared with N-TTA-SD. The monounsaturated FA 18:1n-9 had a tendency to be lower in the liver of the TTA-SD, whereas 20:1n-9 had a tendency to be higher. The increased percentage of 20:4n-6 made the sum of n-6 FAs significantly higher in the liver of TTA-SD.

At S6 as shown in table 4.4, the FA 18:0 was found to be significantly increased in the liver of TTA-SD, whereas the FAs 20:1n-9, 20:2n-6 and 20:3n-3 was found to be significantly reduced. The increased percentages of the FAs 18:0 and 16:0 made the sum of the saturated FAs significantly higher in the TTA-SD. The monounsaturated FAs had a strong tendency to be lower in the TTA-SD. Although only 20:1n-9 was significantly lower, both 18:1n-9 and 22:1n-9 showed a tendency to be lower in TTA-SD. The n-3/n-6 ratio was found to have tendency to be higher in the TTA-SD.

As a general observation in S2 and S6, the substrates for synthesis of the longer PUFAs (18:2n-6 and 18:3n-3) were significantly or numerically reduced, whereas the products of the elongations and desaturations (C20-22) were numerically increased in the TTA-SD group.

Table 4.3 FA composition of total lipid from liver of Atlantic salmon fed TTA supplemented diet (TTA-SD) and not TTA supplemented diets (N-TTA-SD) from sampling point S2 (June 2009).

	Dietary treatment		**Dietary effect**	
***FAs* (% of total)**	**N-TTA-SD**	**TTA-SD**	***P***	R^2
14:0	2.42 ± 0.03	2.32 ± 0.05	0.14	0.20
16:0	19.06 ± 0.20	19.54 ± 0.10	0.22	0.15
18:0	4.59 ± 0.09	4.36 ± 0.06	0.18	0.17
20:0	0.16 ± 0.02	0.21 ± 0.02	0.24	0.13
22:0	0.67 ± 0.04	0.69 ± 0.05	0.75	0.01
24:0	0.44 ± 0.00	0.46 ± 0.01	**0.008**	0.52
∑ **saturated**	27.80 ± 0.27	27.99 ± 0.10	0.61	0.03
18:1*n*-9	14.95 ± 0.16	14.40 ± 0.12	0.08	0.26
20:1*n*-9	3.17 ± 0.05	3.46 ± 0.21	0.06	0.30
22:1*n*-9	1.36 ± 0.05	1.53 ± 0.16	0.17	0.18
∑ **monounsaturated**	19.49 ± 0.20	19.40 ± 0.45	0.84	0.004
18:2*n*-6	3.69 ± 0.04	3.55 ± 0.03	**0.05**	0.34
20:2*n*-6	0.46 ± 0.01	0.45 ± 0.01	0.46	0.06
20:4*n*-6	2.53 ± 0.03	2.98 ± 0.05	**0.0001**	0.83
22:4*n*-6	0.15 ± 0.02	0.13 ± 0.04	0.67	0.02
∑***n*-6**	6.83 ± 0.05	7.10 ± 0.05	**0.02**	0.43
18:3*n*-3	1.30 ± 0.02	1.17 ± 0.02	**0.005**	0.56
20:3*n*-3	0.16 ± 0.01	0.16 ± 0.00	0.89	0.002
20:5*n*-3	7.37 ± 0.11	7.73 ± 0.46	0.27	0.12
22:5*n*-3	2.23 ± 0.08	2.20 ± 0.07	0.81	0.006
22:6*n*-3	27.93 ± 0.21	28.06 ± 0.22	0.75	0.01
∑***n*-3**	38.99 ± 0.19	39.31 ± 0.53	0.48	0.05
∑ **polyunsaturated**	45.82 ± 0.21	46.41 ± 0.49	0.22	0.15
***n*-3/*n*-6**	5.71 ± 0.05	5.54 ± 0.11	0.12	0.22

The FAs were extracted from pooled liver tissue homogenate. The quantity of each fatty acid is given as the percentage of total fatty acids. Values are means ± SEM (TTA-SD: n = 3, N-TTA-SD: n = 9).

Table 4.4 FA composition of total lipid from liver of Atlantic salmon fed TTA supplemented diet (TTA-SD) and not TTA supplemented diets (N-TTA-SD) from sampling point S6 (May 2010).

	Dietary treatment		**Dietary effect**	
***FAs* (% of total)**	**N-TTA**	**TTA**	***P***	R^2
14:0	2.01 ± 0.05	2.01 ± 0.05	0.96	0.003
16:0	11.45 ± 0.37	12.72 ± 0.25	0.09	0.26
18:0	4.15 ± 0.09	4.65 ± 0.05	**0.02**	0.46
20:0	0.24 ± 0.01	0.26 ± 0.01	0.29	0.11
22:0	1.74 ± 0.04	1.70 ± 0.00	0.64	0.02
24:0	0.25 ± 0.01	0.27 ± 0.00	0.40	0.07
∑ **saturated**	20.08 ± 0.44	21.87 ± 0.30	**0.05**	0.34
18:1*n*-9	24.62 ± 0.78	22.09 ± 0.11	0.09	0.25
20:1*n*-9	4.14 ± 0.16	3.29 ± 0.30	**0.03**	0.41
22:1*n*-9	1.07 ± 0.05	0.91 ± 0.03	0.13	0.21
∑ **monounsaturated**	29.83 ± 0.98	26.30 ± 0.79	0.07	0.29
18:2*n*-6	5.73 ± 0.25	4.65 ± 0.80	0.11	0.23
20:2*n*-6	1.23 ± 0.04	1.08 ± 0.04	**0.05**	0.32
20:4*n*-6	1.76 ± 0.07	1.98 ± 0.05	0.14	0.20
22:4*n*-6	0.22 ± 0.01	0.22 ± 0.00	0.97	0.0001
∑***n*-6**	8.94 ± 0.23	7.93 ± 0.87	0.13	0.22
18:3*n*-3	2.40 ± 0.06	2.33 ± 0.02	0.57	0.04
20:3*n*-3	0.50 ± 0.02	0.41 ± 0.02	**0.05**	0.32
20:5*n*-3	8.71 ± 0.22	8.96 ± 0.03	0.54	0.04
22:5*n*-3	3.53 ± 0.07	3.62 ± 0.13	0.57	0.03
22:6*n*-3	18.51 ± 0.70	20.58 ± 0.11	0.13	0.22
∑***n*-3**	33.64 ± 0.87	35.90 ± 0.21	0.18	0.17
∑ **polyunsaturated**	42.58 ± 0.77	43.83 ± 1.08	0.42	0.07
***n*-3/*n*-6**	3.79 ± 0.17	4.65 ± 0.55	0.07	0.29

The FAs were extracted from pooled liver tissue homogenate. The quantity of each fatty acid is given as the percentage of total fatty acids. Values are means ± SEM (TTA-SD: n = 3, N-TTA-SD: n = 9).

5 Discussion

5.1 Production parameters

Growth rate and specific feeding rate was observed to be significantly lower in the TTA fed fish during the 6 weeks after sea transfer (table 4.1). The feed conversion rate was however, not significantly different between the two dietary groups. The lower growth in the fish fed TTA is therefore most likely a result of the low feed intake. All the dietary groups had the same initial weight and an equal sea transfer procedure, so the observed difference is not likely a result of this. The observed differences in feed intake after sea transfer might indicate that dietary TTA induces responses in the smolt that may reduce the appetite. The growth and feed intake during the first 6 weeks after sea transfer was also strongly influence by block. This indicates that other factors at the fish site may strongly influence the growth and appetite during this stage.

The body weight of the TTA-SD group was significantly lower than of the N-TTA-SD group at first and second sampling after sea transfer (fig 4.1 and 4.2). As mentioned before, both the growth and feeding rate were significantly lower in the TTA-SD group the first 6 weeks after sea transfer. This may have influenced the body weight of the TTA fed fish at the two following sampling points. At the third sampling in July the body weight was not significantly different between the fish groups. During the period prior to this sampling the fish fed dietary TTA had a numerically higher TGC. At the forth sampling in September the body weight was again significantly higher in the N-TTA-SD group. During the fourth period from late July to mid September, both the fish fed Arg and Glu diet had a numerically higher SFR and TGC than both the fish fed control and TTA-SD diet. The body weight of the fish fed Arg and Glu diet were also numerically higher at the end of this period (results not shown). This may have influenced the body weight of the N-TTA-SD group at the forth sampling. In a recent study the combination of dietary arginine and glutamate was shown to significantly increase both SFR and TGC during the period from mid July until the end of September (Oehme et al. 2010). In that study the final body weight had a trend ($P = 0.08$) to be higher in the fish fed the arginine and glutamate supplemented diet. The study was conducted at the same research station and under similar conditions with 1^+ Atlantic salmon.

At the final sampling in May the body weight of the TTA-SD group was significantly lower than of the N-TTA-SD group (fig 4.2). Body lipids in juvenile Atlantic salmon has previously been reported to be significantly reduced by dietary inclusion of 0.6 % TTA fed for 8 weeks in seawater (Moya-Falcón et al. 2004). In that study juvenile salmon fed 0.3 % and 0.6 % of dietary TTA had a significantly lower final body weight, thermal growth coefficient and relative feed intake compared with the fish not fed TTA. The supplementation of TTA also resulted in a higher mitochondrial β-oxidation capacity in the liver. It was suggested that the lower weight and lipid content was a combined effect of lower growth, feed intake and increased β-oxidation capacity in fish fed TTA. In our study, the TTA fed fish had significantly reduced lipid content in the muscle at the final sampling (fig 4.9b). No investigation has been conducted on lipid content in carcass or in relation to the β-oxidation capacity of the fish in our study, however, we detected a significantly lower muscle fat in the fish fed TTA. No good correlation or linear relationship was found between the level of muscle fat and body weight (results not shown). During the period prior to final sampling the TTA-SD group had also a slightly higher TGC than the N-TTA group (table 4.1), and the lower body weight is therefore not likely a result of low growth rate. It is suggested that the lower final body weight of the fish fed TTA may be combined result of the removal of fish for sampling and changes in body weight at earlier stages during the trial. This is in line with the observation of significant differences in final body weight, but no differences ($P = 0.5$) in mean TGC. The thermal growth coefficient is therefore a better indicator for the performance of the fish than the final bodyweight in this study. In order to calculate the theoretical estimated final body weight, the mean TGC value, initial body weight (105 g) and the sum of day degrees in celsius (8.7 ºC x 398) during the experiment was taken in to the TGC equation. The results showed that the TTA-SD group with a mean TGC of 3.07 would have an estimated final body weight of 3650 grams, whereas the N-TTA-SD group with a mean TGC of 3.10 would have an estimated final body weight of 3725 grams. With these estimated final mean body weights, the two groups would have had a weight difference of 75 grams, which is not far from the actual observed difference of 126 grams.

Both the TTA-SD and N-TTA-SD group showed seasonal variation in growth rate and feeding rate (table 4.1). The growth rate was low during the first ten weeks after sea transfer. The specific growth rate for salmon smolt have been reported to be generally low in the first 3-6 weeks after seawater exposure, and variation in the time when the fish resumes normal appetite has also been observed (Handeland et al. 2000; Jobling et al. 2002a; Jørgensen & Jobling

1994; Usher et al. 1991). The rate of growth (TGC) of 1^+ salmon has also been shown to be significantly lower during the first 8 weeks after sea transfer compared to the next 8 weeks (Alne et al. 2011). This corresponds well to the growth rate observed in our study. The FCR was increased with increasing body weight and age (fig 4.3). However, during the periods of good growth (TGC > 3.2) from June to July and from September to December, FCR increased relatively less than in periods of reduced TGC. This indicates that the fish utilized the feed efficiently for building new tissue and accumulate fat during these periods. Small and young fish is known to utilize the feed more efficiently for growth than larger and older individuals, which often require more energy for body maintenance (Robinson & Li 2010).

The overall growth, feed intake and feed conversion ratio of the fish in this trial was considered as good and dietary TTA had no statistical significant negative effect on overall mean growth rate and feed intake. This coincides with other studies performed on cod, rainbow trout and Atlantic salmon (Alne et al. 2009a, b; Arge et al. in press; Kennedy et al. 2007a, b).

5.2 Liver parameters

Feeding of TTA in the present study significantly increased the fat content in the liver, HSI and lipid liver index, 10 weeks after sea transfer (table 4.2 and fig 4.11a). Juvenile Atlantic salmon fed TTA at 0.6 % of the diet for 8 weeks resulted in a significantly higher HSI and liver mitochondrial FA oxidation together with an increase in liver lipid content (Moya-Falcón et al. 2004). Tendencies towards an increase in HSI has also been observed in 1^+ Atlantic salmon smolt fed the same dietary inclusion level of TTA as in this study (Alne et al. 2009b). Administration of TTA to rats (male Wistar rats) was shown to increase the HSI and it was proposed that the increase in liver mass might partly be explained by the increase in total liver protein by mitochondrial and peroxisomal proliferation (Asiedu et al. 1996). In that study the hepatic triacylglycerols was found to be significantly lower in the TTA treated rats, as compared to control. In contrast, the hepatic cholesterol levels were found to be significantly increased. No determination of the different lipid classes or protein level was conducted in our experiment. TTA is known to stimulate mitochondrial FA oxidation in the liver of both mammals and fish (Gudbrandsen et al. 2005; Moya-Falcón et al. 2004), and increase other enzymes involved in lipid metabolism (Berge et al. 2002; Kennedy et al. 2007a, b). This indicates that TTA induces an increased capacity for FAs oxidation in the liver and studies have

proposed that these responses of TTA may drain FAs form blood/plasma and extrahepatic tissues, known as the *hepatic FA drainage hypothesis* (Berge et al. 2005). As muscle fat was reduced at this point, it is not unlikely that some of these responses of TTA may increase the liver lipid content and relative lipid weight, which again may partly influence the HSI. It should be noted that a reduction in muscle fat within the fish fed TTA may also indicate a higher FA oxidation in the muscle, as previously reported in Rørvik et al. (2007). At the final sampling in May 2010 however, the HSI of the fish fed TTA (fig 4.11b), arginine and glutamate was found to be significantly lower than the fish fed the control diet. At this sampling no difference in lipid content was detected. The obtained results from our study indicate that TTA may have a different influence on HSI and lipid content in relation to season and size of the salmon. More research is needed to understand the mechanism involved in the regulation of HSI in samlonids and how TTA and amino acids may influence this. Dietary TTA supplementation to cod has shown to significantly lower the HSI (Kennedy et al. 2007b). Cod is known to store large amount of fat in the liver (Lie et al. 1986), and a decrease in relative liver weight is considered as beneficial, as high HSI and lipid content are often related to fatty liver diseases (Kennedy et al. 2007b).

The FAs composition of the liver seems generally to be quite similar to previous results obtained by Kennedy et al. (2007a) with rainbow trout, and Moya-Falcon et al. (2004) with Atlantic salmon, where slight differences between the dietary treatments were found (table 4.3 and 4.4). TTA has been found to up-regulate the activity of Δ^9 desaturase in the liver of rats, by mainly increasing the amount of oleic acid,18:1n-9 (Madsen et al. 1997). In contrast to this, the fish fed TTA in our study had a trend towards lower levels of oleic acids in the liver compared to the fish not fed TTA, both after the first and second TTA feeding period. This reduction mainly decreased the total sum of the monounsaturated FAs. In Kennedy et al. (2007a) the TTA fed fish had significantly lower oleic acids levels and sum of monounsaturated FAs in the liver compared to the fish fed control diet. In Moya-Falcon et al. (2004), however, TTA did not influence the level of oleic acid, but the sum of the monounsaturated FAs in the PL and TAG fractions of the liver, was here found to be significantly higher in the fish fed TTA compared with the control. In our study, the sum of saturated FAs was found to be numerically higher after the first TTA feeding period and significantly higher after the second TTA feeding period. The substrates for synthesis of the longer PUFAs were also significantly or numerically reduced in the fish fed TTA, whereas the products of elongations and desaturations pathways (mainly EPA, DPA, and DHA) were numerically increased. This

might indicate that dietary TTA up-regulates elongations and desaturations in the fish, and may partly be a reason why the n-3/n-6 ratio was found to have a tendency to be higher in the fish fed TTA. The digestibility of saturated FAs has been shown to be significantly lower in fish fed TTA supplemented diets, and increased percentages of n-3 FAs (especially DHA) by dietary TTA has been reported in the gills and heart of Atlantic salmon (Moya-Falcón et al. 2004). No increase was however observed in the liver in that study, but in Kennedy et al. (2007a) numerically increased percentages of EPA and DHA, and a significant increase in DPA was found in the liver of TTA fed fish when compared with the fish fed control diet. In rats the percentages of hepatic n-3 FAs was shown to be decreased by dietary TTA supplementation, especially the percentages of EPA and DHA (Frøyland et al. 1997).

5.3 Sex-specific differences in condition factor and lipid content

From sea transfer in April and until June, the CF decreased significantly for both fish groups, before it significantly increased during the later summer and autumn (fig 4.4). This agrees with previously reported results obtained by Alne et al. (2011), where CF decreased from sea transfer in May until late June, before it significantly increased during August. The muscle fat did also significantly increase during the autumn, showing that the fish accumulated fat during the period. Accumulation of fat has been observed during the autumn for 1^{+} salmon (Alne et al. 2011; Mørkøre & Rørvik 2001; Måsøval et al. 1994; Roth et al. 2005), and it has been suggested that this is natural response in preparing for a cold winter season (Alne et al. 2011). At the second sampling in June, 9-10 weeks after sea transfer, we detected sex-specific differences within the fish fed the TTA-supplemented diet. This was at the end of the first TTA feeding period and here the female fish had significantly lower condition factor than the male fish (fig 4.6 a). The muscle fat had also a tendency to be lower in the female fish and this contributed partly to that the fish fed TTA-supplemented diet in general had a significantly lower CF and muscle fat than the fish not fed TTA (fig 4.5a, 4.8a and 4.9a). The reduction in muscle fat of the TTA fish agrees with results obtained from Alne et al. (2009a). In that study however, no gender determination of the experimental fish for muscle fat analysis was preformed, and it was therefore not possible to decide about the effect of TTA in relation to gender. The administration of TTA in that study resulted in a significantly reduced percentage of sexual mature males during the first autumn in sea. This indicated that TTA had an effect on the

energy status of the male fish. The difference in muscle fat between the fish not fed TTA, and fish fed TTA was 0.4 % (3.4 vs. 3.0) (Alne et al. 2009a). It was suggested that the reduced incidence of sexual mature males may partly be explained by the effect TTA had on body fat reserves. The current findings in our study does not contradict this suggestion, as the TTA did also reduce the muscle fat content among the males with 0.3 % (4.3 vs. 4.6) when compared to the fish not fed dietary TTA, where no difference between male and female fish was observed. The current finding therefore shows that TTA has an effect on lipid metabolism in both male and female fish, but to a larger extent in females at first spring/summer in sea. From late autumn and during the winter the CF was more or less stable for both the TTA-SD and N-TTA-SD group. From December to May however, the CF significantly increased for the N-TTA-SD group. This coincides with observations made by Mørkøre & Rørvik (2001), where the CF of the 1^+ salmon was found to significantly increase from March to May. In contrast to this, the fish fed TTA had a slight decline in CF and a significant reduction in muscle fat from December to the final sampling in May, after the second TTA feeding (fig 4.7). At this final sampling, we again detected sex-specific differences within the fish fed the TTA-supplemented diet. In contrast to the sampling in June, the male fish was now found to have a significantly lower muscle fat and CF than the female fish (fig 4.6b and 4.9b). No significant differences were detected between the female and male fish not fed dietary TTA. This is an interesting finding and it seems that dietary TTA provokes a different response in female and male salmon depending on the time of year, size of fish and/or status of the fish in terms of fat content and when the fish is ready for the onset of sexual maturity.

The proportion of female and male fish taken from each net-pen for analysis varied, as it was sampled 10 random fish from each net pen at the different sampling points. At the sampling in June 2009, the most uneven distribution was 3 males and 7 females in one of net pens with fish fed the TTA supplemented diet. At the sampling in May 2010, we sampled 3 males and 7 females in two of three net pens with fish fed the TTA supplemented diet. Regardless of the distribution, significant effects of gender was detected and as both muscle fat and CF coincide, the obtained results strongly indicates that TTA provokes sex-specific differences in fat for 1^+ Atlantic salmon during the first and second spring in sea. The detection of sex-specific differences reveals that sex-determination may be important in studies where components that may have an effect on energy status of the fish shall be evaluated.

In the present study the TTA fed fish had significantly lower muscle fat content after 10 weeks. This agrees with the results presented by Rørvik et al. (2007), where the fish fed TTA for 9 weeks had a significantly lower muscle fat content. After the second TTA feeding period the muscle fat content was again found to be significantly reduced, and this supports the suggestion that the FA oxidation capacity and degradation of lipids was increased in the fish fed TTA supplemented diets. Lipids are generally a cheap source of energy and high inclusion levels of lipids are often utilized in feeds for fish farming (Hillestad & Johnsen 1994). High amounts of dietary lipids are known to increase the deposition of lipids in Atlantic salmon (Torstensen et al. 2001a, b). This increase may have negative influence of the filet quality and also more importantly the fish health (Sargent et al. 2002). Reduced muscle fat by TTA-feeding may therefore be beneficial for Atlantic salmon fed high amounts of lipids over a longer period of time.

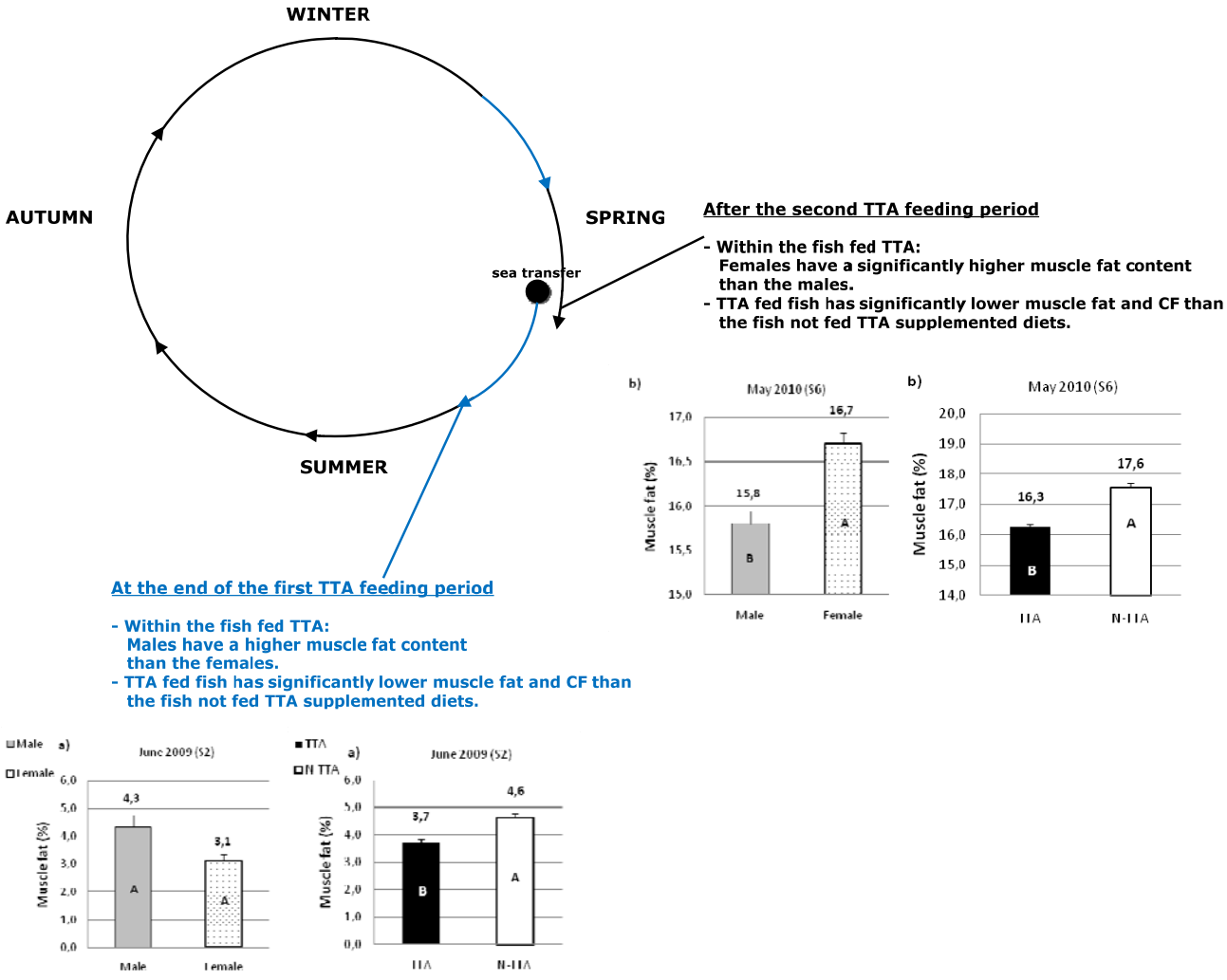
5.4 Why sex-specific differences? – A possible hypothesis

Studies have indicated that the levels of stored fat and energy in the fish body during the late winter and spring may be of great importance for the onset of sexual maturation (Kadri et al. 1996; Rowe & Thorpe 1990; Thorpe et al. 1990). As mentioned before, the strategic supplementation of TTA during the weeks prior to the first spring for 0^+ salmon and during early spring for 1^+ salmon, significantly reduces the incidence of sexual mature males (Alne et al. 2009a; Arge et al. in press). In both studies the muscle fat of the fish was significantly reduced after the TTA feeding period during the spring. As hardly any females become sexual mature during the first autumn, it may be assumed that females have a higher energy threshold in the spring to be able initiate the maturing process. Atlantic salmon females are known to become sexual mature later than males in the wild, and invest more energy in offspring production than males (i.e. gonads/eggs) (Fleming 1996). A higher energy cost of reproduction has also been observed for Sockeye salmon females when compared with males (Hendry & Berg 1999). These mentioned factors may be possible explanations to why we detected sex-specific differences during the present study. As the male fish has the ability to become sexual mature during first autumn, it will acquire a certain fat and energy threshold in the body during the spring. When the male fish is feed TTA, it may to be more persistent in the terms of not using of its own fat reserves, because of its “desire” to maintain fat reserves

and become early sexual mature. The female fish, however, which do not have the same ability to start the onset of sexual maturity at this time point, is more easily influenced by TTA and the muscle fat is more strongly reduced among the females. During the next spring, however, the opposite occur. Here the salmon is bigger and have a larger portion of energy to utilize and it is therefore possible for the female salmon to become early sexual mature the following autumn. The male salmon will also have the ability to become sexual mature at second autumn in sea, however, it may not display the same "drive" and necessity of keeping high energy stores, as the females. If so, the males may be more easily influenced by TTA, and the muscle fat is significantly reduced among the males. It is important to emphasize that this is only a hypothesis, and that there may be many reasons for the observed sex-specific differences. Several biotic and abiotic factors are known to influence the time of sexual maturation, and to understand more about the different underlying factors and triggers that may influence the observed sex-specific differences, more research is needed.

6 Concluding remarks

The main new finding in this study was that dietary TTA provokes sex-specific differences in fat metabolism for 1^{+} Atlantic salmon observed the first and second spring in sea. It is hypothesized that the obtained differences may be closely linked to energy and fat status, and the desire to maintain fat reserves to be able to become early sexual mature. We further observed that the fish fed TTA had an significantly increased HSI and liver lipid content at the end of the first TTA feeding after sea transfer, whereas after the second feeding of TTA during the last spring in sea, the HSI was significantly decreased in fish fed TTA. Dietary TTA supplementation also seems to reduce the percentages of oleic acid and increase the percentages of saturated FAs and n-3 PUFA in the liver of 1^{+} Atlantic salmon during the seawater phase. An overview of the main new finding is illustrated in figure 6.1.



Figur 6.1 The sex-specific differences provoked by dietary TTA during the present study.

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