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Effects of vaccination and dietary treatment on development and melanin deposition of Atlantic salmon (*Salmo salar* L.)

Marina Piña Jafelice

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Abstract

Visual appearance is an essential quality property of food products. For salmonids the red color of the flesh is a main characteristic noticed by consumers, and fillets with discolored patches are downgraded. During recent years, dark melanin pigmentation has achieved great attention. In particular dark fillet spots are a costly problem for the salmon industry as such fillets cannot be sold as high quality products. The main goal of the present study was to investigate the effect of vaccination and dietary supplementation of zinc or vitamin E on appearance of Atlantic salmon (*Salmo salar* L.), starting before vaccination in freshwater (March 2013) until the fish reached 1.9kg in seawater (March 2014). The focus was on melanin deposition in abdominal organs, abdominal wall and fillets. Also overall fillet and liver coloration, occurrence of gaping and body conformation were evaluated additionally. Organ adhesions and the relative weight development of viscera, muscle, liver and heart were monitored throughout the experiment. The results showed that changes in melanin deposition differed between the tissues studied, with increasing incidence in fillets showing the clearest development. Melanin deposits were consistently higher in organs (significant) and abdominal peritoneum (numerically) of vaccinated compared with unvaccinated salmon. At the final sampling, the melanin score in fillets was significantly higher in the vaccinated (23% of the fillets) than unvaccinated salmon (10% of the fillets). Vaccinated fish also had higher scores for organ adhesions, smaller hearts during the early seawater phase (Sept-Dec), paler livers and higher liver% (HIS) immediately after vaccination, and larger livers at the final sampling, paler fillets but less gaping immediately after sea transfer. Compared with the control feed, dietary Zn supplementation resulted in higher fillet yield in December but lower yield in March, higher melanin score in organs and less adhesions in the early seawater phase, less visceral fat in December but higher in March, darker liver color, except immediately after vaccination.

Keywords: *Atlantic salmon, melanin, vaccination, zinc, vitamin E.*

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1. Introduction

Aquaculture began in China more than 2400 years ago, where a large-scale production started in 1949. In Norway, the first records are from 1850 with a hatchery of brown trout (*Salmo trutta*), whereas the native Atlantic salmon (*Salmo salar*) appears as a cultivated specie in the beginning of the 1960s. Nowadays, aquaculture is one of the biggest industries in Norway and the Atlantic salmon is the main specie, responsible for more than 80 percent of the total production (FAO, 2003).

In Norway the wild salmon has a historical high importance, not only economically, but also socially and culturally (Porter, 2005). Although there is currently an increased demand for fish, there is also a high quality, stability and reliability demand from the consumers (FAO, 2003). Moreover, there is more knowledge about the consequences of fish consumption, leading not only to the search for taste preferences, but also regarding its health benefits. Salmon is an excellent source of a large variety of indispensable nutrients which include high-quality proteins, vitamins (especially vitamins A and D), minerals and omega-3 fatty acids. It is documented that the nutrients of salmon have protective effects against chronic diseases in humans, in particular cardiovascular diseases (Børresen, 2008).

The aim in intensive aquaculture is production of fast growing, healthy fish with a final flesh quality according to consumers preferences. To obtain these criteria, main approaches are: domesticating the cultured specie, controlling the production environment, feed manipulation, adoption of optimal harvest practices, utilization of opportunities for preharvest conditioning as well as exploitation of the convenient logistics of farm and factory during the postharvest processing and handling (Paterson *et al.*, 1997). There is no general definition of good flesh quality, and consumers usually do not recognize whether the seafood product they eat has been caught in the wild or raised in a farm (Paterson *et al*., 1997). Furthermore, consumers are generally unable to explain exactly why they have a preference for one product over another (Greenhoff & MacFie, 1994), but those who have experienced seafood that has been sourced from the wild, often prefer them due to their firmer texture and organoleptic properties (Sylvia *et al.*, 1995).

The most significant quality factors in fish are texture, color, fillet gaping, taste and flavor. Visual appearance of the food product is a very important property in the industry (Kiessling *et al.*, 2006), and for salmonids the red color of the flesh is one of the main characteristic noticed by consumers who are willing to pay more for salmon with intensively colored flesh (Anderson, 2000). The red color of salmonid flesh results from the deposition of carotenoid pigments that are supplemented to the diet, with astaxanthin being the predominant carotenoid (Nickell & Springate, 2001). The presence of discoloration, recognizable either as bloodspots or uneven color, white stripes or defects such as melanin spots are important quality problems of salmon (Koteng, 1992). Nowadays intra-muscular melanin deposits are a major quality problem of in Atlantic salmon fillets (Berg *et al.*, 2012).

Melanocytes are the cells that produce melanin and they are responsible for the dark pigmentation of fish (Hearing *et al.*, 1991). The reason why fish produce melanin in a dark spot pattern is not totally clear yet. A relationship between pathogens and dark coloration in fish has been observed after a bacterial infection where melano-macrophages were seen at the site of the lesion on the skin (Ribelin & Migaki ÅRSTALL). Pigment-producing granulomas in the muscle were identified as an inflammatory reaction response form in Atlantic salmon, associating the immune system to pigmentary systems (Hilde *et al.*, 2012). Dark pigmentation changes are frequently observed in organs as a reaction to vaccination, and vaccination has also been suggested as one possible reason for grayish and black patches in salmon fillets (Koppang *et al.*, 2005).

The main goal of this study was to investigate appearance of organs and fillets of vaccinated and unvaccinated Atlantic salmon fed diets supplemented with zinc or Vitamin E. The focus was on melanin deposition in abdominal organs (visceral peritoneum), abdominal wall (parietal peritoneum) and fillets (skeletal muscle), but also fillet and liver coloration, occurrence of slits or holes between the muscle segments (gaping) and body conformation were evaluated. Additionally biometric traits were studied, including the relative growth development of viscera, muscle, liver and heart.

2. Theoretical Background

2.1 General I: health

Dark melanin spots (Figure 1) decrease the quality of fish fillets (Koteng, 1992). It has been acknowledged that these marks were the result of an inflammatory condition most often induced by vaccination, due to the use of vaccines with oil-based adjuvants. However, the vaccination of salmon occurs in the posterior part of the abdominal cavity whereas melanin spots are most frequently found in the anterior part of the fillet (from the dorsal fin towards the head) (Reidar *et al*., 2007). Recent studies have also showed that a similar melanization pattern can occur in unvaccinated salmon (Norwegian School of Veterinary Science, 2013). However, melanin may appear in locations of injury or infection in many different species, leading to the general conception that melanin has anti-infection properties (Fagerland *et al*., 2013). Also, it has been shown that melanocytes (melanin-producing cells) produce several inflammatory mediators, suggesting that they are a part of an inflammatory response process (Poole *et al*., 1993). In Atlantic salmon the melanogenesis occurs in muscle-located granulomas, which represents an association between the immune and pigmentary systems (Larsen *et al*., 2012).

Figure 2.1: Melanin spots on salmon fillet. The location of the spot on the upper right fillet is the most frequent (Mørkøre, 2012).

The success in gaining control of the health problems in the Norwegian salmon industry and a dramatic reduction in the use of antibiotic (Figure 2.2) was to a large extent due introduction of efficient vaccines (Poppe, 2006). However, it turned out that the vaccines caused some side effects for the fish in different ways. Many factors may affect the development of those effects, such as temperature, that shouldn't be too high; fish size, that should be 35g or larger at vaccination and other biological factors, for example: light regime, growth, water quality, fish density, feeding, handling or sorting. Reduced appetite and poor growth of salmon are two of the side effects that may occur during a shorter or longer period (two to six weeks). Salmon injected with saline do not get this growth reduction or loss of appetite as fish injected with oil-based vaccines. In some cases, however, the vaccinated fish catch up the lost growth and it is as big as unvaccinated fish by seawater transfer. Also, vaccinated fish usually grow slower in seawater than unvaccinated fish; however, it depends on the vaccine and the vaccination date. Under normal conditions, or during periods of low growth, there will be no difference between vaccinated and unvaccinated fish. Vertebral deformations can occur in different parts of the vertebral column and at different life stages of farmed salmon as a result of vaccination. In a study by the Marine Research Station in 2004, radiographs revealed that there was no higher incidence of fused vertebrae among vaccinated fish than among unvaccinated ones, but the proportion of compressed vertebrae was clearly higher in vaccinated fish compared to the unvaccinated ones. However, other factors such as rapid growth, low phosphorus content and bioavailability in feed, breeding, contaminants and high incubation temperature are also shown to increase the risk of such damages. Deformations can have many causes, and vaccination is therefore only one of several factors that, in certain situations, can trigger or intensify the development of deformation in salmon. It has been shown that vaccination date, temperature by vaccination, size at vaccination and vaccine type has affected the degree of vertebral deformation. This means that deformation can occur and affect large parts of the life cycle, not just in the early stages during the vortex formation.

Vaccination can induce reactions in the abdominal cavity. All vaccinated fish get inflammation on the injection spot and also adhesions frequently seen - either between organs or between organs and the abdominal wall. There is a clear correlation between immune response and adhesions; the immune reaction occurs when oil adjuvant and antigen together cause irritation to tissues and inflammation that provides protection against diseases. After vaccination, there is an influx of melano-macrophages and other

macro professionally cells. As a result of a normal immune response, they will have a deposit of black pigment on the viscera, or the peritoneum. Studies have shown that increased melanin in the internal organs and muscles can be linked to certain vaccines and vaccine strategies. In fact, an adjuvant, often based on mineral oil, is added to the vaccine, in order to provide long-term protection for fish. Studies suggest that vaccines based on mineral oils can increase the deposition of melanin, but the quality of vaccination, such as injection point and penetration depth are also important. A large Norwegian salmon slaughterhouse noted significant differences in the amounts of melanin between salmon from fish farms that had received fish from the same smolt supplier where the fish had received the same vaccine treatment. This suggests that there may be interactions between different factors. The vaccine has been designated as the main cause of dark spots in fillet for many years, but based on experiments it seems very likely that the dark pigmentation of organs and fillets can have different causes, and the vaccine does not appear to be the main one. It is unlikely that physical trauma caused by vaccination is the major cause of the problem with fillet spots in harvest fish. Project records and in-depth analysis suggest that there are may be different reasons for the occurrence of dark pigmentation in organs, the abdominal wall and fillets. Additionally, dark spots in different parts of the fillet may possibly have different determinants. These are indications that should be followed up in future studies (Berg *et al*., 2007 and Mørkøre, 2012).

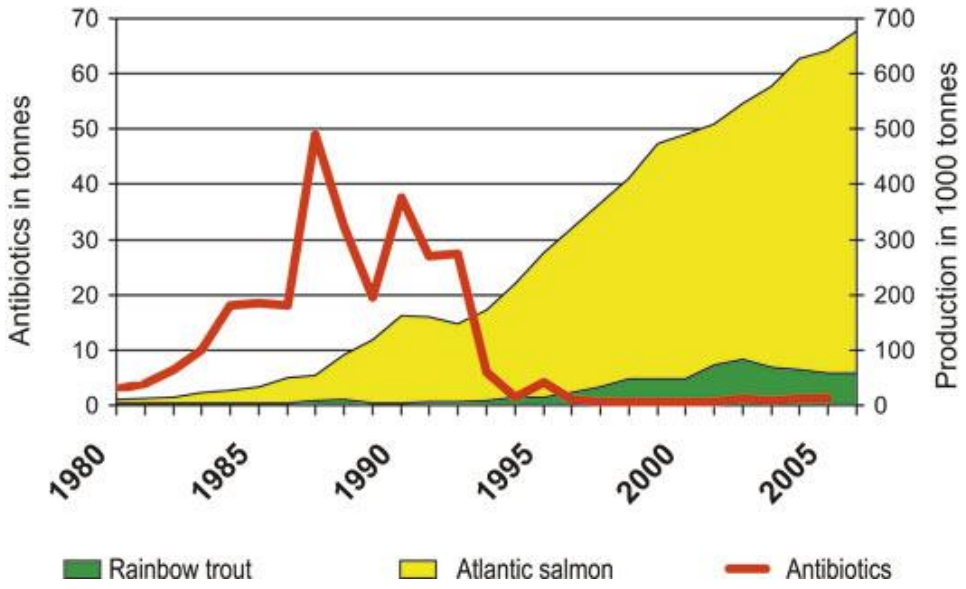


Figure 2.2: The use of antibiotics compared to Norwegian aquaculture production (Poppe, 2006).

Pancreas disease (PD) is another factor that can contribute to melanin deposition of salmon muscle, even though the feedback from the industry is not clear in terms of correlations between increased levels of melanin in fillet and PD (Norsk Fiskeoppdrett, 2008). PD is a contagious viral fish disease caused by salmonid alphavirus (SAV) that has had significant impact on the Norwegian salmonid aquaculture, affecting on average 90 sites with PD each year since 2006 (Jansen *et al*., 2010 and Norwegian Veterinary Institute, 2006). PD may be related to the discoloration of fillets (Figure 2.3). There may be several reasons for it, but if the muscle is damaged, it is not possible to obtain sufficient colour through increased level of pigment in the feed. The presence of dark spots and pale fillet color can occur simultaneously, but PD infected salmon can also have dark spots fillets without the overall color being affected (Mørkøre, 2012).

Figure 2.3: Pale Atlantic salmon fillets which were infected with PD (Larsson, 2012).

Stress can in a general sense be related to mechanical / disease / environmental / nutritional reasons. When it comes to feed, foreign substances can give adverse effects and can cause macrophages to have increased levels of the components hemosiderin and lipofuscin (Mørkøre, 2012). Hemosiderin is a golden brown pigment derived from breakdown of hemoglobin present in red blood cells, and lipofuscin is found in many

cells throughout the body, and its pigment provides an indicator of free radical damage and consists of phospholipids complexes with proteins (Krause, 2005). If the macrophages from the kidney (which can include high contents of melanin, hemosiderin and lipofuscin) migrate to the bleeding area, they can provide massive deposition of dark pigments (blood / iron and melanin). In carp it is shown that melanin deposition can occur as a result of toxic compounds from the feed leading to accumulation of melanomacrophages, hemosiderin and lipofuscin in the anterior part of the kidney. One hypothesis is that these can migrate from the kidney to the bleeding sites / damaged areas of the abdominal wall and give rise to dark spots on the fillet. It is possible that undesirable feed components will increase the deposit of dark pigments in injured areas in muscles (melanin, hemosiderin, lipofuscin) and that feed components that strengthen the blood vessels wall and the immune system can reduce the problems with melanin deposition. It has been hypothesized that zinc and vitamin E are dietary components that may have this effect, therefore have the possibility to reducing the deposit of dark pigmentation when taken by fish (Mørkøre, 2012).

2.2 General II: quality

Melanin spots are found in a large percentage of fillets. They do not disappear when smoking and they are a big cosmetic problem (Norsk Fiskeoppdrett, 2008). In Norwegian processing plants in 2007 it was estimated that 8-20% of all fillets had melanin spots and, as a consequence, 4% of the entire production was discarded (Reidar *et al*., 2007). In 2013 data it was reported that approximately 12% of Norwegian salmon fillets had lightly stained spots smaller than 3cm in diameter and 2% of the fillets had darker spots that were over 3cm on average (FAQ, 2013). Even higher losses due to melanin spots in muscles of Atlantic salmon are reported, causing up to 30% loss in some processing plants back in 2006 (Thorsen, 2006). Geographically, the highest rate of melanin spots presence seems to be in southern Norway (22%) and the lowest one in Northern Norway (12%), being 15% in Mid-Norway. Different temperatures do not seem to explain the differences between regions (Mørkøre, 2012). Although the melanin is as a natural component of many foods with no side effects and without any toxic or allergenic consequences (NPS, 2013), the consumers associate any discoloration of fillets with lower product quality. Since the presence of melanin spots reduces the

quality of the fillet and consequently the fillet price, they are downgraded in the production line. As a result, the portions that contain the dark spot must be cut, since they cannot be sold as whole fillets (Reidar *et al.*, 2007).

Dark discoloration of salmon fillets is mostly due to melanin deposition, but dark spots can also contain blood pigments (causing red spots) and scar tissue or a combination of melanin, blood and scar tissue, which can be difficult to differentiate (Figure 2.4) (FAQ, 2013).

Figure 2.4: Similarity between red spots and dark spots on salmon fillet (Mørkøre, 2013).

It is known that the dark pigments in the fillets are a response to tissue damages or local inflammatory conditions and it is a part of the fish's immune system (FAQ, 2013). Melanin often appears on the surface of the abdominal wall, but it can also appear elsewhere on the fillet or deeper in the muscles. The melanin spots are usually 1-4 cm of diameter, but they may also be larger (Norsk Fiskeoppdrett, 2008). On average, the rate of melanin appears to increase with the size of the fish. This is interesting as it indicates that melanin deposition in salmon filet is not a phenomenon that can be associated only with vaccination or vaccine type, but that the problem can also occur later in the fish's life, possibly getting worst with time (Mørkøre, 2012).

Defining the underlying cause of melanin spots in salmon fillet is a complex subject and not related to only one single cause. It is not known whether vitamin E levels in the diet affects the deposition of melanin in fish muscle, but it is an hypothesis as in humans it have been showed that vitamin E can inhibit bleeding tendencies. In the summer of 2011 experiments (FHF / NFR project) showed that increased levels of vitamin E in feed for salmon before harvest made them more robust so that the stress associated with slaughter gave less effect on stress markers in blood. In the same

experiment the salmon which had increased vitamin E in the diet also improved intestinal health and had greater muscle strength (Mørkøre, 2012). Vitamin E is helpful to improve the flesh quality and storage shelf life of fish as it is involved in defense against free radicals and has protective effects on the oxidation of highly polyunsaturated fatty acids (Baker, 2001).

2.3 Melanin

Melanins are polymorphous and multifunctional biopolymers of high molecular weight and they are among the most stable and insoluble biochemicals (Jacobson 2000). They are a group of natural pigments that can be found in most plants and animals, the primary determinant for skin color in humans and a strong antioxidant (Mørkøre & Prytz 2013). The term "melanin" (μέλας = black) is a purely descriptive one, which simply denotes a black pigment of biological origin (Swan, 1974). Melanins are synthesized at the bottom of the epidermis in humans, in a region termed the basal layer. Special cells located in this basal layer, named melanocytes, produce melanin containing packets, called melanosomes. This process of melanin production is termed melanogenesis, and is initiated once the nuclei of skin cells begin to become damaged from ultraviolet radiation (UVR), emitted by either the sun, or an artificial source. The melanosomes are then spread to separate keratinocytes (skin cells) throughout the epidermis and carried by tentacle-like projections, termed dendrites. Once the melanosomes reach the end of the projections they are squeezed out, into the keratinocytes. The melanin containing packets spread out above the nucleus, where they stay, protecting the DNA inside the organelle from harmful UVR. The skin cells will eventually rise to the top of the epidermis where they die and are desquamated (shed away) (Chedekel *et al.*, 1994). They can belong to three basic types: eumelanin, pheomelanin, and neuromelanin, but only eumelanin has been identified in teleosts (Bagnara & Matsumoto 1998; Adachi *et al.*, 2005). Eumelanin (Figure 2.5) is the most common type and it is also the one that is brown or black (Hearing & Tsukamoto, 1991). It is primarily a light-absorbing pigment and the major pigment recruited for three critical adaptive mechanisms of proximate morphological color changes in humans and animals such as: photoprotection, camouflage and visual communication (Leelercq *et al.*, 2010).

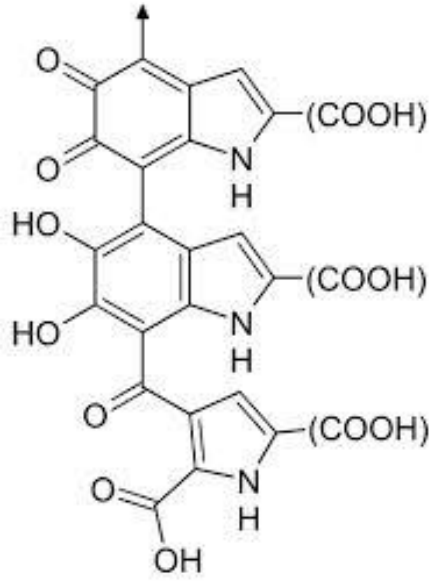


Figure 2.5: Structural unit of eumelanin.

Melanin can be used as an additive for taste, color and good health effects. The natural black pigments of foods like Caviar and Truffles are melanins. In Venice cuttlefish ink (melanin) has been used since the 14th century to flavor seafood dishes and today Italian black macaroni (pasta-neri) has melanin as the major ingredient in it. Nowadays it is well known that melanins, which are naturally present in many herbal foods, do contribute to easy digestion and to overall good health (NPS, 2013). Nowadays in our western society it is very popular that people want to increase the melanin production on their bodies, usually for a cosmetic reason. With certain types of food it is possible to induce the body to produce more melanin. Food stuffs that may stimulate melanin production include eggs, apricots, legumes, beans, soy (for a nutrient called L-tyrosine that is an amino acid used to build proteins in the body), copper containing food like oysters, organ meats (particularly the liver) and shellfish. Other animal products containing nutrients lending themselves to melanin production include chicken, turkey and fish, as well as dairy products like cheese and milk. B vitamins are also taken for the same reason (Mørkøre, 2012 and Rose, 2013).

The function of melanin is defined by their physical and chemical properties. It has been shown that melanins are photoprotective pigments; this action is related to its high efficiency to absorb and scatter photons, particularly the higher energy photons from the UVR and blue part of the solar spectrum (Meredith & Sarna, 2006). Melanin is considered the most powerful protector against UVR and HEV (High Energy Visible)

light. It is nature's answer to the undesirable effects of sunlight and therefore melanin is mostly used as an active photo-protective ingredient in cosmetics and sunscreens. UVR is known to easily and quickly spoil oil, fat and milk products by breaking down the fatty molecules, which makes many foods susceptible to damage by this radiation, acquiring bad taste and smell. This rancidity process is carried out through free radical intermediaries. Through its intrinsic property of efficient absorption of UVR and its ability to capture free radicals, melanin is able to preserve foods; giving them a longer shelf-life by slowing the damage or stopping it completely. In this respect melanin is ideal for using as a food additive resulting in a delayed expiry date (NPS, 2013). Melanin is also responsible for the dark color in skin, hair, eyes, fur and feathers. Gives the feathers more strength to it, may promote drying of feathers by absorbing radiant heat and there is some evidence that melanin may also inhibit bacterial degradation of feathers (Figure 2.6). Melanin also protects against parasites, and it is a powerful antioxidant and considered an “anti-secretory agent” acting against excessive secretion of acids in the stomach (Mørkøre, 2013 and NPS, 2013). An antioxidant is a substance capable of preventing or slowing the oxidation of other molecules. Oxidation is a chemical reaction involving transfer of an electron from electron rich to electron deficient unit. The electron deficient molecule is named an oxidizer or oxidizing agent. Heavy metals due to the presence of vacant d-orbital behave as potent oxidizing agents. Normally, an antioxidant can protect against metal toxicity by trapping free radicals, thus terminating the chain reaction by chelating metal ion and preventing the reaction with reactive oxygen species or by chelating metal and maintaining it in a redox state, leading to its incompetency to reduce molecular oxygen. Substances which protect biomolecules from free radical-mediated damage both in vivo and in vitro fall under this category (Flora, 2009).

Figure 2.6: Melanin in bird feathers (North Coast Diaries, 2013).

2.4 Vitamin E

Vitamin E was discovered and characterized as a fat-soluble nutritional factor during studies with rats in 1922 (Ronald *et al.*, 2006). It functions as a lipid soluble antioxidant and protects biological membranes, lipoproteins and lipid stores against oxidation, having the protection of unsaturated fatty acids against free radical-mediated oxidation as a main function (Hamre *et al*., 1998). This vitamin contains two compounds: the tocopherols and the tocotrienols, including a variety (alpha, beta, gamma and delta), being the alpha as the most used since it presents the major biological activity, therefore presenting better absorption. Commercially, the *dl*-*α*-tocopherol and *d*-*α*-tocopherol (also called *RRR*-tocopherol) are available in purified forms or in different dilutions, being used exclusively in feeds. Vitamin E can be found naturally in vegetable oils, eggs, liver, green vegetables and plants. Compared to other vitamins, vitamin E is found to be relatively nontoxic, although studies showed that a dose of 5000 mg of *dl*-*α*-tocopherol/kg of diet for trout caused reduced packed-cells volumes (McDowell, 1989).

Vitamin E is known to be one of the most important indispensable nutrients influencing the fish immune system, since its supply can reduce mortality, improve fish performance, increase specific and nonspecific immune responses (Ispir *et al.*, 2011 and Halver, 2002), maintain flesh quality and normal resistance of red blood corpuscles to haemolysis and permeability of capillaries (Halver, 2002). Several deficiency

symptoms in fish, such as erythrocyte fragility, anemia, muscular dystrophy and depigmentation have been caused by a diet intake which was deficient in vitamin E (NRC, 1993). The requirement of vitamin E is different between species, and it varies according to the developmental stage. It has been shown that the dietary requirement is 120 mg/kg of dry diet for Atlantic salmon. However, there are numerous factors that can influence the turnover of vitamin E in fish, such as water temperature, levels of other biologically active antioxidants, dietary level of selenium, levels of other antioxidants and the quality of dietary fat with respect to peroxidation (Hamre & Lie, 1995).

2.5 Minerals and zinc

Similar to other animals, fish require minerals to have a normal life process. They are able to take these inorganic elements from food and environmental water. Homeostatic mechanisms operating on the fish facilitate the right ranges from concentrations and functional forms of the minerals, which are responsible for the animal's ordinary metabolic activity in cells and tissues. Minerals have the function of skeletal formation, maintenance of colloidal systems, regulation of acid-base equilibrium and also for biologically important compounds like hormones and enzymes. When the mineral intake does not reach the minimum level required for that animal, biochemistry, structural and functional pathologies can be caused, depending on how low the intake was and the duration of mineral deprivation. On the other hand, an excessive intake and assimilation of those components can be toxic (Watanabe *et al*., 1997).

The mineral zinc participates as an active component or cofactor in important enzymatic systems with a vital role in the metabolism of lipids, proteins and carbohydrates. It is active in the synthesis and metabolism of nucleic acids (RNA) and proteins, it is an essential component in over 80 metalloenzymes and it also plays a key role in the action of hormones such as insulin, glucagon, corticotroph, follicle stimulating hormone and luteinizing hormone (Tacon, 1990).

Zinc should be an essential component in manufactured feeds as it is an important trace element in fish nutrition, involved in numerous metabolic pathways. The gills and gastrointestinal tract are involved in its the uptake (Takeshi *et al.,* 1997). The zinc requirement for Atlantic salmon is of 37-67 mg/kg of dry feed (Maage &

Julshamn, 1993). The average range of zinc in salmon diets was known to be 80-118 mg/kg (Tacon & De Silva, 1983) and nowadays it is usually 150 mg/kg of zinc in commercial diets (Nutra Olympic, 2014) with the maximum limit being 200 mg/kg (EFSA, 2014). Its deficiency has been found to impair immunological responses in rainbow trout (Kiron *et al.,* 1993), and affecting significantly the mineral composition of common carp gonads (Takeshi *et al.,* 1997). In fish, it can also lead to growth retardation, lower digestibility of protein and carbohydrate, causing eye lens cataract and erosion of fins and skin (Ogino & Yang, 1978). Studies on pigs suggest that zinc acts effectively on controlling some pathogenic bacteria and enhances animal performance when used in high doses (Hahn & Baker, 1993). However, high concentrations of zinc in fish feed can cause chelating effect with some minerals, such as iron and copper, which participate directly in the formation of red blood cells, thus determining deficient erythropoiesis (Knox *et al.,* 1984).

2.6 Vaccination

The first documented disease prevention in fish using vaccine was by the Polish Snieszko and collaborators', who published a paper in 1938 about protective immunity in carp immunized with *Aeromonaspunctata*. As the entire paper was written in Polish, it did not spread much towards other parts of the world. Then, in 1942 a report in English was written by Duff, who had worked with trout immunized by parenteral inoculation and by oral administration against the bacteria *Aeromonas salmonicida* (Gudding & Muiswinkel, 2013). The first report on vaccination of fish against a viral disease must have been from the Russian fish pathologist Goncharov in 1951 (Goncharov & Mikriakov, 1968). After a slow start since the 19th and early 20th centuries, fish immunology ended up developing as a promising and independent scientific field after 1945. A great advance in activities at the cellular and molecular level occurred during the 1950s and 1960s. Fish began to be considered more like other vertebrates, and owners of a sophisticated immune system showing specificity and memory, allowing the application from the basic data on immunization of fish for large scale vaccination in aquaculture. If compared to animal husbandry, fish farming is still relatively new in many countries (Muiswinkel, 2008). It has improved year by year and nowadays an important amount of the problems in aquaculture that lead to the use of antibiotics or chemotherapeutics can be prevented with vaccines and better knowledge

in farming techniques. Some of them are: improvement of the diet, effluents treatment, mortalities and unconsumed feed collection systems, better selection of spots for ongrowing and ectoparasites control.

Vaccines are various preparations of antigens which derived from specific pathogenic organisms that are rendered non-pathogenic, acting as a preventive measure against future diseases. They stimulate the immune system of the organism and increase the resistance to disease. The vaccine can be water or oil based. The oil provides adjuvant qualities increasing the effectiveness of the vaccine and duration of the protection. Vaccines can be applied orally, with immersion or injection to the fish. In oral vaccination, the vaccine is either mixed with the feed, coated on top of the feed (top-dressed) or bio-encapsulated. Immersion vaccination depends on the mucosal surfaces to recognize pathogens they had been in contact with. After being immersed in water containing the diluted vaccine, the suspended antigens from the vaccine may be absorbed by the skin and gills. Then, they will be transported to specialized tissues where the systemic immune response builds up. Anesthesia is needed for the injection vaccination, since it decreases the stress for the fish, prevents mechanical injuries and helps it to recover faster from the handling. This kind of vaccine can be administrated by intramuscular or intraperitoneal injection; the intraperitoneal being the most prevalent, where the needle penetrates the abdominal wall of the fish by 1 to 2 mm (Komar *et al.,* 2004). The most recommended position of the injection point for vaccination is in the midline of the abdomen, one pelvic fin length in front of the base of the pelvic fins (Figure 2.7), where the deviation in the point of injection should not exceed 0.1 %. This is very difficult to achieve in practice, hence the deviation shall be kept as close as possible to 0.1%. A deviation in the injection point occurs when the vaccine is deposited in a way where it does not float freely in the abdominal cavity, meaning that the injection point was outside the recommended injection area. Other reasons can be that the depth or angle of the needle was not correct. A vaccination that is not optimal can lead to damage and higher moratlity (Intervet International B.V, 2005).

Injection vaccination has some advantages that make it a preferred method. In fact, it provides a long duration of the protection and the responsible professional for vaccination at the farm can be sure that every fish in the population has received the vaccine according to the correct dose, which can be difficult to know by other vaccination methods (Komar *et al.*, 2004).

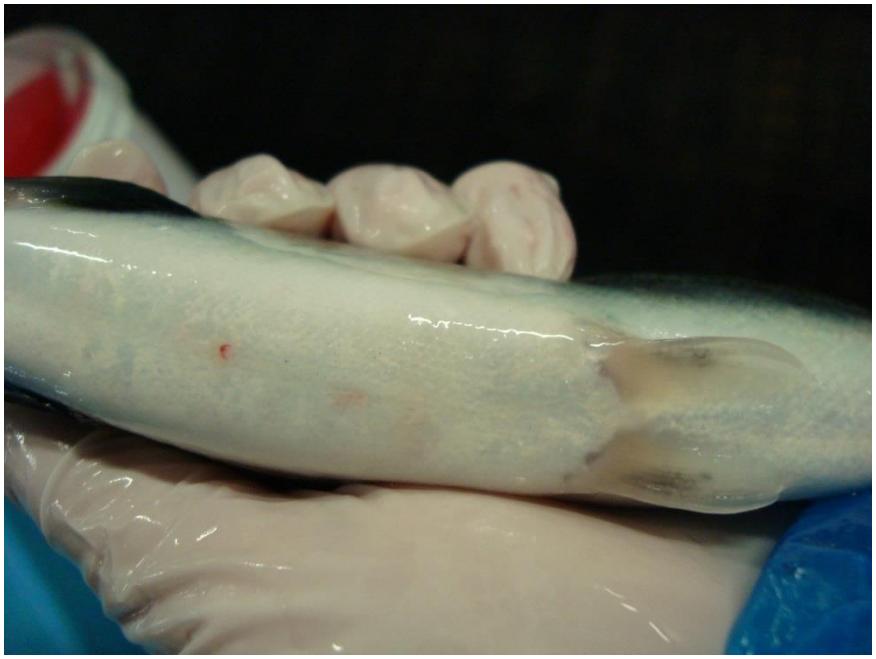


Figure 2.7: Right point of injection vaccination of salmon.

In salmonid fish the transfer of maternal antibodies seems to be at very low levels, being too low to provide protection of the offspring against diseases and infections (Lillehaug *et. al.*, 1996). However, this disadvantage is compensated by the early maturation of the fish immune system and Salmonid fish as small as 2 g can be protected after being exposed, for example in immersion vaccination (Johnson *et. al.*, 1982).

Overall, the history of fish vaccination has been successful; there have been obstacles regarding the use of the fish immune system for disease prevention, but it has been shown that the basic mechanisms of immunity in fish, birds and mammals are similar. However, studies have also proven that there are great differences between species with huge influence on the strategy and methods for immuno-prophylaxis. In Norway, the use of vaccines has been progressive for many reasons, such as innovative scientists in public and private institutions and companies. Also a good cooperation among the scientific community, authorities and the industry has been an important factor that contributed to this progress (Gudding & Muiswinkel, 2013). Moreover, the approval of vaccines by the authorities without much bureaucracy has contributed to make it a fast process in Norway (Midtlyng *et al.*, 2011), making it possible for the vaccines to be developed, tested experimentally in the field and implemented at a high speed (Gudding & Muiswinkel, 2013).

2.7 Fillet gaping

The term gaping in aquaculture is used to characterize the undesirable separation of muscle blocks in a raw fillet. Fish fillets consist of small muscle blocks, mostly appearing to be in a rectangular shape bordered by thin shiny membranes of connective tissue. The block of muscle, or myotomes, consists of thousands of parallel threadlike muscle cells, so thin that they can be compared to the thickness of a human hair (FAO, 2001). Each cell is encased in a tiny tube of connective tissue called myocommata or myosepta, consisting of collagenous connective tissue, adipocytes and non-adipose cells. Their function is to anchor the whole axial muscle to both the skeleton and the skin and they can be recognized as repeating white bands separating the "salmon-colored" myotomes (Pittman et al., 2013). The tubes merge at both ends with a sheet of connective tissue, resulting on firmly attached muscle cells, and the break of the junctions between these tubes and sheets results into gaping. The gaps appear as slits between muscle blocks, and they can range from slim separation at the cut surface to complete separation down to the skin of a fillet.

A fish fillet that has gaping (Figure 2.8) is difficult to sell, as the gaps spoil the appearance of fillets and make skinning and cutting them into slices difficult or even impossible. Usually, round fish gape more than flatfish and each species are different when it comes to how much gaping they form. For example, haddock and cod are known for being particularly vulnerable in terms of gaping, whereas catfish and ling never seem to gape at all (FAO, 2001). The major problem that gaping in fillets brings is the rise to lace-like slices and irregular shapes in the muscle that significantly detract from the attractiveness of the final product. It represents one of the most important quality threads in the salmon industry and it can decrease up to 38% of its value (Michie, 2001).

Figure 2.8: Atlantic salmon fillet with gaping (Pittman, 2013).

3. Material and Methods

3.1 Fish material and sampling

Freshwater phase

6,765 Atlantic salmon (*Salmo salar* L; Aquagen) fry were kept in a tank (volume 10,500 l; height 1.70m and diameter 2.80m) (Figure 3.3) with recirculating freshwater (5.4^{o}C) for å period of 2-3 weeks before they were randomly distributed into six to the experimental tanks 20/03/13. The fish were fed *Skretting Nutra Olympic 3.0* until the feeding experimental started March 27th 2013. The diets used were a standard commercial diet manufactured by Skretting AS, Averøy, Norway (Control diet) (Control group) or the same diet coated with Vitamin E (Vitamin E group) or Zinc (Zinc group). Zinc sulphate ($ZnSO_4$) was diluted in water and coated on the feed pellets in 25kg batches. Vitamin E was mixed into rapeseed oil and coated onto the pellets in a blender. The Control feed and the Zinc feed were also coated with rapeseed oil. The pellets were spread on a tray and dried for two days before they were fed to the fish. The diets were fed to fish in duplicate tanks until sea transfer in October 2013.

The fish were vaccinated by hand (Vaccinated) or injected with saltwater (1% NaCl) (Unvaccinated) April 4th 2013 using a 6-component injection vaccine from MSD Animal Health (Norvax Minova 6); 0.1 ml dose, mineral oil adjuvance, and protection against furunkulosis, vibriosis (O1, O2), cold water vibriosis, winter ulcers (*Moritella viscosa*) and infectious pancreas necrosis IPN (sub unit VP2 of the IPN virus), immunity development after 500 day degrees. Minimum body weight of the fish at vaccination was 35 g. Starvation time before vaccination was 3 days. After injection, the vaccinated and unvaccinated fish were mixed and transferred back to their respective tanks. In order to distinguish between vaccinated and unvaccinated fish, the fish were marked by clipping the adipose fin (most posterior dorsal fin) of the unvaccinated fish. Fish were sampled for analyses (Figure 3.1) before vaccination or saltwater injection (April 5th), May 5th and just before sea-transfer May 30th 2013. The quality of the vaccination was controlled April 4th 2013 by MSD Animal Health (see Appendix 8.2). See Table 3.1 for an overview of the initial number of fish used and dietary treatments.

Figure 3.1: Sampling of fish in fresh water phase.

Table 3.1. Initial number of fish used in the experiment and dietary treatments.

Control group	4 201 fish. Diet: Commercial diet produced by Skretting AS, with 300 mg Vitamin E and 150 mg Zn kg^{-1} The following feeds were used: March 2013: Nutra Olympic 3.0 June 2013: Spirit ST 75-70A 3mm August 2013: Spirit PL ST 150-50 A 4.5mm October 2013: Spirit Pluss 600-50A 7mm November 2013: Spirit Pluss 600 50A 7mm January 25th 2014: Optiline V 1200-20A 9mm
Vit E group	1 011 fish. Diet: Control feed coated with 400mg Vitamin E per kg D,L-alpha-tocopherol acetate (*vitamin E*) from *Sigma* (*97*% purification)
Zinc group	1554 fish. Diet: Control diet coated with 100mg zinc per kg (Zinc sulphate, $ZnSO_4 \cdot 7H_2O$ from VWR International)

Fish were fed to satiation and uneaten feed was collected after each meal and pumped up into wire mesh strainers as described by (Einen *et al*. 1999). Each diet was tested for recovery of dry matter under the environmental conditions present during the experiment as described by (Helland *et al*. 1996). The weight of uneaten feed was corrected for water absorption during feeding and collection.

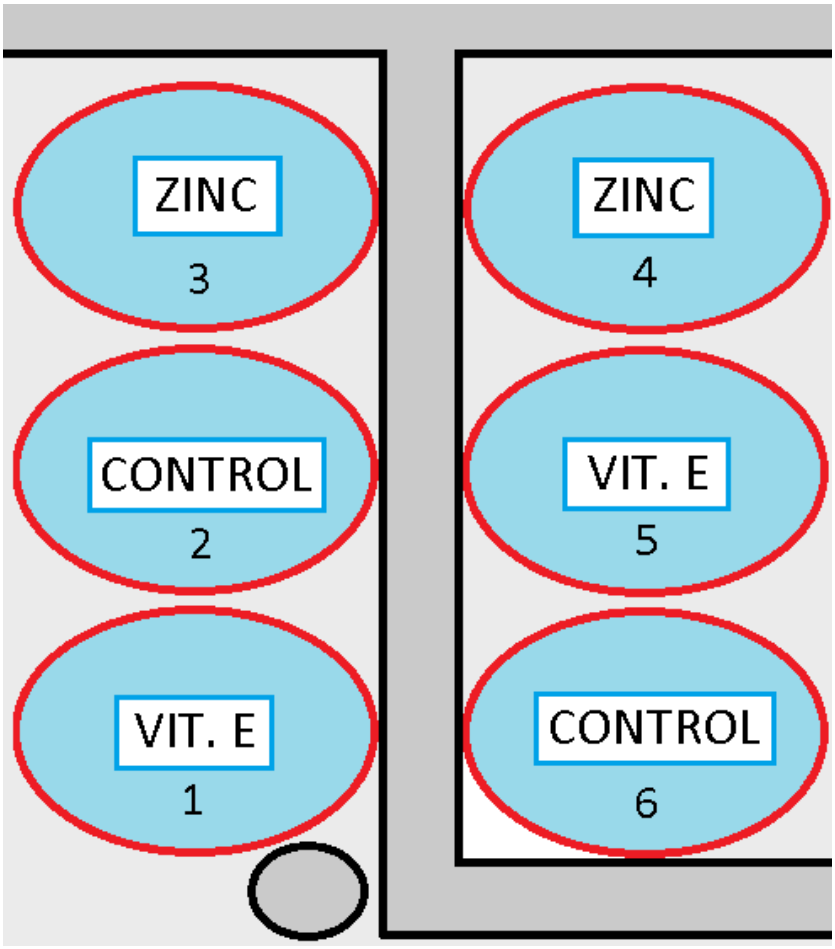


Figure 3.2: Experimental design used in the fresh water phase.

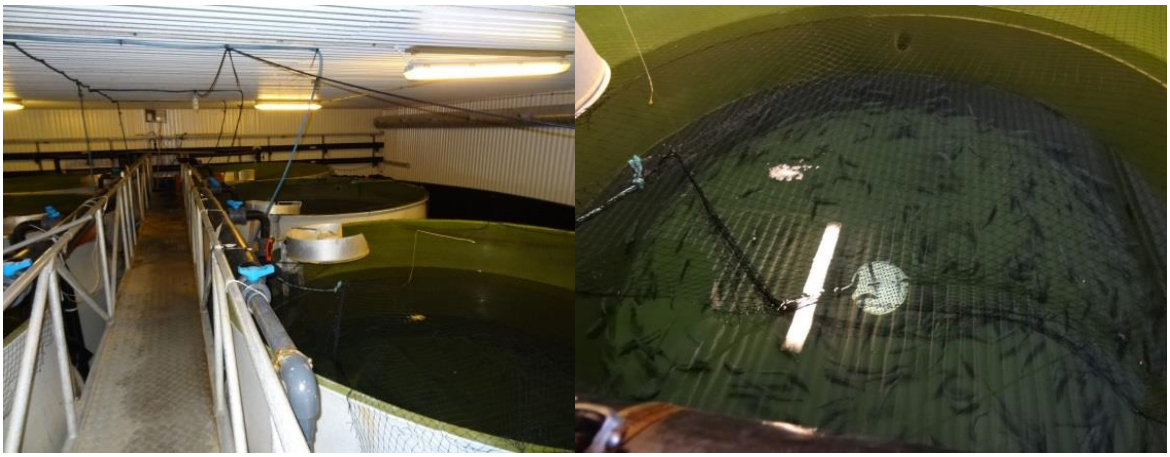


Figure 3.3: Tanks used for the fresh water phase.

Seawater phase

Vaccinated and unvaccinated fish from the Control group were distributed randomly into three seawater cages (125m^3). Vaccinated fish fed zinc or vitamin E in freshwater were mixed (Vitamin E group fin clipped) and distributed randomly into two 125m^3 cages (Fig 3.4). The fish were fed to satiation by automatic feeders and uneaten feed was collected as described for the fish in freshwater. Seawater temperature was recorded daily at 3m depth (Fig 3.5). The average temperature during the whole seawater phase (May 30th – March 26th) was 9.7 °C. Preparation of the diets was the same as in freshwater (the Vitamin E diet was excluded in the seawater phase). The fish were sampled for analyses at March 26th 2014. At sampling (Figure 3.6) the fish was slaughtered and gutted according to standard commercial procedures at the processing plants. The fish was killed by percussive stunning. Both gill arches were cut and the fish were bled in circulated water at ambient temperature. The salmon were gutted, cleaned and immediately filleted by hand by experienced workers. The time from slaughtering until filleting was less than one hour. For an overview of the experimental design and sampling dates, see Figure 3.7.

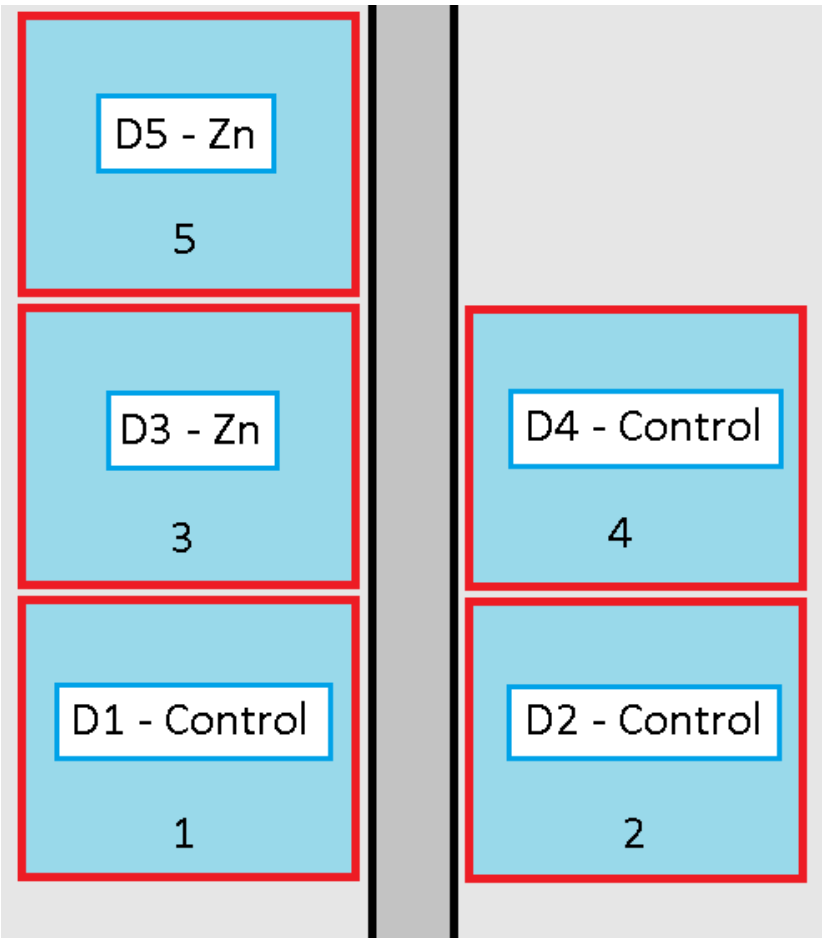
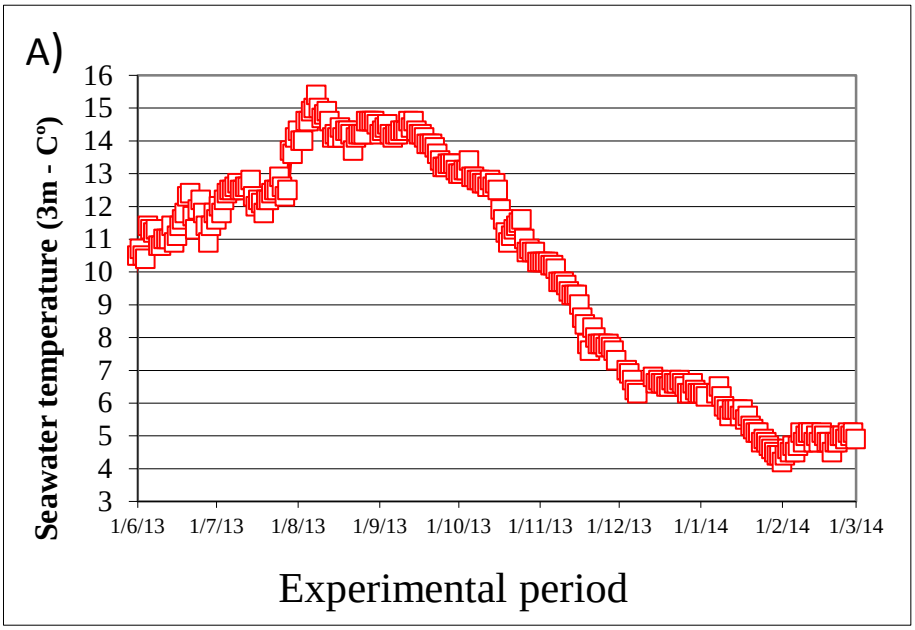


Figure 3.4: Experimental design used in the seawater phase.



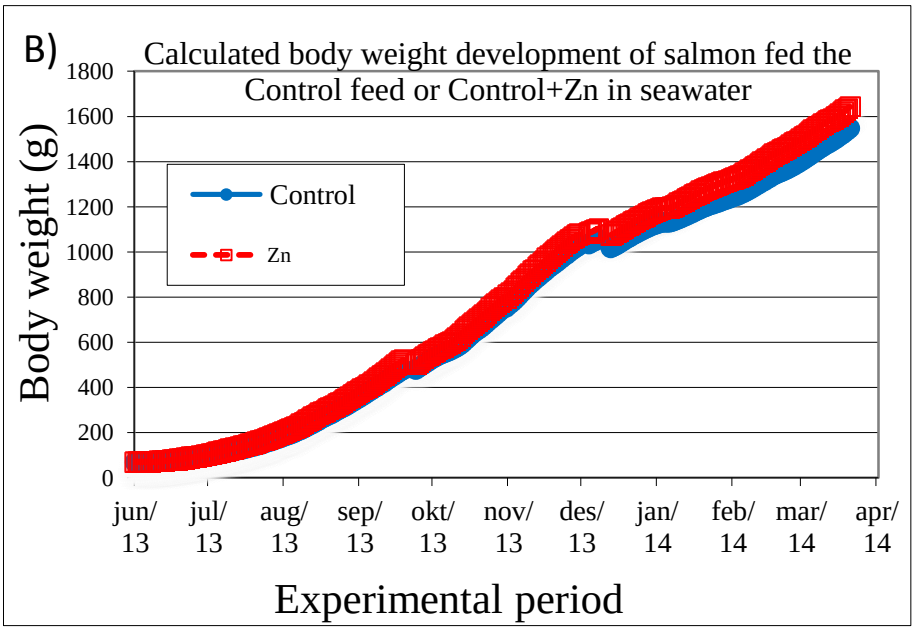


Figure 3.5: Development in sea water temperature (A) and body weight of the total population (B) during the experiment. The overall average (calculated) body weight of the salmon fed the Control feed and Zn feed was 1547g and 1644g, respectively.

Figure 3.6: Sampling of fish in seawater phase.

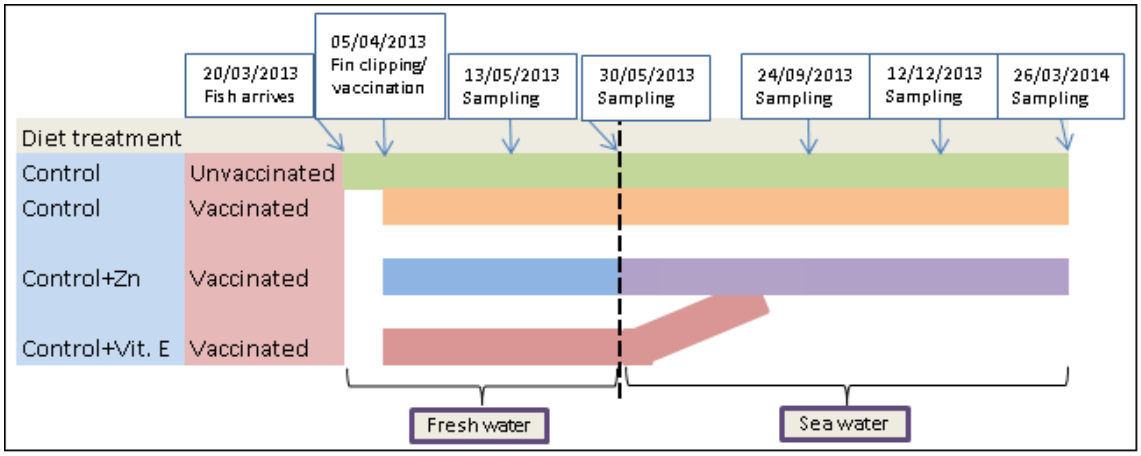


Figure 3.7: Overview of the experimental design and sampling dates.

3.2 Organ and fillet analyses

3.2.1 Melanin in Fillet

Dark spots on the salmon fillet, presumably due to melanin deposition, were graded visually according to a scale that went from score 0-8. The localization of the melanin found on the fillets (Figure 3.7) was recorded according (Mørkøre, T., 2012).

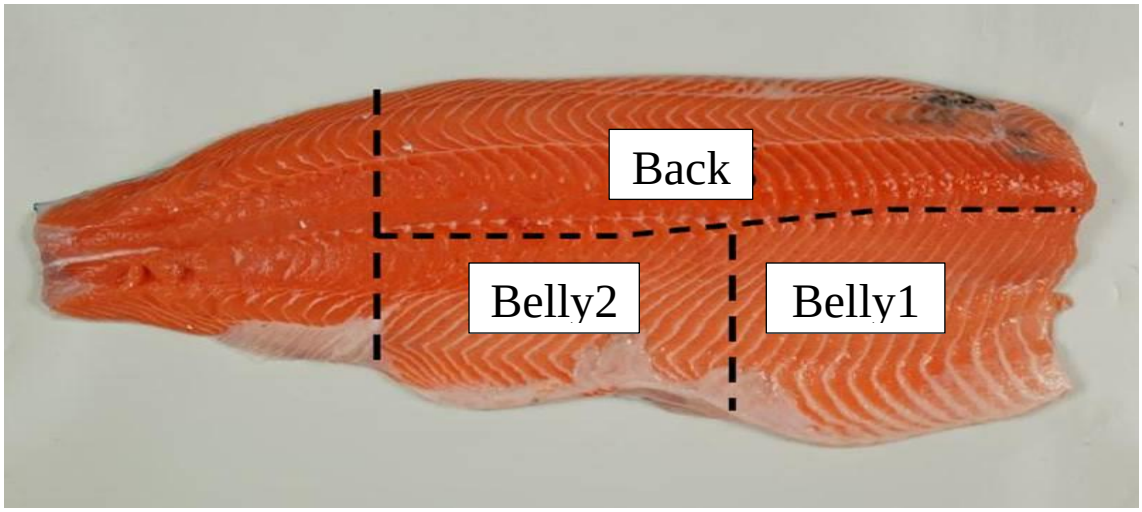


Figure 3.8: Scale used to identify the localization of the dark spots found in the salmon fillets (Mørkøre, T., 2012).

3.2.2 Fillet Color

For visual color evaluation the fillets were compared against the *Salmo*Colour Fan™ (DSM) (Figure 3.8) which has a scale ranging from 20-34, where score 20 is the palest color and score 34 is the most intense color. The color card readings were performed between the posterior part of the dorsal fin and the gut (Norwegian Quality Cut, NQC).

Figure 3.9: *Salmo*Colour Fan™ used for color evaluation on Atlantic salmon fillets (Burros, M., 2003).

3.2.3 Adhesions

Organ adhesions were classified according to a standardized scoring system by using a scale from 0 to 6, where 0 equaled no adhesions and 6 the highest possible degree of adhesions (Midtlyng et al., 1996).

3.2.4 Melanization of Abdominal Organs and Wall

The degree of melanization was classified by macroscopic examination of the abdominal organs (visceral peritoneum) and abdominal wall (parietal peritoneum) scored on separate (0–3) VAS scales (Taksdal et al. 2012).

The scale was used as follows:

0 = no melanin;

1 = pin points or small spots;

2 = considerable amount of melanin;

3 = melanin covering large areas of the abdominal wall/ abdominal organs.

3.2.5 Visceral Fat

The visceral fat (Figure 3.9) was measured by using a scale from score 1-5 (Mørkøre et al., 2013).

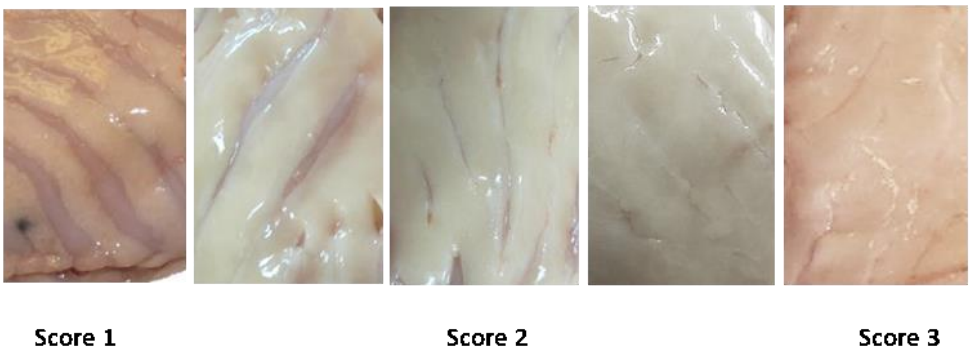


Figure 3.10: Scale used for the measurement of visceral fat scale (Mørkøre et al., 2013).

3.2.6 Liver Color

The liver color (Figure 3.10) was measured by using a scale that went from score 1-5, where

1 = light; 2 = light brown; 3 = brown; 4 = dark brown; 5 = dark (Mørkøre et al. 2013).

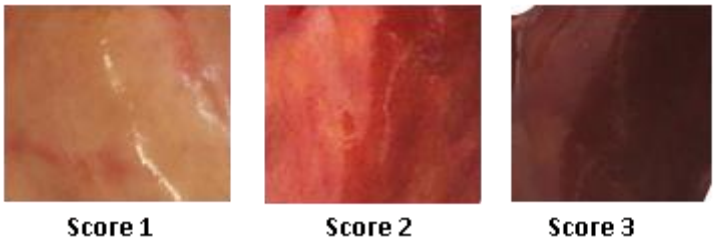


Figure 3.11: Scale used for the measurement of liver color (Mørkøre et al. 2013).

3.2.7 Fillet gaping

The fillet gaping was evaluated according to a scale from score 0-5, where 0 represents no gaping and five is extreme gaping that makes the fillet fall apart (Andersen et al. 1994).

3.2.8 Data analyzes

Statistical analysis was performed by the Statistical Analyses System 9.1(SAS Institute Inc.). The results are represented as LSmean (± SEM) and the level of significance was set at 5% ($P<0.05$). The results were ranked using pdiff.

3.2.9 Calculations

Feed conversion ratio, FCR:	(feed intake, g) x (wet weight gain, g)$^{-1}$
Condition factor, CF:	W (g) x (fork length, cm)$^{-3}$ x 100
Weight gain, WG:	W_1 (g) – W0 (g)
Hepato somatic index, HIS:	Liver weight (g) / Body weight (g) x 100
Cardio somatic index, CSI:	Heart weight (g) / Body weight (g) x 100
Carcass yield, CY:	Gutted weight (g) / Body weight (g) x 100
Fillet yield, FY:	Fillet weight (g) / Body weight (g) x 100

4. Results

Results will be presented in two sections, the first section include biometric traits that describes: weight and length registrations, fillet and carcass yield and organ indices. The second section describes tissue evaluation, including: melanin in organs, abdominal wall and fillet, visceral fat, liver color, fillet color and fillet gaping. Results for each parameter will be presented with regard to dietary treatment first (presented in tables at the end of each section), and thereafter vaccination (presented in figures).

4.1 Biometric traits

4.1.1. Body weight

Whole body weight

The average body weight of the collected salmon increased from 55.5g at the first sampling in May to 1867.5g at the last sampling in March 2014 (total range 38-2510g). The body weight of the salmon sampled for analyses showed no significant difference between dietary treatments (Table 4.1).

The body weight showed no significant difference between the vaccinated and unvaccinated fish (Figure 4.1). However, the weighing of all fish in December showed that vaccinated fish had significantly lower body weight compared with the vaccinated fish.

Gutted weight

The average gutted weight of the collected salmon increased from 441.5g at the sampling in September to 1668.8g at the last sampling in March 2014 (total range 335-2236g). The gutted weight showed no significant difference between dietary treatments (Table 4.1).

The gutted weight showed no significant difference between the vaccinated and unvaccinated fish.

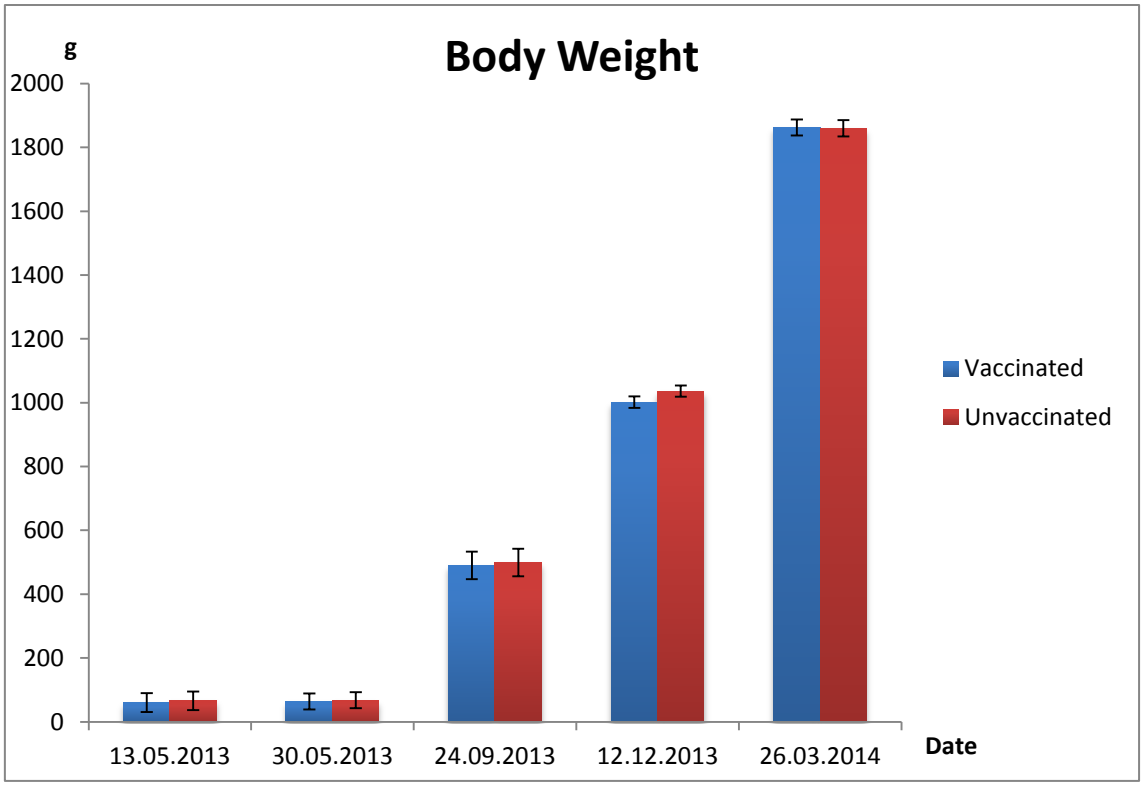


Figure 4.1: Body weight (g) development of vaccinated and unvaccinated Atlantic salmon. Results are presented as LSmeans ± SE.

4.1.2. Body length

The average length of the collected salmon increased from 16.7cm at the first sampling in May to 52.7cm in the last sampling in March 2014 (total range 16.0-59.0cm). The length showed no significant difference between dietary treatments (Table 4.1). However in December the 0.6 cm longer fish length of the salmon fed the Zn supplemented diet tended to be significant compared with the Control (P=0.08).

The length showed no significant difference between the vaccinated and unvaccinated fish (Figure 4.2).

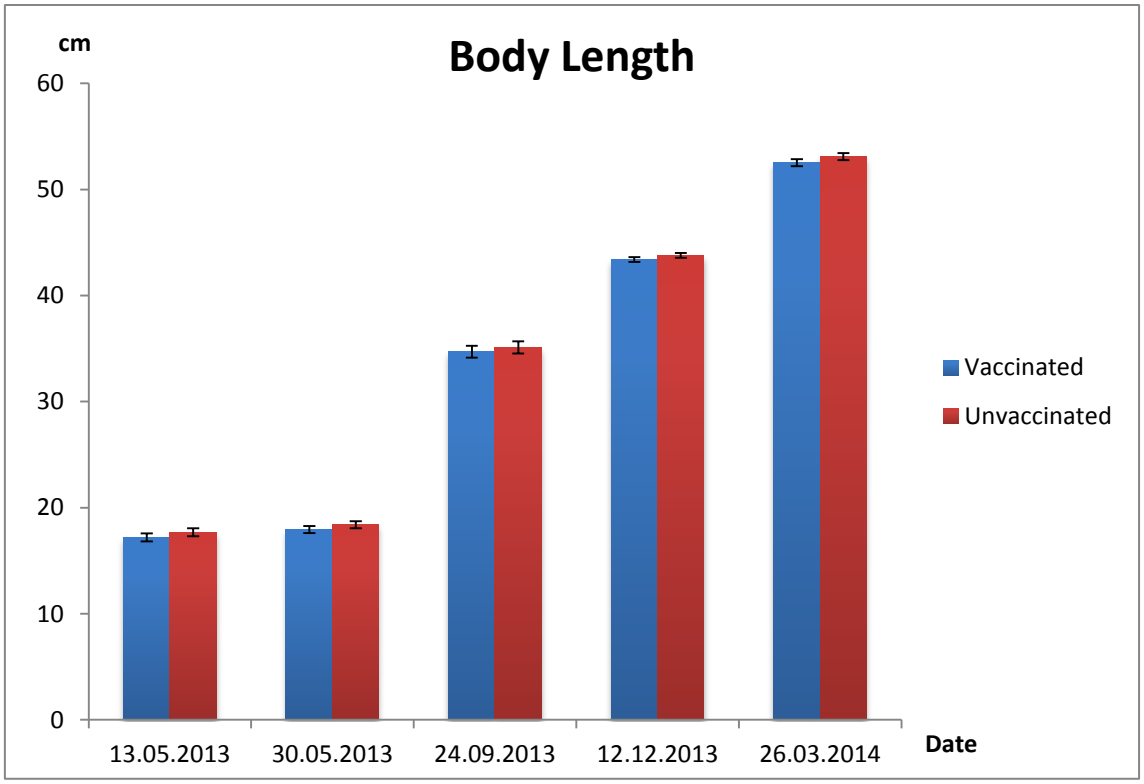


Figure 4.2: Body length (cm) development of vaccinated and unvaccinated Atlantic salmon. Results are presented as LSmeans ± SE.

4.1.3. Carcass yield

The average carcass yield of the collected salmon increased from 88.1% at the sampling in September to 90.3% at the last sampling in March 2014 (total range 59.4-93%). The carcass yield showed no significant difference between dietary treatments (Table 4.2).

The carcass yield showed no significant difference between the vaccinated and unvaccinated fish (Figure 4.3).

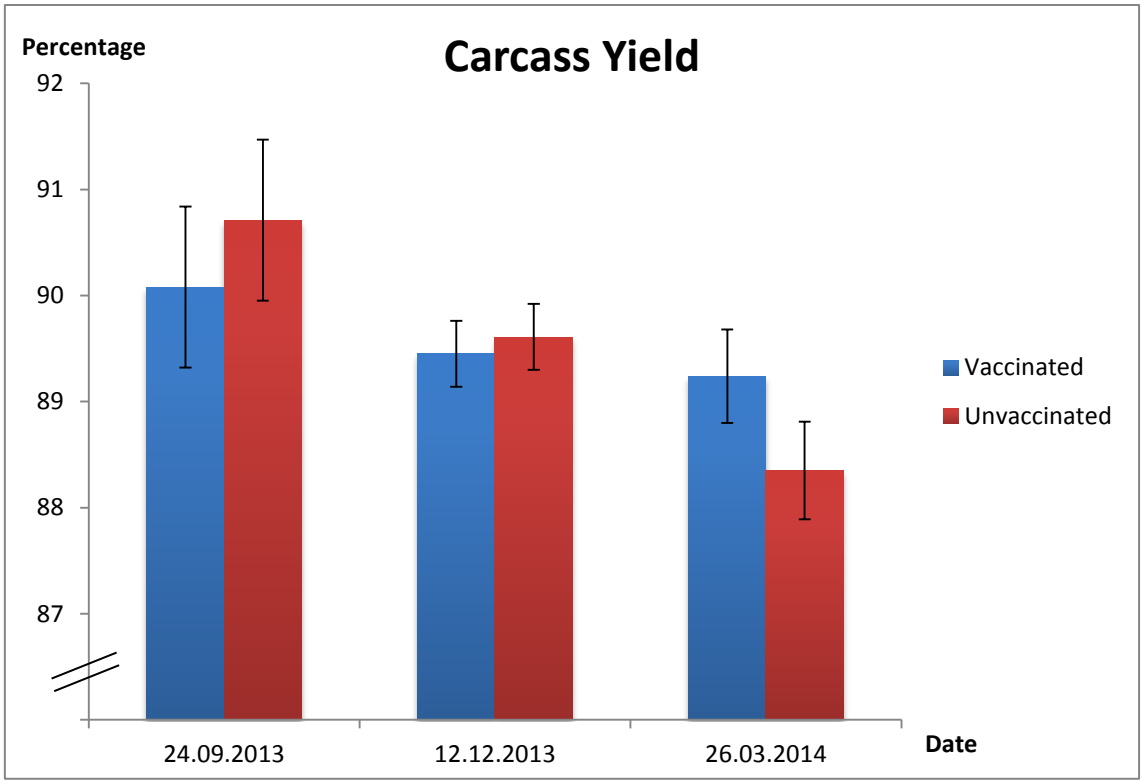


Figure 4.3: Carcass yield (%) of vaccinated and unvaccinated Atlantic salmon. Results are presented as LSmeans ± SE.

4.1.4. Condition factor

The average condition factor of the collected salmon varied from 1.08 to 1.32 (total range 0.87-1.42). The condition factor showed no significant difference between the dietary treatments (Table 4.2).

The carcass yield differed significantly between the vaccinated and unvaccinated salmon at the last sampling of the experiment, where the vaccinated group presented the highest value of 1.29 and the unvaccinated one presented the lowest value of 1.24 (Figure 4.4).

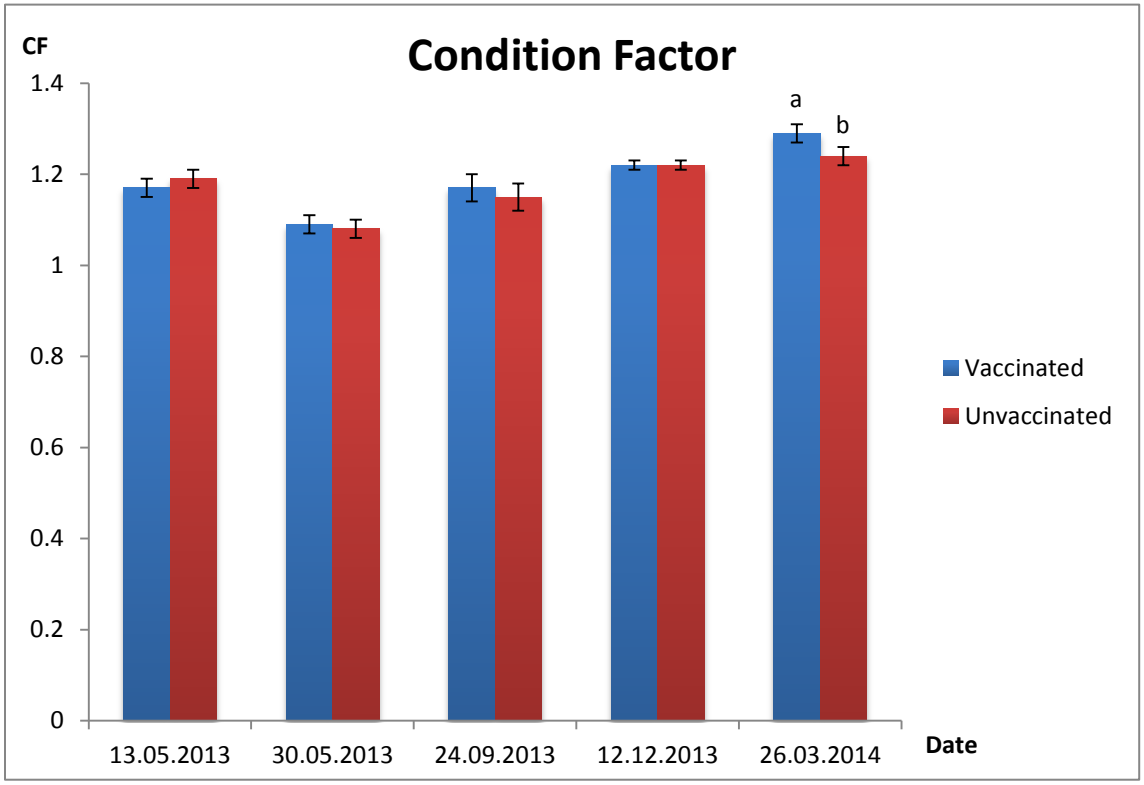


Figure 4.4: Condition Factor of vaccinated and unvaccinated Atlantic salmon. Results are presented as LSmeans ± SE. Different super scripts indicate significant differences between groups within sampling date (P<0.05).

4.1.5. Fillet

Weight

The average fillet weight of the collected salmon increased from 289.5g at the sampling in September to 1198.1g at the last sampling in March 2014 (total range 220-1702g). The fillet weight showed no significant difference between the dietary treatments (Table 4.1).

The fillet weight showed no significant difference between the vaccinated and unvaccinated fish.

Yield

The average fillet yield of the collected salmon showed an overall increase from 58.2 to 64.2% (total range 39.5-73.5 %). Significant differences were observed between the dietary treatments in December and March. In December, the 1.2% units higher fillet yield of the Zn diet compared with the Control diet was significantly different. In March

the fillet yield of both Zn and Zn_E diets were significantly higher compared with the Control group (Table 4.2).

The fillet yield showed no significant difference between the vaccinated and unvaccinated fish (Figure 4.5).

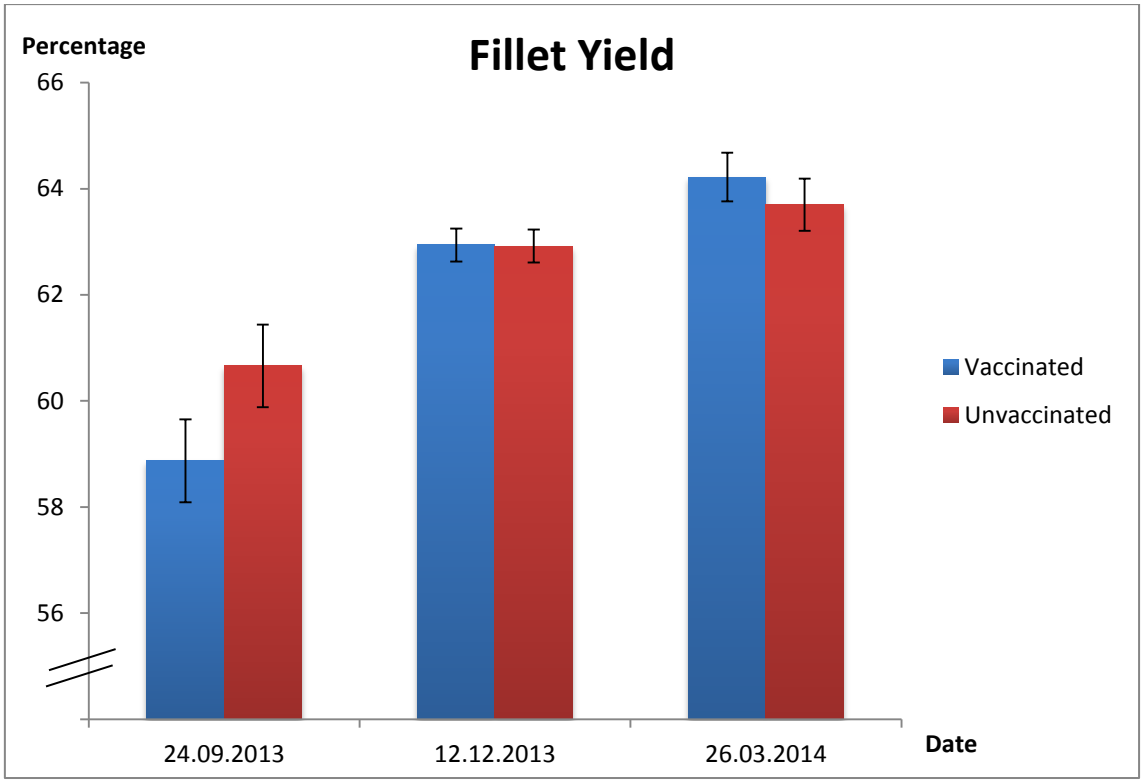


Figure 4.5: Fillet yield (%) of vaccinated and unvaccinated Atlantic salmon. Results are presented as LSmeans ± SE.

4.1.6. Liver weight

Liver weight

The average liver weight of the collected salmon increased from 0.5g at the first sampling in May to 18.8g at the last sampling in March 2014 (total range 0.6-20.9g). Significant differences were observed between the dietary treatments in March with significantly larger livers of the Control group compared with the Zn and Zn_E groups

(Table 4.1). In December the Zn_E diet tended to have a larger liver than the Control diet (P=0.08)

The liver weight showed a significant difference at the sampling from March 2014, where the vaccinated fish presented a greater value (Figure 4.6).

Hepato Somatic Index (HSI)

The average HSI of the collected salmon varied from 0.82 to 1.36. The HSI of the salmon fed the Control diet was significantly highest in the first two samplings on May. In September the HSI of the salmon fed the Control diet was significantly lowest (Table 4.2). In March the HSI of the salmon fed the Control diet tended to be higher compared to the HSI of the salmon fed the Zn diet (P=0.08) or Zn_E diet (P=0.059).

The HSI showed a significant difference at the first sampling in May 2013, where the vaccinated fish presented a greater value (Figure 4.6).

The HSI and liver color from all the analyzed fish presented a correlation of -0.21, with P<0.0001.

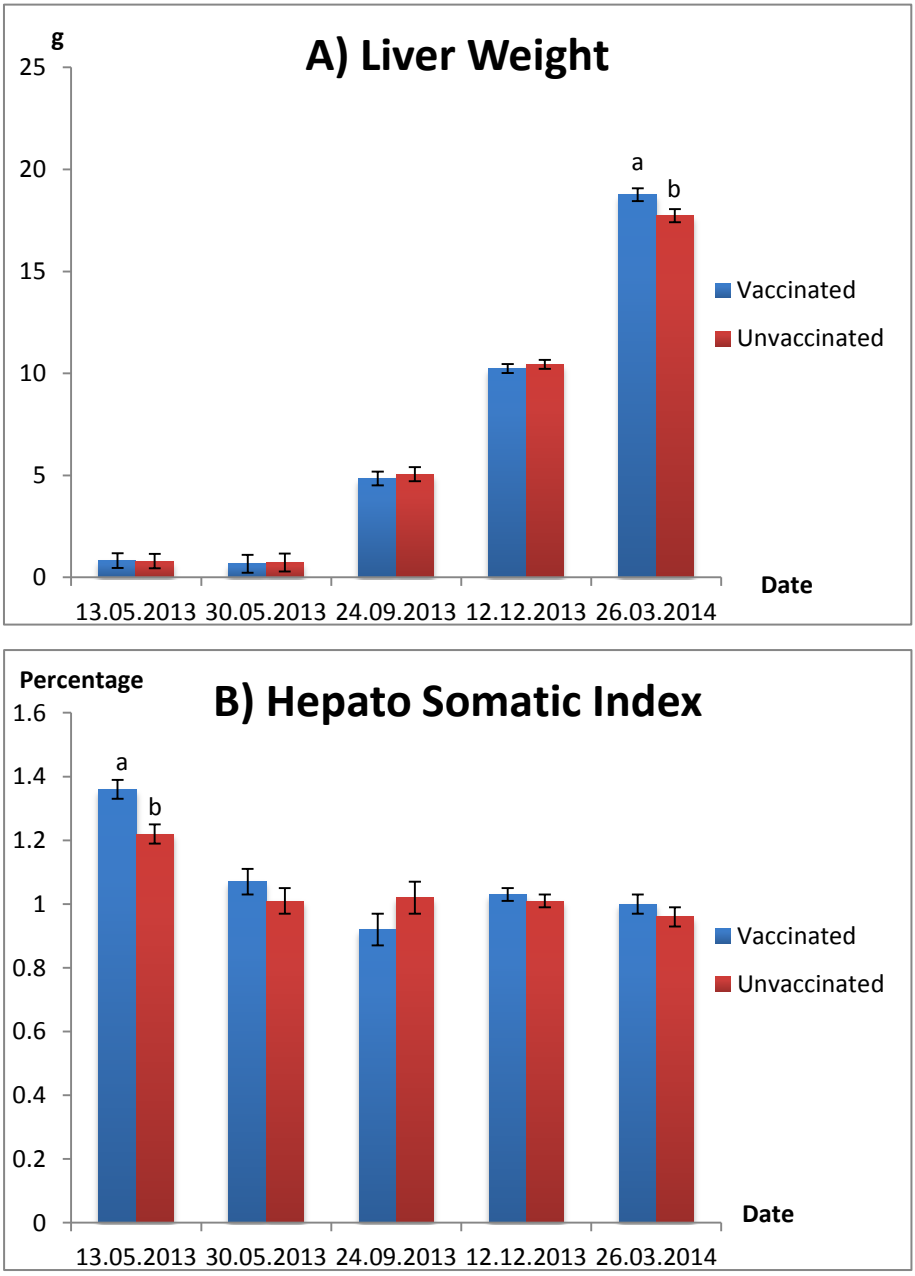


Figure 4.6: Liver weight (A) and HSI (B) of vaccinated and unvaccinated Atlantic salmon. Results are presented as LSmeans ± SE. Different super scripts indicate significant differences between groups within sampling date ($P<0.05$).

4.1.7. Heart

Weight

The average heart weight of the collected salmon increased from 0.08g at the first sampling in May to 2.38g at the last sampling in March 2014 (total range 0.06-3.39g). In December the heart was significantly lowest of the Control group (1.5g) and significantly largest of the Zn_E group (1.8g). In March the heart weight of the Control group was significantly lower (2.2g) compared with the Zn group (Table 4.1).

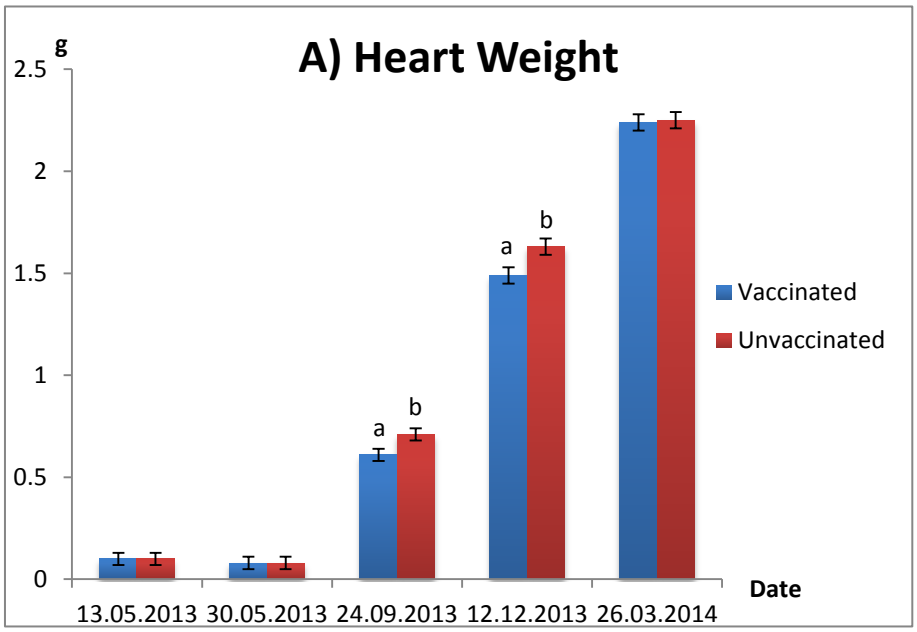
The heart weight was significantly higher of the vaccinated fish in September and December (Figure 4.7).

Cardio Somatic Index (CSI)

The average CSI value of the collected salmon increased from 0.12 to 0.17 (total range 0.09-0.24). In the second sampling at May the CSI was significantly lowest of the Control group (0.13) and significantly largest of the Zn group (0.15). In December the CSI of both the Control and Zn diet was significantly lower compared with the Zn_E group (Table 4.2).

At the second sampling in May the CSI of the salmon fed the Vit E diet tended to be higher compared to the CSI of the salmon fed the Control diet (P=0.09), and the CSI of salmon fed Zn diet tended to be higher compared to the CSI of the salmon fed Vit E (P=0.12).

The CSI showed no significant difference between the vaccinated and unvaccinated fish (Figure 4.7). However, in September the unvaccinated salmon tended to have a significant higher CSI compared with the vaccinated group (P=0.09).



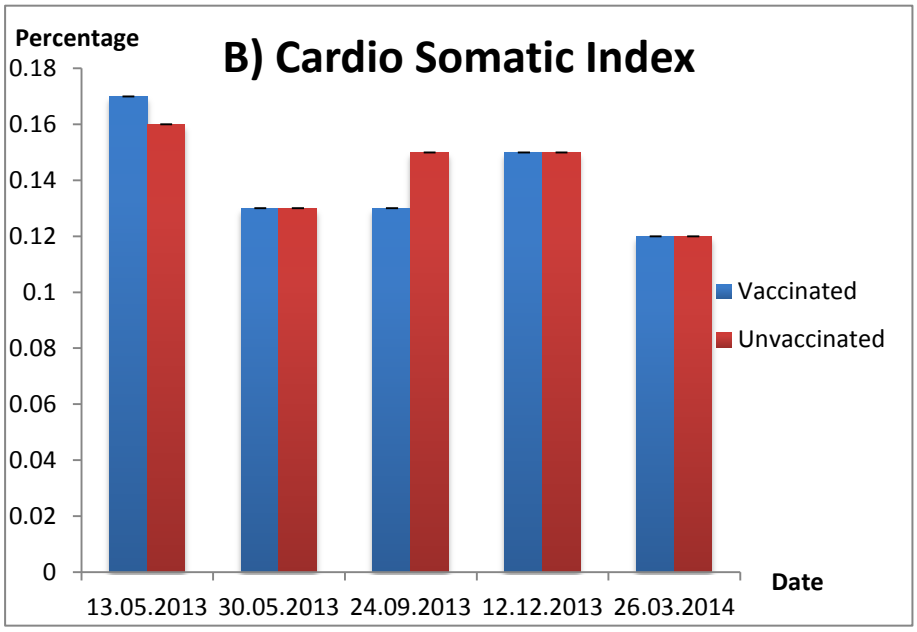


Figure 4.7: Heart weight (A) and CSI (B) of vaccinated and unvaccinated Atlantic salmon. Results are presented as LSmeans ± SE. Different super scripts indicate significant differences between groups within sampling date ($P<0.05$).

Table 4.1: Data from biometric parameters for vaccinated Atlantic salmon fed a standard commercial diet (Control) or the same diet supplemented with zinc (Zn) or vitamin E (Vit E). Diets used in fresh water were Control, Zn and Vit E. In seawater, the Control and Zn groups continued on the same diet, whereas the Vit E group was fed the Zn diet (Zn_E).

Phase		Fresh water		Seawater			SEM
Parameter	Diet	13_05_2013	30_05_2013	24_09_2013	12_12_2013	26_03_2014	
Body weight	Control	60.1 ^{d}	63.5 ^{d}	490.0 ^{c}	1001.7 ^{b}	1862.2 ^{a}	28.1
	Zn	63.7 ^{d}	65.8 ^{d}	488.4 ^{c}	1041.5 ^{b}	1867.4 ^{a}	26.9
	Zn_E	.	.	460.9 ^{c}	1029.7 ^{b}	1841.2 ^{a}	26.7
	Vit E	55.5	63.4	.	.	.	35.1
Gutted weight	Control	.	.	441.5 ^{c}	900.9 ^{b}	1662.7 ^{a}	35.9
	Zn	.	.	443.3 ^{c}	938.1 ^{b}	1668.8 ^{a}	40.8
	Zn_E	.	.	443.5 ^{c}	926.5 ^{b}	1616.0 ^{a}	40.2
	Vit E	.	.	.	.	.	.
Lenght	Control	17.2 ^{d}	17.9 ^{d}	34.7 ^{c}	43.4 ^{b}	52.5 ^{a}	0.4
	Zn	17.5 ^{d}	18.2 ^{d}	35.1 ^{c}	44.0 ^{b}	52.7 ^{a}	0.4
	Zn_E	.	.	34.5 ^{c}	44.0 ^{b}	52.1 ^{a}	0.4
	Vit E	16.7	18.0	.	.	.	0.5
Fillet weight	Control	.	.	289.5 ^{c}	631.3 ^{b}	1198.1 ^{a}	26.3
	Zn	.	.	290.0 ^{c}	668.1 ^{b}	1166.1 ^{a}	29.1
	Zn_E	.	.	295.0 ^{c}	659.7 ^{b}	1150.0 ^{a}	29.1
	Vit E	.	.	.	.	.	29.1
Liver weight	Control	0.8 ^{d}	0.7 ^{d}	4.8 ^{c}	10.2 ^{b}	18.8 $^{a\,A}$	0.3
	Zn	0.8 ^{d}	0.6 ^{d}	5.4 ^{c}	10.4 ^{b}	17.3 $^{a\,B}$	0.4
	Zn_E	.	.	5.2 ^{c}	10.8 ^{b}	17.0 $^{a\,B}$	0.3
	Vit E	0.7	0.5	.	.	.	0.5
Heart weight	Control	0.1 ^{d}	0.1 ^{d}	0.6 ^{c}	1.5 $^{b\,C}$	2.2 $^{a\,B}$	0.0
	Zn	0.1 ^{d}	0.1 ^{d}	0.7 ^{c}	1.6 $^{b\,B}$	2.4 $^{a\,A}$	0.0
	Zn_E	.	.	0.7 ^{c}	1.8 $^{b\,A}$	2.3 $^{a\,AB}$	0.1
	Vit E	0.1	0.1	.	.	.	3.0

Lower case super scripts in the table indicate significant difference over time and capital letter super scripts indicate significant difference between dietary treatments ($P<0.05$). The absence of a letter indicates no significant difference.

Table 4.2: Data from biometric parameters for vaccinated Atlantic salmon fed a standard commercial diet (Control) or the same diet supplemented with zinc (Zn) or vitamin E (Vit E). Diets used in fresh water were Control, Zn and Vit E. In seawater, the Control and Zn groups continued on the same diet, whereas the Vit E group was fed the Zn diet (Zn_E).

Phase		Fresh water		Seawater			SEM
Parameter	Diet	13_05_2013	30_05_2013	24_09_2013	12_12_2013	26_03_2014	
Carcass yield	Control	.	.	90.1	89.5	89.2	0.5
	Zn	.	.	88.9	90.1	89.3	0.6
	Zn_E	.	.	90.3 ^{a}	90.0 ^{a}	88.1 ^{b}	0.6
	Vit E	.	.	.	.	.	.
Condition factor	Control	1.17 ^{b}	1.09 ^{c}	1.17 ^{b}	1.22 b	1.30 a	0.0
	Zn	1.17 ^{bc}	1.08 ^{d}	1.13 ^{cb}	1.22 ^{b}	1.28 ^{a}	0.0
	Zn_E	.	.	1.11 ^{c}	1.20 ^{b}	1.32 ^{a}	0.0
	Vit E	1.18 ^{a}	1.09 ^{b}	.	.	.	0.0
Fillet yield	Control	.	.	58.9 ^{c}	62.9 $^{b\,B}$	64.2 $^{a\,A}$	0.5
	Zn	.	.	58.2 ^{c}	64.1 $^{a\,A}$	62.4 $^{b\,B}$	0.6
	Zn_E	.	.	60.1 ^{c}	63.8 $^{a\,AB}$	62.4 $^{b\,B}$	0.6
	Vit E	.	.	.	.	.	.
HSI	Control	1.37 $^{a\,A}$	1.07 $^{b\,A}$	0.92 $^{c\,B}$	1.03 ^{bd}	1.00 ^{cd}	0.0
	Zn	1.24 $^{a\,B}$	0.92 $^{c\,B}$	1.16 $^{b\,A}$	1.00 ^{c}	0.93 ^{c}	0.0
	Zn_E	.	.	1.09 $^{a\,A}$	1.05 ^{a}	0.92 ^{b}	0.0
	Vit E	1.22 $^{a\,B}$	0.82 $^{b\,B}$	.	.	.	0.0
CSI	Control	0.17 ^{a}	0.13 $^{c\,B}$	0.13 ^{bcd}	0.15 $^{b\,B}$	0.12 ^{d}	0.0
	Zn	0.16 ^{a}	0.15 $^{ab\,A}$	0.14 ^{bc}	0.14 $^{bc\,B}$	0.12 ^{c}	0.0
	Zn_E	.	.	0.14 ^{b}	0.17 $^{a\,A}$	0.12 ^{b}	0.0
	Vit E	0.16 ^{a}	0.14 $^{b\,AB}$	.	.	.	0.0

Lower case super scripts in the table indicate significant difference over time and capital letter super scripts indicate significant difference between dietary treatments ($P<0.05$). The absence of a letter indicates no significant difference.

4.2 Tissue Evaluation

4.2.1 Melanin in organs

The average melanin score in organs of the collected salmon varied from 0.6 at the first sampling to 1.3 at the last. In the first sampling at May the melanin in organs was significantly lowest of the Control group (0.6) and significantly largest of the Vit E group (0.8). In December the melanin in organs of both the Zn and Zn_E diets was significantly highest compared with the Control group (Table 4.4).

The melanin in organs showed a significant difference between the vaccinated and unvaccinated fish at all the five sampling dates, showing a higher score in vaccinated salmon (Figure 4.8).

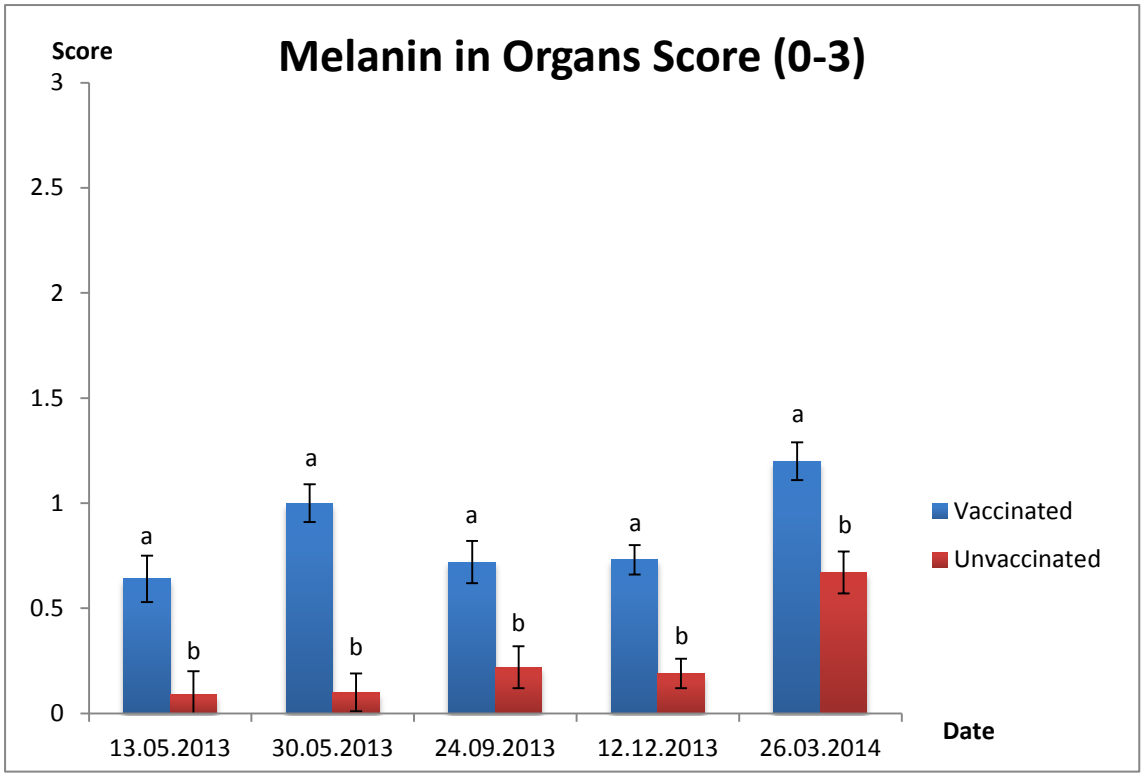


Figure 4.8: Melanin in organs of vaccinated and unvaccinated Atlantic salmon. Results are presented as LSmeans ± SE. Different super scripts indicate significant differences between groups within sampling date (P<0.05).

4.2.2. Melanin in abdominal wall

The average melanin score of the abdominal wall of the collected salmon varied from 0.5 to 1.1. The overall incidence of salmon with no melanin in the abdominal wall was 13.4%. There was no significant difference between dietary treatments (Table 4.4).

The melanin in abdominal wall showed no significant difference between the vaccinated and unvaccinated fish (Figure 4.9).

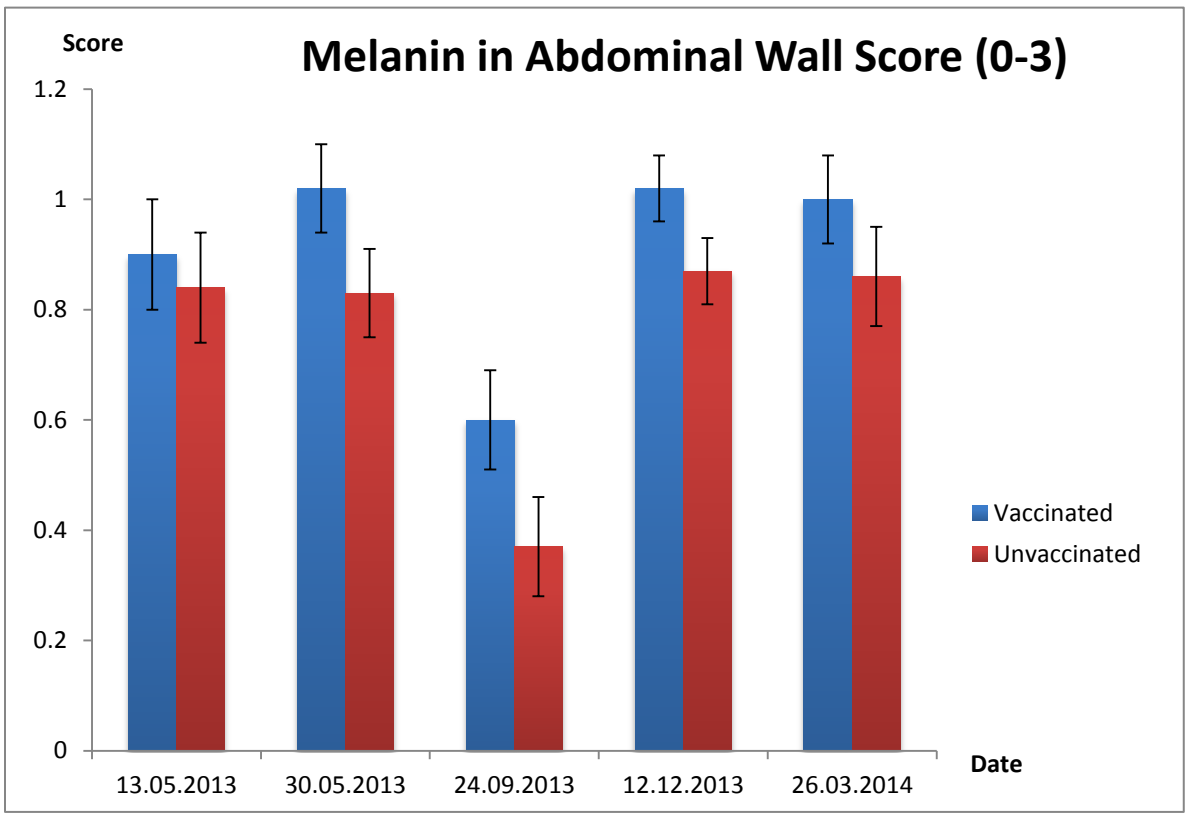


Figure 4.9: Melanin in the abdominal wall (score 0-3) of vaccinated and unvaccinated Atlantic salmon. Results are presented as LSmeans ± SE.

4.2.3. Melanin

Melanin in fillet

There was no significant difference between dietary treatments for melanin in fillet. Over time the melanin in the salmon fillet from the Control group increased throughout the experiment, showing a significant difference between the sampling in September and December (Table 4.4). In March the Control diet tended to have a higher melanin in fillet than at December (P=0.1).

Significant differences were observed between the vaccinated and unvaccinated salmon at March. The vaccinated salmon presented significantly more melanin in fillet (score 0.2) when compared to the unvaccinated group (score 0.1) (Figure 4.12). The percentage of salmon that presented melanin spots was significantly highest in the vaccinated (23.3%) than the unvaccinated salmon (10.3%) (Figure 4.10).

Location and percentage of melanin in fillet

The total number of fish analyzed for melanin in the fillet was of 398, being 100 in September, 199 in December and 99 in March. The percentage of salmon that presented dark spots on the fillet surface was 11.0, 9.0 and 16.2% for the three last samplings, respectively (Table 4.3). The location of the dark spots was consistently highest on the B1 (anterior part of the belly) (Table 4.4), except for the unvaccinated salmon from the last sampling (Figure 4.11).

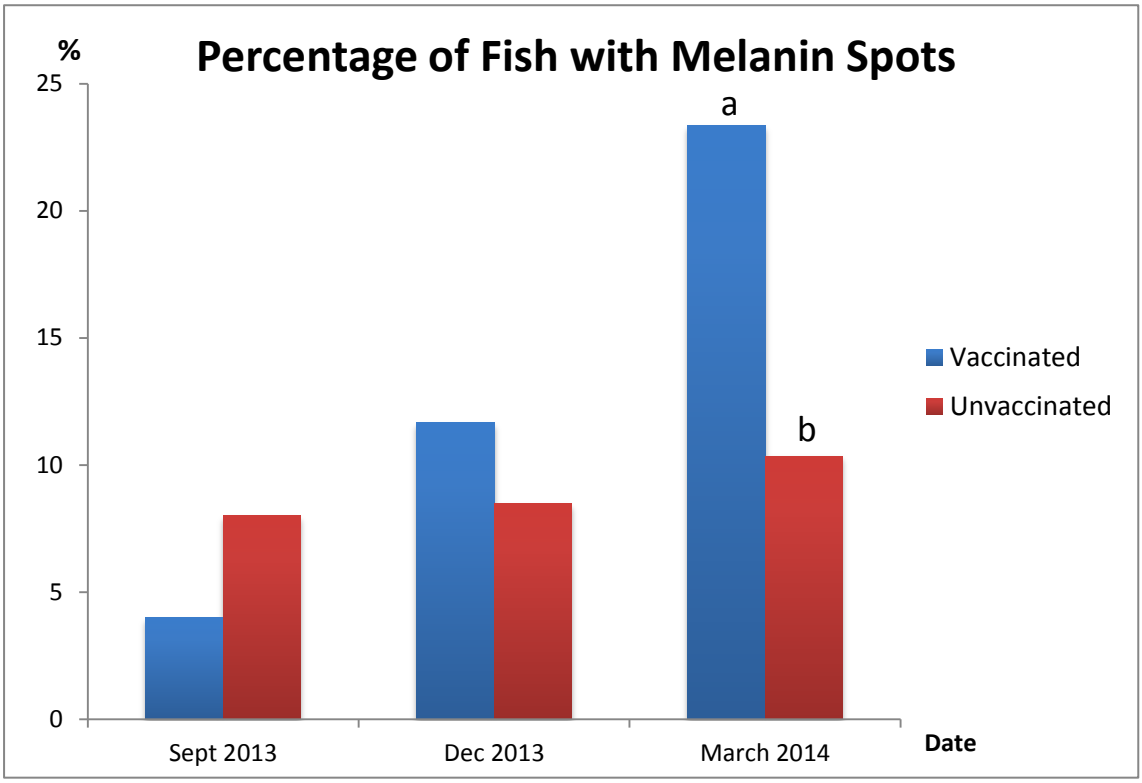


Figure 4.10: Percentage of fish with melanin spots of vaccinated and unvaccinated Atlantic salmon. Results are presented as LSmeans ± SE.

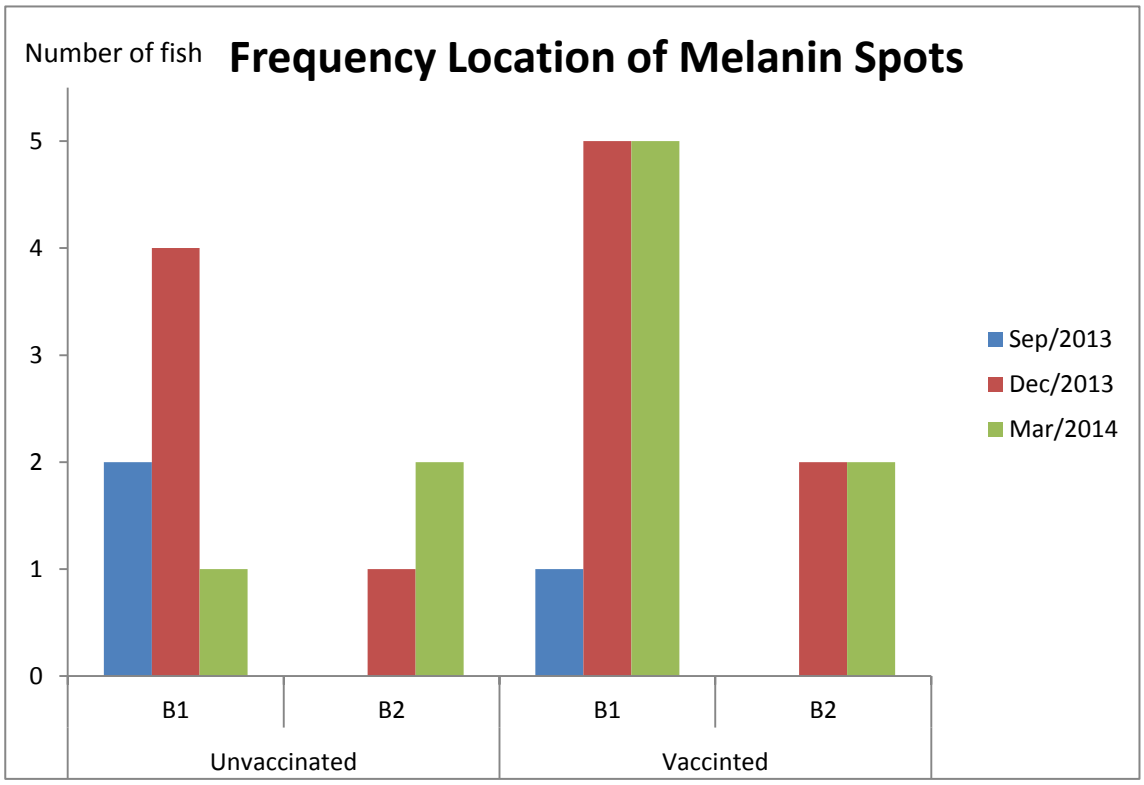


Figure 4.11: Frequency location of melanin spots found on vaccinated and unvaccinated Atlantic salmon.

Table 4.3: Location and percentage of melanin in fillet of all Atlantic salmon analyzed

Melanin in Fillet			
	Sept/2013	Dec/2013	Mar/2014
	(n=100)	(n=199)	(n=99)
Percentage of fish with dark spots	11.0	9.0	16.2
Fillet, number			
Right	4	6	8
Left	7	12	8
Both	0	1	1
Location, number			
Belly anterior (B1)	8	12	10
Belly posterior (B2)	3	5	6
B1 and B2		2	1

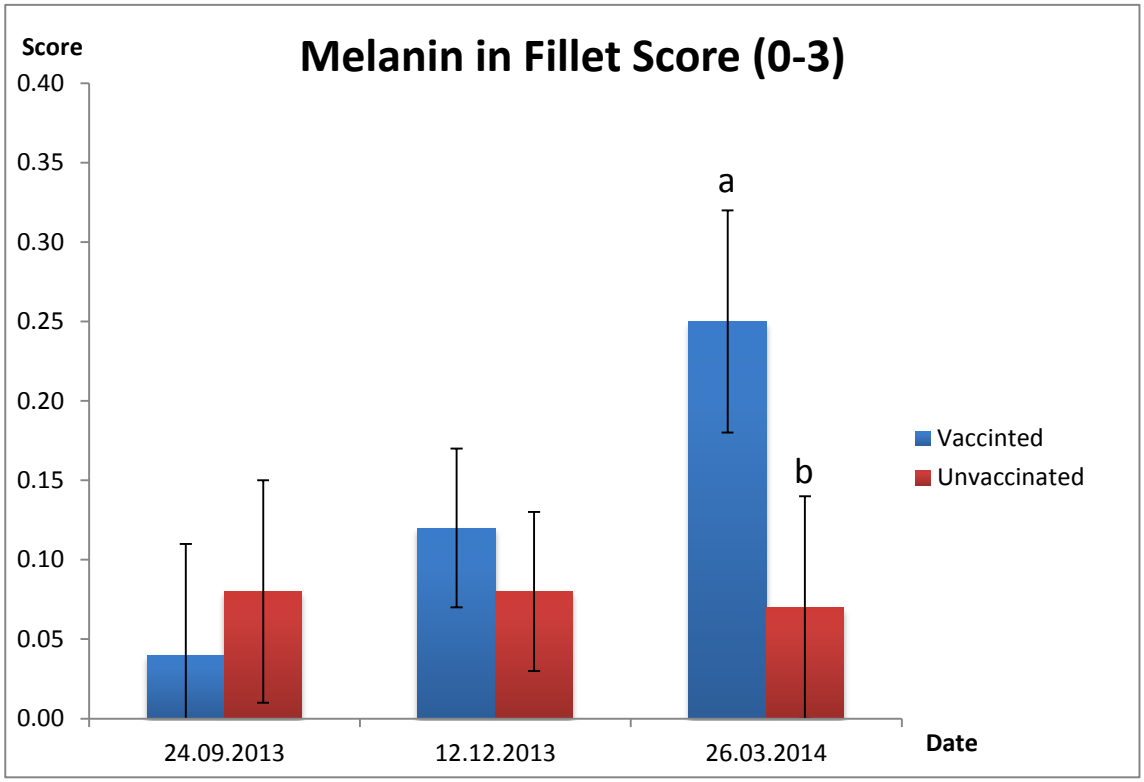


Figure 4.12: Melanin in fillet (score) of vaccinated and unvaccinated Atlantic salmon. Results are presented as LSmeans ± SE.

4.2.5. Adhesions

The average score for adhesions (score 0-6) of the collected salmon varied from 0 to 1. Significant differences were observed between the dietary treatments in the first sampling in May and in December. In May, the Zn group had a significantly higher score (0.4) than the Control group (0.0). In December the Control group had a significantly higher score (1.0) than the Zn group (Table 4.4)

The adhesions showed a significant difference between the vaccinated and unvaccinated salmon from the second sampling at May, December and March, where the vaccinated fish presented higher score (Figure 4.14).

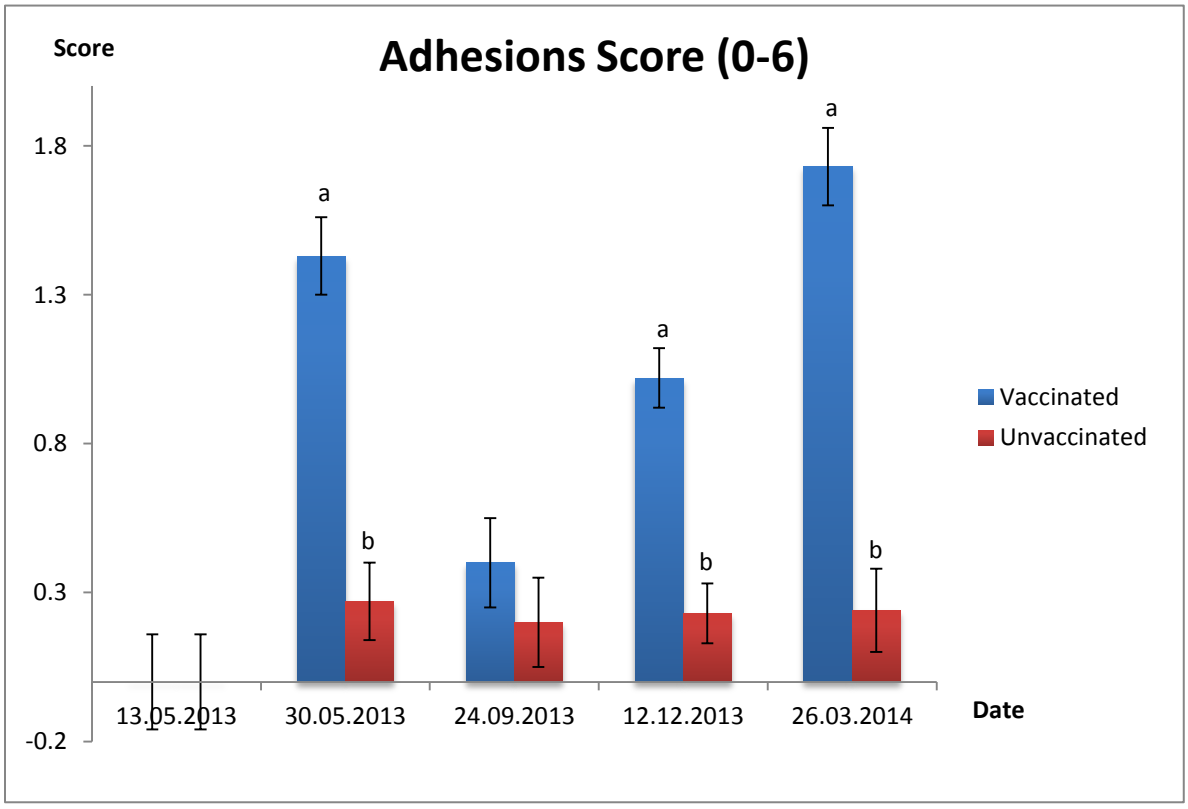


Figure 4.14: Adhesions of vaccinated and unvaccinated Atlantic salmon. Results are presented as LSmeans ± SE. Different super scripts indicate significant differences between groups within sampling date (P<0.05).

4.2.6. Visceral fat

The visceral fat score (0-5) of the collected salmon varied from 1.8 to 2.8. Significant differences were observed between the dietary treatments in December between the Control group to the Zn and Zn_E groups, where the visceral fat of the Control group was significantly highest (Table 4.4). However in December the salmon fed the Zn supplemented diet tended to have significantly more visceral fat compared with the Zn_E (P=0.10). Significant differences were observed between the dietary treatments in March between the Control group to the Zn and Zn_E groups, where the visceral fat of the Control diet was significantly lowest (Table 4.4).

The visceral fat score showed no significant difference between the vaccinated and unvaccinated fish (Figure 4.15).

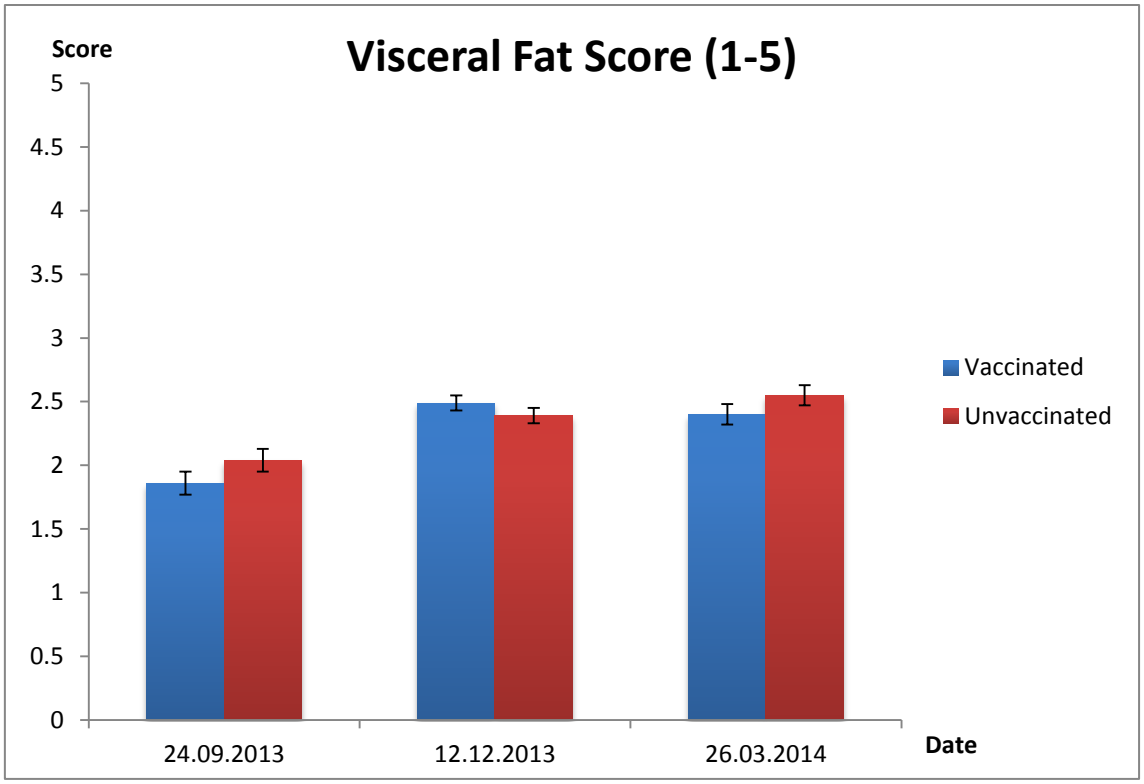


Figure 4.15: Visceral fat of vaccinated and unvaccinated Atlantic salmon. Results are presented as LSmeans ± SE.

4.2.7. Liver Color

The average score for liver color of the collected salmon varied from 2.4 to 3.94. Significant differences were observed between the dietary treatments in the first two samplings in May, December and March. At the samplings in May there was a significant difference between the Control group and the Zn and Vit E groups. In December there was a significant difference between the Control group and the Zn group. In March there was a significant difference between the Zn group and the Control and Zn_E groups (Table 4.4).

The liver color differed significantly between the vaccinated and unvaccinated salmon at the second sampling in May and in December, where the unvaccinated fish presented a darker liver color (Figure 4.16).

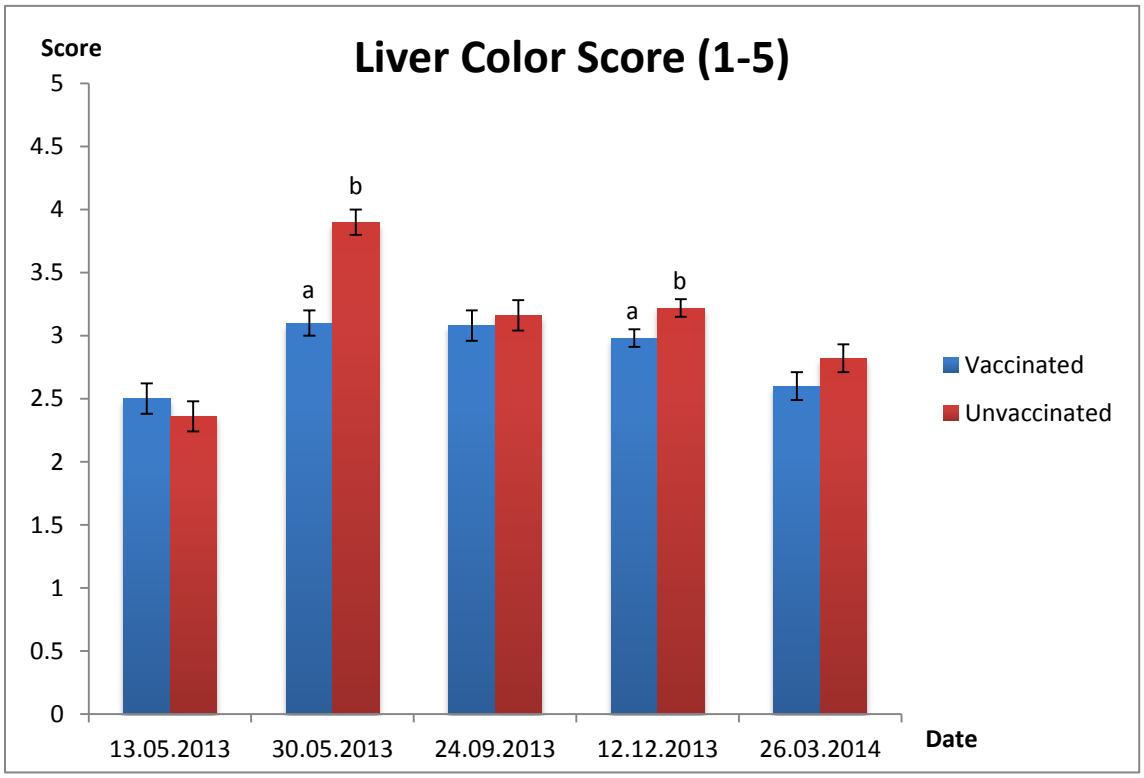


Figure 4.16: Liver color of vaccinated and unvaccinated Atlantic salmon. Results are presented as LSmeans ±SE. Different super scripts indicate significant differences between groups within sampling date (P<0.05).

4.2.8. Fillet color

The average score for fillet color of the collected salmon varied from 21.3 to 26.5. There was no significant difference found in the fillet color between dietary treatments. A numeric difference was observed between diets, where the salmon fed the Zn diet presented higher score for fillet color at all the samplings taken (Table 4.4). In March the Zn diet tended to have a higher fillet color score than the Control group (P=0.07).

The fillet color showed a significant difference between the vaccinated and unvaccinated salmon in March, where the unvaccinated group presented a higher value (Figure 4.17).

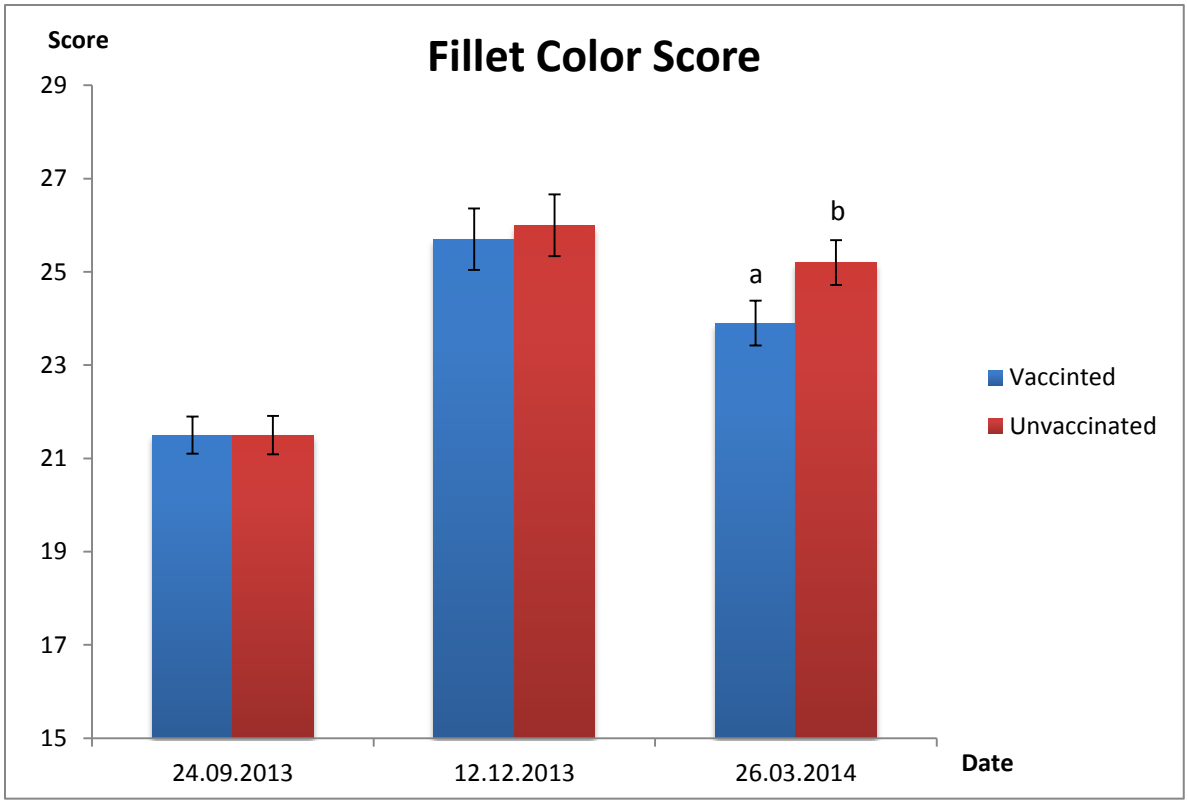


Figure 4.17: Liver color of vaccinated and unvaccinated Atlantic salmon. Results are presented as LSmeans ± SE. Different super scripts indicate significant differences between groups within sampling date (P<0.05).

4.2.9. Gaping

The average score for gaping of the collected salmon varied from 0.3 to 1.9. There was no significant difference found in gaping between dietary treatments. A significant difference was observed over time for all diets, where the gaping score decreased throughout the experimental period (Table 4.4).

There was a significant difference in gaping between the vaccinated and unvaccinated salmon in September, where the unvaccinated salmon presented a higher score (Figure 4.18). In December the unvaccinated salmon tended to have more gaping than the vaccinated group (P=0.08).

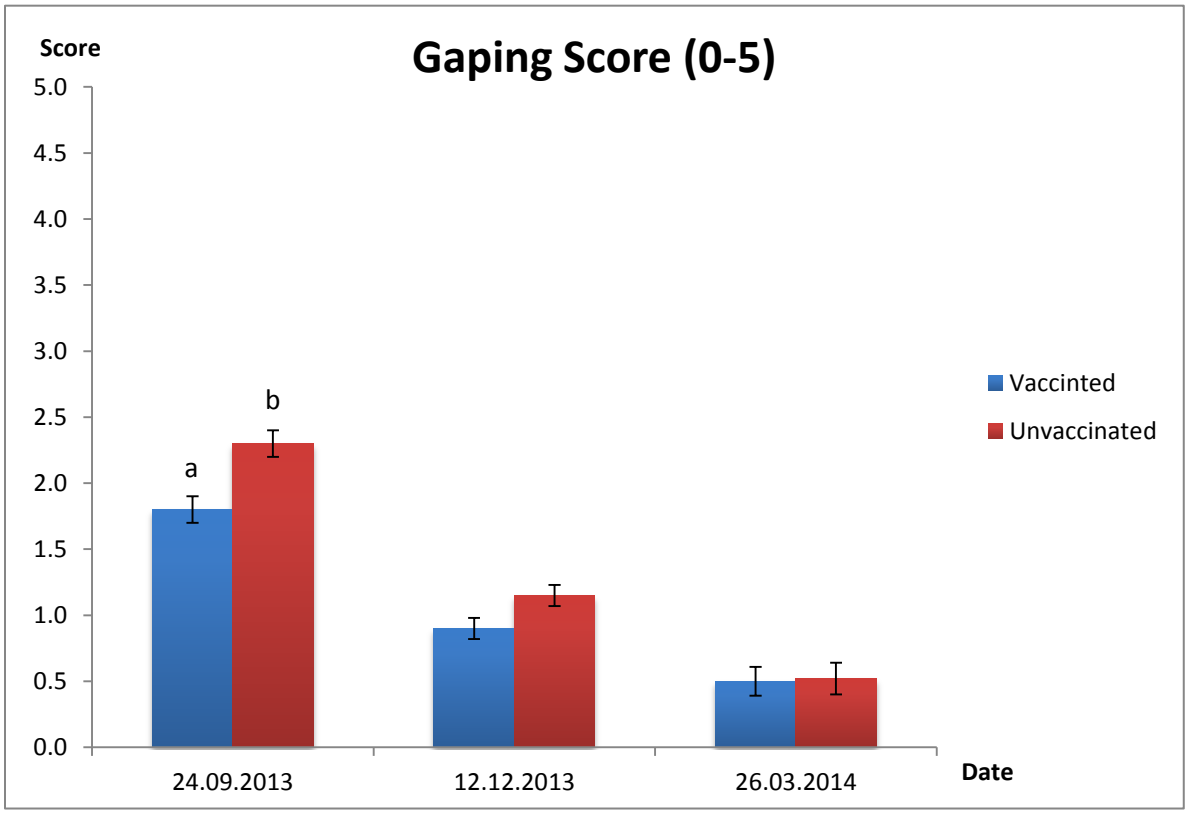


Figure 4.18: Gaping of vaccinated and unvaccinated Atlantic salmon. Results are presented as LSmeans ± SE. Different super scripts indicate significant differences between groups within sampling date ($P<0.05$).

Table 4.4: Data from visual evaluation of fillets and abdominal organs, peritoneum and fat of vaccinated Atlantic salmon fed a standard commercial diet (Control) or the same diet supplemented with zinc (Zn) or vitamin E (Vit E). Diets used in fresh water were Control, Zn and Vit E. In seawater, the Control and Zn groups continued on the same diet, whereas the Vit E group was fed the Zn diet (Zn_E).

Phase		Fresh water		Seawater			SEM
Parameter	Diet	13_05_2013	30_05_2013	24_09_2013	12_12_2013	26_03_2014	
Melanin in organs	Control	0.6 $^{b\,B}$	1.0 ^{a}	0.7 $^{b\,B}$	0.7 $^{b\,B}$	1.2 ^{a}	0.1
	Zn	0.9 $^{ab\,AB}$	1.0 ^{ab}	0.9 $^{b\,AB}$	1.0 $^{ab\,A}$	1.2 ^{a}	0.1
	Zn_E	.	.	1.1 ^{A}	1.0 ^{A}	1.3	0.1
	Vit E	0.8 $^{b\,A}$	1.1 ^{a}	.	.	.	0.1
Melanin in abdominal wall	Control	0.9 ^{a}	1.0 ^{a}	0.6 ^{b}	1.0 ^{a}	1.0 ^{a}	0.1
	Zn	1.0 ^{a}	1.0 ^{a}	0.5 ^{b}	1.1 ^{a}	1.0 ^{a}	0.1
	Zn_E	.	.	0.6 ^{b}	1.0 ^{a}	1.1 ^{a}	0.1
	Vit E	1.0	1.0	.	.	.	0.1
Melanin in fillet	Control	.	.	0.0 ^{b}	0.1 ^{ab}	0.3 ^{a}	0.1
	Zn	.	.	0.1	0.1	0.2	0.1
	Zn_E	.	.	0.2	0.1	0.2	0.1
	Vit E	.	.	.	.	.	.
Frequency of melanin in fillet	Control	.	.	0.0	0.1	0.2	0.1
	Zn	.	.	0.2	0.1	0.2	0.1
	Zn_E	.	.	0.2	0.1	0.2	0.1
	Vit E	.	.	.	.	.	.
Adhesions	Control	0.0 $^{c\,B}$	1.4 ^{a}	0.4 ^{c}	1.0 $^{b\,A}$	1.7 ^{a}	0.1
	Zn	0.4 $^{bc\,A}$	1.6 ^{a}	0.1 ^{c}	0.7 $^{b\,B}$	1.7 ^{a}	0.1
	Zn_E	.	.	0.0 ^{c}	0.9 $^{b\,AB}$	1.8 ^{a}	0.1
	Vit E	0.2 $^{b\,AB}$	1.6 ^{a}	.	.	.	0.2
Visceral fat	Control	.	.	1.9 ^{b}	2.5 $^{a\,A}$	2.4 $^{a\,B}$	0.1
	Zn	.	.	1.9 ^{c}	2.3 $^{b\,B}$	2.7 $^{a\,A}$	0.1
	Zn_E	.	.	1.9 ^{c}	2.1 $^{b\,B}$	2.7 $^{a\,A}$	0.1
	Vit E	.	.	.	.	.	.
Liver Color	Control	2.5 $^{b\,A}$	3.1 $^{a\,B}$	3.1 ^{a}	3.0 $^{a\,B}$	2.6 $^{b\,B}$	0.1
	Zn	2.0 $^{c\,B}$	3.7 $^{a\,A}$	3.2 ^{b}	3.3 $^{b\,A}$	3.0 $^{b\,A}$	0.1
	Zn_E	.	.	3.1 ^{a}	3.1 $^{a\,AB}$	2.4 $^{b\,B}$	0.1
	Vit E	2.0 $^{b\,B}$	3.4 $^{a\,A}$	.	.	.	0.2
Fillet color	Control	.	.	21.5 ^{b}	25.7 ^{a}	23.9 ^{a}	0.5
	Zn	.	.	21.8 ^{b}	26.5 ^{a}	25.2 ^{a}	0.6
	Zn_E	.	.	21.3 ^{b}	24.7 ^{a}	24.7 ^{a}	0.6
	Vit E	.	.	.	.	.	.
Gaping	Control	.	.	1.8 ^{a}	0.9 ^{b}	0.5 ^{c}	0.1
	Zn	.	.	1.9 ^{a}	0.8 ^{b}	0.4 ^{c}	0.1
	Zn_E	.	.	1.9 ^{a}	0.8 ^{b}	0.3 ^{c}	0.1
	Vit E	.	.	.	.	.	.

Data are presented as LSmeans and SEM indicates standard error of mean. Lower case super scripts in the table indicates significant difference over time and capital letter super scripts indicates significant difference between dietary treatments ($P<0.05$). The absence of a letter indicates no significant difference.

5. Discussion

The discussion will be presented in two sections: biometric traits and tissue evaluation measured of the Atlantic salmon studied.

5.1 Biometric traits

The condition factor of a fish is calculated from the relationship between the weight of the fish and its length, where a higher value indicates a voluminous fish. The results from the present study showed that the condition factor of the fish dropped during the freshwater phase towards sea transfer, and increased throughout the grow-out period in seawater. The lower condition factor at sea transfer can be explained by the seawater adaptation where the salmon goes through the smoltification period, as also suggested by Folmar & Dickhoff (1980). In the last sampling, vaccinated fish presented a higher condition factor than the unvaccinated fish. A high condition factor can indicate a greater percentage of muscle in the fillet or a high amount of fat located in the viscera. The vaccinated salmon had numerically higher amount of muscle (0.52%) compared with the unvaccinated salmon, whereas the condition factor of the unvaccinated salmon was lower due to longer fish length (0.57cm) and lower amount of viscera (0.89%). This shows that vaccination did not lower the percentage of muscle, which is the most important part of the fish in terms of economic value. The results from the dietary treatments showed that the condition factor increased over time for all diets tested in seawater, whereas the control group presented a higher fillet yield and lower visceral fat compared with the other dietary groups at the last sampling. Hence, the control group presented higher percentage of muscle and the zinc supplementation seemed to stimulate visceral fat accumulation.

Hepato somatic index (HSI) and Cardio somatic index (CSI) represent the ratio of the liver and heart weight compared to the full body weight. The present results showed decreased HSI and CSI values throughout the experiment for both vaccinated and unvaccinated fish. The first sampling in freshwater showed a significant difference where vaccinated fish presented a greater HSI, but over time the values became similar. There was a reduction in HSI and CSI values among the dietary treatments throughout the experiment. The control diet presented a significantly higher value for HSI at the

first sampling, but over time this pattern did not persist and no difference was observed among diets at the last sampling. As presented by Larsson et al. (2014) bigger and paler livers can indicate excess of fat, implicating in metabolic disturbances. The fat of the liver was not measured in this experiment but the liver color had a pattern behavior, with increasing darkness right before seawater transferred and lightening towards the end of the experiment. On the sampling at May 30th and December 12th there was a significant difference showing that unvaccinated fish had darker liver color, which could mean that the numerical higher HSI values presented by vaccinated fish is due to fat accumulation. The Zn diet showed a significant difference compared with the other dietary treatments, where it presented a darker liver color at the second freshwater sample and the last two seawater samples, and a numerical difference at the first seawater sample. However, in seawater phase the HSI of the salmon fed the Zn diet did not differ significantly from the salmon fed the Control diet. This can indicate as supported by Hjeltnes & Julshamn (1992), that the livers from dietary treatments were not overly fat, as all the HSI values presented were within normal range.

5.2 Tissue evaluation

Organ adhesions in fish are seen between internal organs and the abdominal wall. In the present study there was a significant difference between vaccinated and unvaccinated fish, where the vaccinated presented a higher adhesion score at three of the sampling times and a numerical difference at one sample. As showed in previous studies by Poppe (1997), Haugarvoll et al. (2010) and Drangsholt et al (2011) the use of oil-adjuvant vaccines has led to considerable side effects such as extensive adhesions between individual visceral organs or between visceral organs and the body wall caused by an inflammatory response. Dietary treatments had no effect on organ adhesions.

The flesh color of Atlantic salmon is one of its main quality traits (Gormley, 1992) as consumers associate a deep pink color with superior flesh quality (Clydesdale, 1993). In this study, vaccinated and unvaccinated fish presented a low color score at the first color registration in September when compared with those in December and March. This can be explained by the positive correlation presented between body weight of in salmonids and color, where increased body weight will result in an increase in the desirable

coloration of the flesh (Johnston. et al., 2006). The color intensity of vaccinated fish had significantly lighter coloration of 24 on the SalmoFan scale compared with 25.2 of unvaccinated fish. This color difference is considerable, but the lighter color of the vaccinated fish was not below a lower critical level of score 23 in most markets (Mørkøre, 2010). There was no difference in fillet color between the dietary treatments and the effect over time was the same as presented by the unvaccinated fish. The average fillet color did not change significantly from March to December, even though the body weight increased. This could indicate the beginning of a stabilization of the flesh coloration.

Gaping is an undesirable separation of muscle blocks in the raw fillet and it is also an important quality factor in salmon (FAO, 2001). In the present study, both vaccinated and unvaccinated fish showed the same pattern regarding gaping score, with decreasing incidence over time. At the first gaping analyzes in September, there was a significant difference between the two groups, with unvaccinated fish showing more gaping. In December the unvaccinated salmon also tended to have higher gaping ($P = 0.08$), but at the last sampling in March the gaping score was very and similar for vaccinated and unvaccinated fish ($P = 0.92$). This shows that, besides the decreasing of gaping, the vaccinated and unvaccinated fish presented values that became more alike over time. There was no difference between the dietary treatments for gaping. This negative correlation between gaping and body weight is in disagreement with previous study by Love (1979) and Kiessling et al. (2004), where gaping increased with larger body size.

In this study the melanin in organs showed a significant difference between the vaccinated and unvaccinated fish at all the sampling five dates, with vaccinated fish presenting a higher score. According to Koppang et al. (2005), abnormal pigmentation of organs and tissues may be associated with pathological conditions. Granulomas can be formed at the induction site and elsewhere due to the use of mineral oil-adjuvant vaccines. Hence macro components can disseminate from the injection site throughout the body to different organs and tissues, therefore inducing autoimmune reactions of the fish. Consequently, the results seen on dark pigmentation of organs in this study can be explained as a side-effect from the vaccination. Comparing the dietary treatments there was a significant difference at three sampling dates, showing lower degree of melanin

deposition in organs of salmon fed the Control diet. At the first sampling in freshwater, the control differed from the Vit E group, in September the control differed from the Zn_E and in December the control differed from both Zn and Zn_E. It has been documented that these two dietary components (Zinc and Vitamin E) can stimulate immune responses in fish (Kiron *et al.,* 1993. Ispir *et al.,* 2011 and Halver, 2002). The increased melanin deposition of the salmon fed diets supplemented with vitamin E or zinc may therefore reflect an upregulated immune response.

Melanin deposition of the abdominal wall for vaccinated and unvaccinated fish did not show any difference throughout the experiment. However, vaccinated fish presented numerical higher values on every sampling, which is in accordance with a previous study from Koppang et al. (2010), who linked melanization of the abdominal wall to a response to vaccination. The authors based their assumption on their finding of oil content in intraperitoneal granulomas, that was consistent with mineral oil adjuvant used in the vaccine. Fish fed different diets did not present significant difference in amount of melanin in abdominal wall at any sampling.

Melanin in fillet is a major quality issue for salmon. Consumers tend to associate any discoloration of the muscle with lower product quality, which leads to a reduced price or even rejection of the fillets that present melanin spots (Reidar et al., 2007). In this study the incidence of melanin in fillet was documented at the sea water phase, but not in freshwater. A significant difference between vaccinated and unvaccinated fish was observed at the last sampling, where the vaccinated fish presented a higher score. The vaccinated fish also presented a numerical higher value at the sampling in December. While the values of the unvaccinated fish were almost constant throughout the experiment, the vaccinated showed a clear increase on each sampling. The same result was found numerically for the percentage of fish with melanin spots. This shows that vaccination had a negative effect on pigment deposition in the salmon muscle. The relationship between vaccination and increased dark pigmentation of Atlantic salmon flesh has been supported by other studies. According to Koppang et al. (2005), pigmentation of white muscle of farmed salmon has been reported in vaccinated fish from British Columbia, Canada, Scotland and Chile, but not in countries like Tasmania and Australia, where salmon were not subjected to intraperitoneal vaccination and no flesh pigmentation has been found in wild fish. The former authors also stated that the

pigmented changes in the white muscle of vaccinated Atlantic salmon could be classified as a granulomatous inflammatory condition, similar to that of foreign-body type, and the absence of known pathogens or other explanations leaves intraperitoneal vaccination followed by a foreign body reaction as the most probable cause for these colorational changes. Similarly, Larsen et al. (2012) associated dark staining of salmon skeletal muscle to immune and pigmentary systems of the fish, concluding that the pigment-producing granulomas are an inflammatory reaction. The vaccine strategy is one of the factors that could contribute to these reactions by increasing melanin formation in internal organs and muscles, with an incorrect hitch at vaccination, resulting on melanin spots under the peritoneum (Norsk Fiskeoppdrett, 2008). However, in this study the rate of melanin appear to increase with the size of the fish. As suggested by Mørkøre (2012) this is interesting as it indicates that melanin deposition in salmon fillets is not a phenomenon that can be associated only with vaccination or vaccine type, but that the problem can also occur later in the fish's life, and possibly worsen with time. The increase of melanin with size of vaccinated fish showed a similar pattern as organ adhesions in sea water, while unvaccinated fish values continued constant. The higher scores for organ adhesions at the sampling before sea transfer (May 30th) may be due to a personnel factor, as the evaluation was done by the same person throughout the whole experiment, except at this particular sampling date. In contrast with melanin deposition in organs, the different dietary treatments had no significant effect on dark staining of the fillets or organ adhesion. The difference in development, and also regarding vaccine and dietary effects, indicates that organ adhesions and melanin deposition in organs, abdominal wall and fillet may have different underlying causes.

The location of melanin spots for both vaccinated and unvaccinated fish was similar. The pigmentation appeared mostly superficially on the anterior part of the belly (B1), having only on one date presented a higher value for the posterior part of the belly (B2), and only for unvaccinated fish. No dark pigmentation was found on the dorsal part of the fillet. These results are in agreement with Mørkøre (2012), who reported that most of the dark spots in salmon fillets are found in the anterior part of the abdomen, and rarely seen in the dorsal fillet part.

6. Conclusion

The present study demonstrated significant variation in biometric traits and quality parameters for vaccinated and unvaccinated Atlantic salmon.

Melanin in the abdominal wall was found in freshwater before vaccination. Organ adhesions were observed after vaccination in freshwater, while dark pigmentation appeared only in seawater. Melanin depositions in organs appeared after vaccination.

The vaccinated fish presented a higher condition factor at the end of the experiment, with more muscle (0.52%), more viscera (0.89%) and shorter fish length (0.57cm). Vaccination of the salmon did not have a negative impact on the amount of skeletal muscle present in the fish. Vaccination seemed to influence fat accumulation in liver, as they were periodically lighter and larger numerically.

Melanin deposition in organs and organ adhesions were found as a side effect after vaccination. The vaccination of the salmon also increased the amount of melanin deposition in the salmon muscle.

The dietary treatments did not affect positively the condition factor of the salmon as the group with more muscle was the Control and the Zn diet seemed to induce visceral fat accumulation.

The diets with zinc and vitamin E supplementation have increased the immune response from the salmon increasing melanin deposition in organs and did not seem to increase the fat in the liver.

Dietary treatments had no effect on organ adhesions, gaping and melanin in abdominal wall.

The size of the fish was also a factor that contributed to increase muscle pigmentation, as more melanin spots was found on larger fish.

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8. Appendix

8.1 Instruks Fôrcoating til Laks

Förtype: Nutra Olympic 3.0 (Skretting) fra storsekk, market ‹‹Mørke Flekker››.

Prosedyre 1: 25 kg fôr tilføres sementblander. Under omrøring tilsettes 600 mL vann.

Prosedyre 2: Fôr samles opp fra plastdekke til sementblander. Under omrøring tilsettes 250 mL rapsolje.

Prosedyre 3: Fôr samles opp fra plastdekke til fôrsekker.

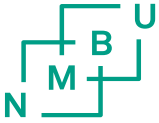
Dag	**KONTROLL**	**VIT. E**	**ZINK**
1	Prosedyre 1	Prosedyre 1	Prosedyre 1 +11,2g sinksulfat
Fôr legges på plastdekke over natten			
2	Prosedyre 2	Prosedyre 2 + 15,45g Vitamin E	Prosedyre 2
Fôr legges på plastdekke over natten			
3	Prosedyre 3	Prosedyre 3	Prosedyre 3
Samle fôr i fôrsekker			

8.2 Vaksinasjonskontroll: Nofima Rapport

Settefiskanlegg	**Informasjon**
Oppdrettsselskap	Nofima AS
Lokalitetsnavn	Sunndalsøra
Kontaktperson	Per Brunsvik, Valeria Ivanova
Adresse lokalitet	Sjølseng, 6600 Sunndalsøra
Telefon lokalitet	64 97 01 00
E-mail	valeria.ivanova@nofima.no
Medlem av gruppering	Ingen
Antall smolt som produseres	Konsesjon på 480 000
Ansvarlig for oppfølging MSDAH	Olaf Skjærvik
Personer tilstede ved vaksinasjonskontrollen	Valeria Ivanova
Dato vaksinasjonbesøk	04.04.13
Vaksinasjonsperiode	04.04.13 – 05.05.13
Fiskehelsetjeneste	Fiske-Liv, Cecilie Skjengen
Fiskehelsetjeneste tilstede ifm vaksinering	Nei
Dato utsett	Satt til uke 22- 2013
Mottaker av fisken	Nofima Averøy
Vaksine og vaksinasjonsregime	
Vaksinetype	Norvax Minova 6
Batch nr vaksine	C329A02
Utløpsdato	10-2013
Vaksinerer anlegget mot PD?	-
*Dato vaksinasjon PD komponent	-
*Dato vaksinasjon kombinasjonsvaksine	-
Kombinasjonsvaksine	-
Lagringssted	Kjøleskap
Lagringstemperatur	+ 4 grader celsius.
Om fisken	
Antall fisk som vaksineres	Iht til forsøksoppsett, ca 4000
Art, stamme og generasjon	AquaGen, Ikke oppgitt.
Sortering	Fisken var usortert.
Dager siden siste sortering	-
Snitt, min og max vekt (oppgitt av anlegget)	Oppgitt til 54 gram, maks, min ble ikke oppgitt.
Dager med faste	3-4 dager.
Helsestatus og helsehistorikk	Ingen tidligere sykdomshistorikk.
Dato for siste helsebesøk	20.03.13
Smoltifisering	Nei
Supersmolt	Nei
Fiskens generelle tilstand (finneslitasje etc)	Det var noe gjelleforkortning på fisken.
Fra kar nr (fisk som undersøkes)	I henhold til forsøksoppsett.
Til kar nr (fisk som undersøkes)	I henhold til forsøksoppsett.

Miljøforhold, fôr og vannkvalitet					
Type anlegg (gjennomstrømning / resirkulering/merdanlegg etc)			Gjennomstrømning.		
Vannkilde, vannforhold			Grunnvann.		
Sjøvannsinnblanding hvis gjennomstrømming			Nei.		
Evt. system for desinfisering av sjøvann			-		
Type filter hvis resirkulering			-		
Vanntemperatur			5,4 grader Celsius		
Luftemperatur			Ca 2 grader Celsius		
pH			6,9		
Beskrivelse av lysregime			12/12 vaksineres til 24/0		
Sulting, antall dager (døgngrader)			Sultet fra og med 02..04.13		
Fôrleverandør			Skretting		
Helsefôr før vaksinering (ja/nei/ evt. type)	Ja -		Nei -		Iht Forsøksoppsett
Helsefôr etter vaksinering (ja/nei/ evt. type)	Ja -		Nei -		
Fôringsregime frem mot utsett			Ikke oppgitt		
Evt. annet om miljøforhold, fôr og vannkvalitet			-		
Metodikk, utstyr og prosedyrer					
Har anlegget egne skriftlige prosedyrer (SOP) for vaksinasjonsprosessen som følges (ja, nei, evt. beskriv)	Ja X		Nei		
Gode rutiner for bestilling av vaksine, team/mannskap, bedøvelsesmiddel, helseundersøkelse, opplæring av uerfarent personell etc.			Ja.		
Beskrivelse av vaksinasjonsmetode (maskin, manuell, egne folk, team)			Fisken ble vaksinert manuelt av egne folk.		
Forsøksleder			Valeria Ivanova		
Hastighet på vaksineringen			-		
Transport av fisken fra kar til ventekar (pumpetype, lengde på slange)			Fisken ble håvet.		
Ventekar (volum, vannutskifting og oksygenering, oppholdstid, temperatur)			Ca 800 l		
Overføring til bedøvelseskar (mekanisk, manuell)			Fisken ble håvet.		
Bedøvelsesmiddel (type, holdbarhet)			Finquel 09/2014		
Vannkvalitet i bedøvelseskar (klart/grumset)			Klart vann.		
Skifte av bedøvelsesvann (rutiner)			Etter ca 500 fisk.		
Oksygenering i bedøvelseskar			Nei		
Måles oksygen i bedøvelseskar			Nei		
Temperatur i bedøvelsesvann			5,4 grader Celsius		
Innsovningstid (måles)			Ca 75 sek		
Oppholdstid i bedøvelsesvann (måles)			Ca 2 min		

Overføring til vaksinasjonsbord (manuell, mekanisk)	Fisken ble håvet.	
Skylling av fisk før vaksinasjonsbord (ja/nei)	Nei	
Ca. oppholdstid på vaksinasjonsbordet (måles)	Ca 1 min.	
Rennende vann på vaksinasjonsbordet (ja/nei)	Ja	Nei X
Injeksjonsutstyr	Socorex	
Kanyletype, lengde og diameter	Belanox, 0,6 * 5 mm	
Hvordan bestemmes kanylelengde i praksis?	Sjekkes ved å stikke igjennom bukvegg og åpne fisk.	
Er fisken godt nok bedøvet og ligger rolig på vaksinasjonsbordet	Ja X	Nei
System for transport av fisk ut til karet etter injeksjon (pumpe, fritt fall, lengde på transport)	Fisken ble håvet, fra et oppvåkningskar.	
Er fisken våken når den kommer ut i karet eller synker den ned i karet	Ja X	Nei
Prosedyrer for skifte av kanyler	Ja X	Nei
Håndtering og oppbevaring av vaksinen	Oppbevart i kjøleskap, temperert før bruk, det ble brukt feil slange ved injesjon slik at det oppstod vakuum i vaksineflaska.	
Hygieneprosedyrer bord, slanger og injektorer	Få antall fisk, det ble brukt sprit til å desifisere saks ved klipping.	
Nye personer i teamet (fått opplæring)?	Ja -	Nei -
Observasjon av vaksine i karet etter vaksinering	Ja	Nei X
Dødelighet/utgang så langt i vaksineringen	Vaksinering i kun to dager.	
Blir fisken utsatt for unødig stress, håndtering evt. risiko for ytre skade gjennom prosedyren (skarpe kanter, trenging, havner på risten på bunnen av oppvåkningskar etc)?	Fisken ble håntert mye ved håving. Fisken ble håvet 4 ganger før den var tilbake i karet. Dette kunne kanskje vært redusert dersom man kunne vaksinert direkte i karet fisken skulle gå i.	
Hvor ofte kontrolleres stikkpunkt og blødning fra stikk-kanalen?	Gjøres ikke.	
Hvor ofte åpnes fisk for å sjekke at vaksinen er riktig deponert	Gjøres ikke.	
Rapport og oppfølging		
Dato for neste planlagte besøk	Før sjøsetting.	
Rapport sendes til følgende mottakere	Turid Mørkøre, Tore Hovland, Valeria Ivanova.	
Er det avtalt videre oppfølgingsplan frem til slakt?	Etter avtale.	



Norwegian University
of Life Sciences

Postboks 5003
NO-1432 Ås, Norway
+47 67 23 00 00
www.nmbu.no