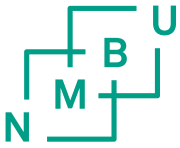
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Fillet quality and health of vaccinated or unvaccinated Atlantic salmon (*Salmo salar* L)

A Master thesis

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ABSTRACT

The prime goal of the present study was to investigate the impacts of vaccine on biometric traits, melanization of tissues and fillet quality of Atlantic salmon (*Salmo salar* L). In the experiment, a total number of 420 salmon, (1+ spring smolt), with an average body weight of 2548 ± 2 g (mean ± standard error) were randomly distributed in three same sized net pens (125 m^3; 100 vaccinated and 40 seawater injected salmon in each net pen). The experimental period was from 5th June to 19th August 2014. The salmon were vaccinated or seawater injected on 4th April, 2013 when body weight was minimum 35 g. The fish were fed a standard commercial feed. Harvesting was performed according to standard procedures or after crowding. There were demonstrated variations in the biometric and quality parameters of the vaccinated and unvaccinated salmon. The condition factor ($P = 0.0014$), fillet yield ($P = 0.0227$), organ adhesions ($P < 0.0001$), fat in viscera ($P = 0.0073$) and fat in fillet ($P = 0.0248$) were significantly higher in the vaccinated salmon compared with the unvaccinated salmon. On contrary, the flesh texture of posterior part of the fillet was significantly softer ($P = 0.0006$). Melanin spots of fillets showed no significant difference between the vaccinated and unvaccinated salmon, but melanin of the abdominal wall and organs were significantly higher of the vaccinated salmon ($P = 0.0169$ and $P < 0.0001$ respectively). In conclusion, the vaccine had significant effects on the condition factor, fillet yield and fat in fillet and had no significant effects on the melanin spots in fillet of the salmon in the experiment.

Key words: Atlantic salmon, vaccine, stress, quality parameters, health.

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1. INTRODUCTION

Atlantic salmon (*Salmo salar* L) is the major economical important species in salmonid families. Norway, Chile, UK, Canada are the major countries for salmon production in world. Among them, Norway ranks at top to produce and export of Atlantic salmons (NSC, 2014). Norway's long coastline and cold, fresh seawater provides excellent conditions for aquaculture activities of salmonids. But for the disease issue, farmers are losing a large amount of fish and income and Norway losing huge amounts of foreign exchanges. Atlantic farmed salmon supply being tighter than expected in 2015 in Norway, as smaller sizes harvested due to sea- lice pressure and disease issues, said the investment bank Nordea.

A successful salmon culture depends on various factors such as fries quality, water and soil quality, culture methods, vaccination and feed quality and regime, operation management etc. Although from the starting of modernized culture methods of Atlantic salmons in 1980 in Norway, every year farmers trying to improve production technologies, but still now, many farms are affecting by various diseases and quite treatments are not possible. Although all farms in Norway using vaccines to prevent diseases, but there have debates to use the safe and effective vaccine. In a survey it was reported that in 2002, 160 million doses vaccines used in the salmon industry (Sommerset et. al. 2005). Despite significant improvement was obtained in the control of infection by vaccination, approximate 10 % of stocked fish still die during the production period (Directorate of Fisheries, 2010).

Vaccination has been successfully applied to combat various fish pathogens (e. g. *Listonella anguillarum*, *Aeromonas salmonicida*,and *Yersenia ruckeri*). Commercial application of vaccines in the salmonid culture industry has resulted, not only in significant reductions in mortalities and disease–associated financial loss to the industry, but also substantial declines in the use of antibiotics (GESAMP, 1996). Nevertheless, while the vaccination represents a major advance in the control of specific diseases, treatments may be stressful and cause detectable side – effects in cultured fish and other animals (e. g., Dohoo and Montgomery, 1996). The factors influence development of side effects of vaccines: adjuvants, antigens, formation of vaccine, dose volume, photo period, temperature, size of fish, hygiene.

Vaccination has also been associated with muscle inflammation and melanin accumulation (Koppang et al., 1998a), granulomatous uveitis (Koppang et al., 1998b), and systemic autoimmunity (Haugarvoll et al., 2010). Intra-muscular melanin deposits are a major

problem in Atlantic salmon farming. In farmed salmon, typically 10 – 20 % of fish display pigmented muscle spots in the fillet (Mørkøre & Heia, 2012). The fillets with melanin deposition are normally discarded from process to sell due to overall quality degradation of the product. The consumer willingness to pay depends on the quality of the products (Alfnes, et al. 2006).

Vaccine induced side- effects and various- diseases lowering quality of salmon fillets. Salmon producing farms and processing industries deducts degraded fillets from selling to market. So for improving of farming of Atlantic salmon, it is essential to manage a high production efficiency and at the same time ability to produce and control the fillet quality according to market demands. The consumer's preferences and interests are always of primary importance for aquaculturists. Texture, colour and fat content of fish fillets are the major parameters that determine the satisfactoriness of the consumer (Haard 1992). Fillets with quality deviations such as gaping, soft flesh, dark spots (melanin), pale and irregular colour and deformities are the main causes for down-grading of the quality of farmed salmon, and hence also economic loss to the industry (Koteng 1992).

Feed nutrition and regime also affect on salmon production and fillet composition. Feed companies usually supply to farmers with expected amounts of feed under different water temperatures and fish sizes. The responsiveness to food varies with the time of day and season (Smith et. al. 1993) and it may be manipulated using artificial photoperiods (Oppedal et. al. 2003). In salmon culture, the feed delivery rate should be taken into account when calculating a feeding regime.

Although there have some experiments on the impacts of vaccines on the quality of salmonids (Larsen et. al. 2014, Berg et al. 2012, Drangsholt 2011, Koppang et al. 2005, Poppe and Breck 1997, Midtlyng et. al. 1996); it is insufficient to invent more tactful, safe and effective vaccine and to find out all adverse effects in Atlantic salmon.

The main goal of the present study was to investigate the impacts of vaccine on biometric traits, melanization of tissues and fillet quality of Atlantic salmon (*Salmo salar* L). The focuses were on the effects of vaccine on the different biometric (condition factor, fillet yield) and quality traits (fillet colour, pH, gaping, fat and texture). The work was also focused to investigate the impacts of vaccine on the melanization (melanin deposit in abdominal wall, abdominal organs and skeletal muscles).

2. THEORETICAL BACKGROUND

2. 1. Water temperature

The water temperature is an important physiological parameter for salmon culture. They normally display best appetite and growth around their temperature optimums. As water temperatures increase, a number of negative effects in salmon may arise. In freshwater, direct biological impacts in salmon include physiological stress, increased depletion of energy reserves, increased susceptibility and exposure to disease and disruptions to breeding efforts. Again in sea water, it has been suggested that many of the food webs of which salmon are a part will be disrupted by the change of temperature. For example, planktonic blooms which are related to climatic factors could cause a scarcity of food at a critical stage of the salmon's life cycle. Warmer ocean temperatures have been shown, in certain areas, to reduce the abundance of other smaller fish into these newly warmed areas. These two factors, when coupled together, could cause a significant rise in the predation pressure on salmon.

A preferred temperature condition in sea cages is about 17°C was suggested by Johansson et. al. (2009), which correspond well with the finding that the Atlantic salmons' selected temperature in a horizontal temperature gradient increased with acclimation (5 – 20°C), showing a final preference at about 17°C (Javaid & Anderson, 1967). In the available range between 11 and 20°C, caged Atlantic salmon individuals and groups clearly avoided water warmer than 18°C as well as water colder than 12°C (Oppedal et. al. 2011a).

2.2. Dissolved Oxygen

The Dissolved Oxygen (DO) is the oxygen that is dissolved in water. It gets there by diffusion from the surrounding air, aeration of water that has tumbled over falls and rapids and as a waste product of photosynthesis. An over simplified formula is given below:

Photosynthesis (in the presence of light and chlorophyll):

Carbon dioxide	+	**Water**	→	**Oxygen**	+	**Carbon-rich foods**
CO_2		**H_2O**		**O_2**		**$C_6H_{12}O_6$**

Oxygen levels are currently declining in oceans and coastal waters around the world in part due to climate change. Warmer surface water absorbs oxygen less easily, and restricts

natural mixing with deeper, colder waters. Warmer water also encourages growth of phytoplankton which uses up oxygen in deep water as they sink and are consumed by bacteria. The core problem is that fish need more oxygen as the water temperature increases. However, as the water temperature increases, the available oxygen and its solubility in water decrease. In salmon pens, soluble oxygen can be adversely affected by the salinity or other factors such as the number of fish, the season, seaweed blooms and so on. When dissolved oxygen is low, it means there's less oxygen available in the water to support aquatic life. Fish show less resistance to disease and lower reproduction rates in hypoxia. If oxygen levels get too low, fish and other animals may die - sometimes resulting in widespread "fish kills."

Stevens et. al. (1998) found that the routine oxygen uptake of juvenile Atlantic salmon in freshwater at 12 – 13°C was not limited by water oxygen saturations above 38 %. This is confirmed from recent studies in sea water (reviewed in Oppedal et al. 2011a) has showed that at 18, 12 and 6°C 400 g salmon post - smolt are not able to maintain routine metabolic rates below approximately 60 %, 40 % and 30 % saturation, respectively. Below these thresholds mortality will commence in farmed salmon if oxygen levels are not improved. The difference between the routine and the maximum metabolic rate (the maximum theoretically possible oxygen uptake under the present conditions) acts as a buffer against factors such as stress, disease and feeding, which narrow this metabolic scope (e.g. Priede 2002). Salmon will therefore migrate vertically in sea cages to avoid hypoxic zones. A summary from several hypoxia trials (WEALTH, 2008) concluded that the immune responses are reduced at levels below 55 % oxygen saturation.

2.3. Stocking density and stress

The stocking density is defined as the total biomass of the fish divided by the sea cage volume, is typically used by authorities to set upper limits for what is allowed in sea cages (e.g. 25 kg m^{-3} in Norway).

Low stocking density has many good effects on salmon growth, water ecology and environment. When stocking densities are low, oxygen in the seabed can break down nutrients more quickly and can easily deal with the organic enrichment in a farm. Low stocking density also allows better oxygen flow through net pen and ensures the water quality is maintained giving fishes a clean, healthy environment that allows them to thrive. When salmon are relaxed and allowed to behave normally in a clean, healthy environment,

they feed better, they grow quicker and when it comes to harvest time, the benefit is seen in the quality of fishes.

Despite its frequent use as a production parameter there are relatively few studies on how different stocking densities affect on salmon in sea cages. Adams et al. (2007) found negative effects on welfare for a stocking density of 35 kg m^{-3} compared with 25 kg m^{-3} and Oppedal et al. (2011b) found reduction of feed intake, growth rate, feed utilization and creation of a greater number of cataracts when the stocking density exceeded 26.5 kg m^{-3}.

Over stocking density also creates stress to farmed fishes. Stress affects the congenital immune defense system of salmon. Stress effects on blood plasma parameters levels (eg. aspartate aminotransferase, cortisol, chloride, glucose, sodium, total bilirubin). Koestan Gadan (2012) discovered that these stress factors lead to increased production of the stress hormone cortisol in the fish. This increment in the level of cortisol affects the immune system of the fish and weaker immunity makes them more susceptible to infections. The fact that stress can trigger an outbreak of Infectious Pancreatic Necrosis (IPN) disease which can increase the mortality rate and lower production of salmon.

2.4. Feed

The feed and feeding strategies aim for growing a healthy fish fast at the lowest possible cost. Standard feeds are designed to give the lowest possible production cost. Premium diets are available in most countries and are being used in certain situations where extra growth rate is profitable. Feeding control systems shall prevent feed waste and assure that the fish get enough feed to grow to its potential. Normally the fastest growing fish show the lowest feed conversion ratio.

During the industry's early phases, salmon feed was moist (high water content) with high levels of marine protein (60 %) and low levels of fat oil (10 %). In the 1990s, the feed typically consisted of 45 % protein, where most of it was marine protein. Today, the marine protein level is lower due to cost optimization and fish meal availability. However, the most interesting development has been happened in the inclusion of fat. This has been possible through the technological development and extruded feed.

The feed intake and feeding behaviour are generally considered to be the reliable criteria for evaluation of health and welfare of farmed fish (Jobling et. al., 2001). Salmon farmers use many different sizes of feed pellets during the grow-out period, and each time the size is changed, new calculations for the optimal number of pellets per fish and delivery must be

done. It would therefore save both time and money if fewer sizes of pelleted feed can be used for larger parts of the grow-out period. The size of the feed pellets and the rate at which they are delivered may affect the amount of feed an individual fish can ingest over a period of time. Pellets of sub-optimal size or pellets that are delivered at a high rate may cause wastage, as fish may be unable to catch large numbers of pellets before they sink through the net pen.

High production efficiency is essential in intensive aquaculture production and defining optimal feeding strategies is receiving considerable attention to the fish farmers. There is limited knowledge on the growth response in fish subjected to cyclic feeding; i.e. when feeding is restricted within a week. Production efficiency, health and quality of the fish can be vary within the same production conditions depending on the genetic origin of the fish (Thodesen & Gjedrem, 2006).

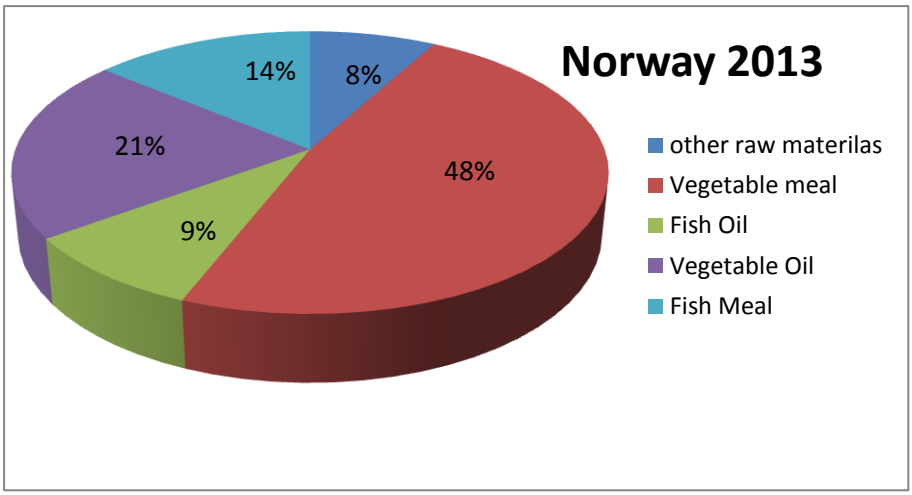


Fig. 2.1. Feed ingredients used in Atlantic salmon farms in 2013 (source: Salmon farming industry handbook, 2014 Marin Harvest ASA).

Previous studies revised the interaction between the feeding biology and feeding regimes in cage reared Atlantic salmon and the possibility of manipulating the feeding intensity in order to reduce costs of the production (Juell et. al. 1994). Manipulation of the feeding regimes through controlled timing and frequency of feed delivering is a way of influencing a number of traits that are of commercial importance. At present farmers are using different feeding strategies, as results on optimal feeding regimes are inconsistent. Feeding a restricted meal sizes can cause competition among fish upon re-feeding and may lead to

increased variability in growth (Jobling et. al. 1995). Feeding to satiation, on the other hand, could neutralize unwanted feeding inequality.

2.5. Vaccination

2.5.1. Vaccine

The vaccine is defined as a preparation of antigen which derived from a specific pathogenic organism that is rendered non-pathogenic, acting as a preventive measure against future diseases. It stimulates the immune system of the organism and increases the resistance to disease. The vaccine can be two types: water or oil based. The oil provides adjuvant qualities increase the effectiveness of the vaccine and duration of the protection. Vaccine can be applied in three ways: orally, with immersion or injection to the fish. Anesthesia is needed for the injection vaccination, since it decreases the stress in 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, where the deviation in the point of injection should not exceed 0.1 %. Injection vaccination has some advantages that make it a preferred method. In fact, it provides a long duration of the protection.

2.5.2. Vaccine invention, development and success against diseases

The Colorado Company, Wildlife Vaccines with Guy Tebbit, John Rohovec and Thomas Goodrich as experts, was the first manufacturer with a licensed fish vaccine. The company produced bacterins for the domestic and international growth market. In case of Europe, the rapid growth of fish production induced to invent and market modern vaccines.

During the early years of aquaculture major viral diseases included Infectious Pancreatic Necrosis (IPN), Viral Haemorrhagic Septicemia (VHS) and Infectious Hematopoietic Necrosis (IHN). For the last two viral diseases, biosecurity has mainly been based on eradication of diseased populations, and research on vaccination was not prioritized. The first successful experiment on vaccination against these diseases included live vaccines, either avirulent or attenuated strains (Hill B. J. et. al. 1980). The live vaccines provided acceptable or even good protection under experimental conditions, but safety considerations stopped further work. Some of the vaccines showed residual virulence to groups of

vaccinated fish at a level which was unacceptable. The safety concern for other fish species in the aquatic environment also contributed to reduced research on vaccines which could be used in commercial operations. Inactivated viral vaccines for fish have provided some effect, especially under experimental conditions. However, the protective immunity in the field by inactivated vaccines has been relatively low compared with the protection achieved by most of the bacterial vaccines. Consequently, the aquaculture industry has not been satisfied with the efficacy (Biering E. et al. 2005).

Table 2. Some common diseases in salmon culture in Norway

Diseases	Causative agents
Infectious Salmon Anaemia (ISA)	ISA Virus
Cold water vibriosis	*Vibrio salmonicida*
Furunculosis	*Aeromonas salmonicida*
Infectious Pancreatic Necrosis (IPN)	IPN Virus
Infectious Haematopoietic Necrosis (IHN)	IHN virus
Bacterial Kidney Disease (BKD)	*Renibacterium salmoninarum*
Pancreatic Disease (PD)	PD virus
Enteric red mouth disease	*Yersinia ruckeri*

In the eighties, a new costly disease initially named "Hitra disease" appeared in salmonid aquaculture in Norway. There was some dispute about the etiology of the disease. It was soon concluded that the disease was an infectious disease caused by a new pathogenic bacterium, *Vibrio salmonicida* (Egidius E. et. al. 1986). Most of Atlantic salmon and rainbow trout in Norway have been vaccinated via injection against this disease which has termed as Cold water vibriosis.

The great challenge for disease prevention in salmonids was Furunculosis caused by *A. salmonicida*. Based on the positive experience with prevention of *Vibrio*- infections using immersion vaccines, there were great expectations for similar effects with a Furunculosis vaccine. However, immersion of Furunculosis bacterins was found to give insufficient protection. Injection of simple whole - cell culture bacterins stimulated a protective immunity, but the magnitude and duration were less than desired. Bacterins produced with

antigens from *Vibrio anguillarum*, *V. salmonicida* and *A. salmonicida* and added mineral oil adjuvants contributed to effective control of diseases which without immunoprophylaxis would have caused great losses to the industry.

Fish vaccinology has shown an amazing development in recent years. Most of the products are first generation vaccines, but a comprehensive scientific production and valuable practical experience are an excellent basis for the development of improved products which will contribute to environmental, social and economical sustainability in global aquaculture. The impact of vaccination to the success of Norwegian aquaculture was expressed by a senior in the Norwegian aquaculture industry, Professor Trygve Gjedrem, as follows: "The industry might have survived with the economic losses due to high mortality, but it could not survive with the negative effects of high use of antibiotics". Vaccination was consequently one of the factors contributing to the development of the salmonid aquaculture industry (Gudding R. et. al. 2014). The low figures for use of antibiotics in Norwegian aquaculture represent a documentation of a success story in the history of vaccinology (Fig. 2.2).

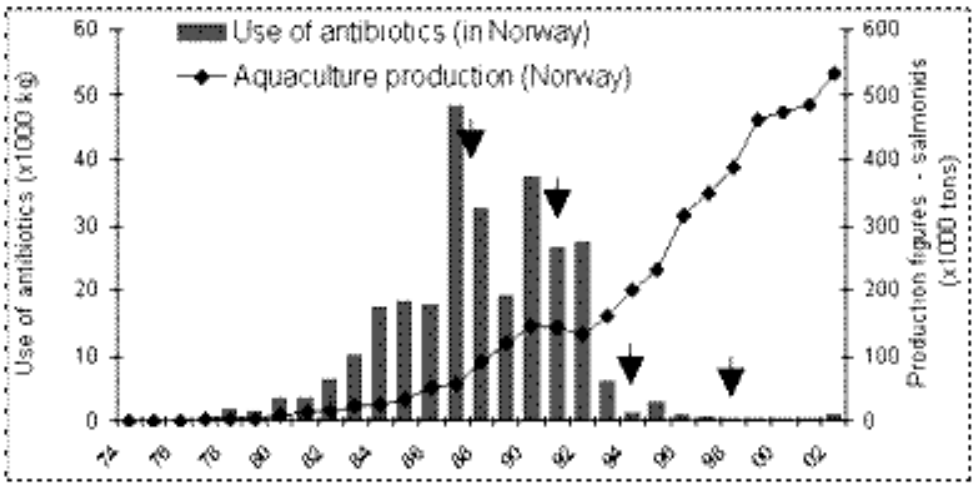


Fig. 2.2. Introduction of 4 generations of vaccine against different bacterial diseases and their impacts on antibiotic usage and productivity in the salmon industry in Norway from 1980 – 2002. (Source: Seafood Norway Report 2003)

2.5.3. Side effects of vaccines

However, it turned out that the vaccines caused some side effects in salmon in different ways. The fish welfare and health are depending on the handling procedures prior to implementing restricted feeding, for example, vaccination procedures. In a research done

by Poppe and Koppang (2014) it was concluded that the acute side - effects of the vaccination can be divided into those resulting from poor handling, anesthesia, contamination of the vaccine, and genuine side-effects are caused by the vaccine itself. Currently, the side - effect profile of the vaccine determines the choice of the vaccine. The vaccine side - effects are mostly being scored based on the rough ordinal scales for vaccine-induced abdominal lesions (adhesions) (Midtlyng et. al. 1996). Earlier studies of injection of oil- adjuvant vaccine side effects have shown that they lead to appearance of adhesions between internal organs that are persistent throughout the production cycles and dependent on time of vaccination and temperature (Berg et. al. 2007; Grini et. al. 2011).

Salmon injected with oil- based vaccines can reduce growth rate and size of fish, loss of appetite. 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 the 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. It has been shown that the vaccination date, temperature by vaccination, size at vaccination and vaccine type has affected the degree of vertebral deformation.

Vaccination can induce reactions in the abdominal cavity. All vaccinated fish get inflammation on the injection spot and also adhesions frequently seen - is 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.

There can be also affects on contents of fatty acids in liver or fleshes in the vaccinated fishes. So it is an important arising issue for vaccination. As essential fatty acids or ω- 3 fatty acids derived products of fishes are very demandable in market for human health.

Furthermore, vaccination may be associated with abnormal pigmentation in the fish tissues and organs (Koppang et. al. 2005). 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 pigmentation 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.

Fig. 2.3. A comparative picture between a healthy fillet and a melanin deposited fillet of Atlantic salmon.

2.6. Growth

Most salmon production in Norway is farmed. Farming takes place in large nets in sheltered waters such as fjords or bays. In Norway, all farmed Atlantic salmon smolts are vaccinated prior to seawater transfer. In recent years, smolts are also being produced for out- of-season (0+) seawater transfer in autumn (August – October), using artificial photoperiod and temperature regimes. These smolts are usually vaccinated only a few weeks prior to seawater transfer (Eggset et. al. 1999). Thus, vaccination is often performed after the parr-smolt transformation has initiated. This accelerated production used for 0+ smolt might induce physiological, endocrine or immunological changes during parr - smolt transformation, which might differ from traditional smolt both in timing and strength of responses. The complex oil- adjuvant vaccines, might also affect the parr - smolt transformation. The use of oil- adjuvanted vaccine close to the start of smoltification has been shown to disturb the smoltification process and cause a delay of approximately two weeks. (Eggset et. al. 1999)

There are conflicting reports on the effect of vaccination on fish growth. Oil adjuvant vaccines have been reported to result in either enhanced growth of fish after vaccination (Ackerman et. al. 2000), no effect on fish growth (Pylkko et. al. 2000) or reduced growth (Melingen, 2001). The contradictory results reported most likely descent from use of different vaccine formulations and use of different protocols to evaluate the effect on feed intake and growth of fish after vaccination.

2.7. Early maturation

Early sexual maturation is detrimental for salmon production, where artificial photo-regimes are used to prevent maturation. Sexually mature parr, precocious males, can be present at sea transfer and their presence is linked to increased mortality (Aunsmo et. al. 2008a). The energy expended for maturation and spawning increases with fish size and females also expend more energy on gonads compared with males (ca 28 % vs. ca 4 % of total energy reserves) (Fleming, 1998). Mature salmon in sea cages to some extent experience osmoregulatory challenges. Besides the energy draining effects of maturation, it has been shown that compared with immature fish mature salmon have a higher prevalence of the parasite *Kudoa thyrsites*, that is a cause of post mortem soft flesh (St-Hilaire et. al. 1998).

In commercial farming, Atlantic salmon has been shown to mature at an early stage in freshwater (parr maturation, Rowe & Thorpe 1990), first autumn in sea (jack maturation, Duncan et. al., 2002), second autumn in sea (grilse maturation, Duston & Saunders et. al. 1999) or in the autumn after two or more sea winters (Duston & Saunders et. al. 1999). The process of initiation of sexual maturation of fish seems to depend on different stimuli gained from both internal factors like age and state of energy reserves (Taranger et. al. 2010) and external factors like photoperiod and abundance of feed (Fjelldal et. al. 2011).

Early sexual maturation in farmed Atlantic salmon results in reduced growth rates and reductions in farm productivity and profitability. Flesh from early maturing fish is of a significantly poorer quality ("downgraded"), which results in considerable losses in market value and farm revenue. Environmental conditions, such as water temperature and day length are known to influence maturation in salmonids. In addition to reduced feed intake and weight gain (Kadri et. al. 19996), sexual maturation in farmed salmon leads to economical losses by downgrading of the fish when slaughtered caused by changes in external characteristics and reduced muscle quality (Aksnes et. al. 1986).

2.8. Fillet colour and appearance

As the Atlantic salmon industry has expanded, meat quality traits have become of increased interest to producers. The quality of flesh depends on factors such as the genetic make - up, age, physical condition, environment, and pre- and post- mortem handling of the fish. In particular, the stress caused during their harvest, transport and slaughter has an important effect on its quality (Gatica et. al. 2008). A study reported that that the fish quality can be affected by a variety of extrinsic factors such as freshness, pre- and post- slaughter handling procedures (Johnston, 1999). The most important intrinsic quality traits are the colour, texture, processing characteristics, fat content, and chemical composition of the fillet (Periago et. al. 2005).

The fillet colour is considered to be an indicator of salmon freshness and quality and processors and retailers will downgrade or even reject product with insufficient colour (Nickell and Springate, 2001). For salmon, consumers prefer a deep pink colour with superior flesh quality. The colour of salmonid flesh results from the deposition of naturally occurring carotenoid pigments present in the diet. Astaxanthin (3-, 3′dihydroxy- β, β- carotene- 4, 4′- dione) is the predominant carotenoid in the muscle of wild Atlantic salmon (Nickell and Springate, 2001). The feeds of farmed salmon are supplemented with astaxanthin and canthaxanthin (β, β- carotene- 4, 4′- dione), only around 10 – 18 % of which is retained in the flesh (Nickell and Bromage, 1998). Another study found that depending on the feeding ration level (0.6 % or 1.2 % of body weight per day); the apparent digestibility of astaxanthin changes from as low as 14.5 % to considerably higher 38 % (Rørvik et. al. 2010).

The carotenoid component of feed represents 6 – 8 % of typical total production costs. Thus colour is one of the most economically important flesh quality traits based on the cost to producers and consumer preference. During maturation of salmon carotenoids migrate from the muscle into the gonads and skin, resulting in a negative correlation between gonadosomatic index and flesh colour (Aksnes et. al. 1986). Paternity analysis of pigment concentration and flesh colour indicates low to medium heritabilities with and only a poor genetic correlation between carotenoid content and perceived colorimetric traits (Norris and Cunningham, 2004).

2.9. Flesh texture and gaping

Softening of the muscle of Atlantic salmon is a quality deterioration that makes the fish unattractive, and depending on its degree may make it unsuitable for further processing. This may lead to rejection of the product and to huge economical losses for the industry. The two most important causes of downgrading during secondary processing were pale or uneven colour and problems associated with soft flesh and gaping, each accounting for around 40% of the loss in value (Michie, 2001). It is important to understand the consequences of production trends for product quality.

Texture is a sensory attribute that is determined by touching the product or when taken in the mouth. The fish flesh consists of numerous muscle segments bound together with the help of the connective tissues. Texture is influenced by both ante- and post- mortem factors (Hyldig and Nielsen, 2001). Ante- mortem factors affecting fillet texture include genetic background (Larsson et. al. 2012), feed and feeding (Einen and Thomassen, 1998), environmental factors (Johnston, 2008) and health status (Lerfall et. al., 2012). Many studies have found that consumers prefer wild caught to farmed fish because of their superior organoleptic qualities and firmer texture e.g. studies with Chinook and Atlantic salmon (Sylvia et. al. 1995).

The phenomena of gaping are referring to the holes appearing in the fish fillets. This occurs when the connective tissues fail to hold the muscle segments together. Fish which have been stressed before death present a considerable amount of gaping that is when myotomes separate from one another (Suzuki, 1981). This is because the intervening threads of connective tissue break causing slits or holes to appear in the fillet. In severe cases, the fillet may even fall apart when skinned. This makes it more difficult to process the flesh, especially in the case of smoked salmon, where thin slices are required. Rough handling of fish can cause damage, which may result in gaping (Love, 1974). The processing temperature is also important with regard to gaping. The connective tissue of newly caught fish is very sensitive to small rises in temperature, so when fish are warm, any handling such as gutting, washing, or moving can result in gaping. However, when warm fish are cooled again in ice, the connective tissue recovers most of its strength, unless the temperature has risen to about 30 °C, in which case the connective damage is irreversible (Love, 1974). Size also influences susceptibility to gaping; smaller fish seem to gape more because the connective tissue is thicker in larger fish (Love, 1974). The season of capture is also important as regards gaping; for instance, when fish begin to feed heavily again after

spawning, there is a general alteration of their biochemistry so that the myocommata are weakened, and the fish are very liable to gape (Mørkøre and Rørvik, 2001).

2.10. Nutrient composition in salmon

Atlantic salmon is nutritious, rich in micronutrients, minerals, marine ω- 3 poly unsaturated fatty acids (Eicosapentaenoic acid and Decosahexaenoic acid), wide variety of vitamins and minerals, including vitamins A and D, phosphorus, magnesium, selenium and iodine and represents an important part of a varied and healthy diet. Food and Agricultural Organization of the United Nations (FAO) highlights "Fish is a food of excellent nutritional value, providing high quality protein and a wide variety of vitamins and minerals, including vitamins A and D, phosphorus, magnesium, selenium and iodine in marine fish". Salmon liver derived oil or food products can reduce the risk for human cardiovascular disease. Data also indicates that EPA and DHA reduce the risk for a large number of other health issues.

The dietary fat content can significantly influence the lipid deposition in flesh or liver of fish. However, the effect of the feed formulation is not only dependent on the individual percentages of the nutrients chosen, but also on their interaction during the digestion. After 9.5 months long study period, where salmon diets contained medium fat level of 32 % or high fat level of 39 %, it was found that fish fed high fat content had more total carcass lipid deposits that correlated positively with the pigment (astaxanthine) content in the flesh (Bjerkeng et. al. 1997). The fish body composition appears to be influenced by the feed ration levels and increasing fish size also results in enhanced adipose deposition (Rasmussen, 2001).

Feed formulations and vaccine can effect a little bit in fat contents in liver of salmon. In addition, the total fat content in the Atlantic salmon flesh varies depending on the season. In a study done by Mørkøre and Rørvik (2001), the fat content increased most substantially from July to November (12 – 13 % units). Carbohydrates are mostly stored in the liver as glycogen that represents an energy reserve used during the periods of low feeding frequency or starvation. In compliance, an experiment done by Einen et. al. (1998) the in vivo glycogen levels increased with the increasing feed ration levels.

2.11. Melanization

2.11.1. Melanin

Any of a group of polymers, derived from the amino acid tyrosine that cause pigmentation of eyes, skin, and hair in vertebrates. The term "melanin" is a purely descriptive one, which simply denotes a black pigment of biological origin (Swan, 1974). Melanin are produced by specialized epidermal cells called melanophores (or melanocytes); their dispersion in these cells is controlled by melanocyte- stimulating hormone and melatonin. There are three basic types of melanin: eumelanin, pheomelanin, and neuromelanin but only eumelanin has been identified in teleosts (Adachi et. al. 2005).

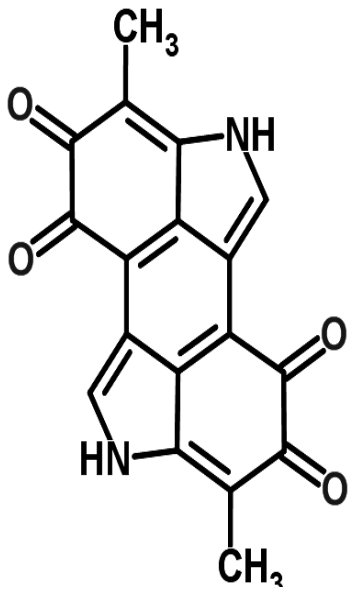


Fig. 2.4. Melanin [Systematic name: 3, 8- Dimethyl- 2, 7 dihydrobenzo (1, 7) isoindolo (6, 5, 4- cd) indole- 4, 5, 9, 10- tetrone; Molecular Formula: $C_{18}H_{10}N_2O_4$].

2.11.2. Causes of creation of melanin

Melanin may occur at sites of injury or infection in a wide range of species, leading to the general conception that melanin and its quinone precursors, have anti-infection properties (Sommerset et. al. 2005). The synthesis of melanin occurs through enzymes encoded by the tyrosinase gene family, of which Dopachrome tautomerase (Dct) is considered to be melanocyte specific (Slominski et. al. 2004). In Atlantic salmon, these genes are expressed in secondary lymphatic organs, where melanin- containing cells, termed melanomacrophages, reside (Mackintosh J.A., 2001). Expression of the tyrosinase

gene family occurs in melanomacrophages during chronic inflammation of Atlantic salmon, indicating a *de novo* melanin synthesis (Larsen et. al. 2012). Histological investigations of pigmented muscle lesions show that they are dominated by inflammation and pigmented cells (Koppang et. al. 2005; Larsen et. al. 2012), frequently termed 'melanomacrophages' in piscine morphological characterization (Agius & Roberts, 2003).

The cause of melanin spot was thought to be linked to the use of vaccines containing oil adjuvants, but other factors such as environmental conditions, genetics and disease also appear to play a role. One particularly interesting finding was the combination of vaccination and temperature / photoperiod smolt production (autumn smolt), which resulted in a larger number of affected fish compared to fish that are vaccinated and then undergo simulated natural smoltification (spring smolt). This may point to a possible cumulative effect of, or interaction between raised temperatures and vaccination. This temperature-related effect was corroborated by the results of a cell experiment, where the synthesis of melanin appeared to be affected by the temperature.

There is a clear association between temperature and fish size at vaccination, and side effects like abdominal adhesions and melanization, where smaller size and higher temperature increases the risk of such side effects (Berg et. al. 2006; Grini et. al. 2011). Temperature at the time of vaccination and in the first period thereafter is perhaps the most important factor that influences the development of these side effects (Berg et. al. 2006). 0+ fish can be exposed to higher ambient water temperatures than spring - smolt (1+ or yearling) around the time of vaccination which can increase the risk of possible side effects (Vågsholm & Djupvik, 1999). Analyses of pathological pigmentation in the hearts of fish suffering from Cardio- myopathy Syndrome (CMS) also found a link between black discoloration and processes of repair and scar tissue formation in the fish. (Fagerland H. A. S. 2013).

The condition has also been reported in captive wild salmon when vaccinated and reared as farmed individuals (Mørkøre, 2012), but importantly, no report exists from unvaccinated wild salmon. Geographically, the highest rate of melanin spots in salmon 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 fish melanin occurrence differences between regions (Mørkøre, 2012).

2.11.3. Role of melanin in immunization

The function of melanin is defined by their physical and chemical properties. It has been shown that melanin are photo protective pigments; this action is related to its high efficiency to absorb and scatter photons, particularly the higher energy photons from the Ultra- violet Radiation (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.

The pigmentary and the immune systems are related each other. There are indications that melanin plays a role in immune functions such as antimicrobial defense, suggesting that immune modulation exerted by the pigmentary system might be an important and underestimated entity (Burkhart C. G. & Burkhart C. N. 2005). Melanocytes respond to cytokines, including interferons, interleukins and tumor necrosis factor (Slominski A. et. al. 2004). Furthermore, they have been shown to produce several inflammatory mediators, suggesting participation in the inflammatory response (Mackintosh J. A. 2001 and Thorsen J. et. al. 2006).

The presence of possible melanin - producing leukocytes in salmon indicates that melanin may play an active role in inflammation in fish, and establishes a collaborative relationship between the pigmentary and immune systems. 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 et. al. 2013 and NPS 2013).

3. MATERIALS AND METHODS

3.1. Field experiment Area and Design

A total number of 420 Atlantic salmon (*Salmo salar* L) (1+ spring smolt), with an average body weight of 2548 ± 2 g (mean ± standard error) were randomly distributed in three net pens (100 vaccinated and 40 SW- injected sexually immature salmon in each net pen) on 5th June and operated until 19th August 2014 for investigating the growth, health and quality parameters performance. On 19th August the rest salmon were transferred into one net pen until 19th September 2014 for investigating the long term stress effects on the health and quality parameters of salmon. The area of each net pen was 125 m³. The field experiments were operated in the Marine Harvest Fish Feed at Averøy and the lab experiments were operated in the marine research station of The Norwegian Food and Aquaculture Research Institute (NOFIMA) at Ås, Norway.

The salmon were marked as vaccinated or unvaccinated (SW- injected) on 4th April, 2013 when body weight was minimum 35 g. In order to distinguish between the vaccinated and unvaccinated salmon, the fish were marked by clipping the adipose fin (most posterior dorsal fin) of the unvaccinated fish (Marina, 2014). A commercial feed of *Skretting Optiline premium 2500-50 (9-mm)* used from June to August 2014.

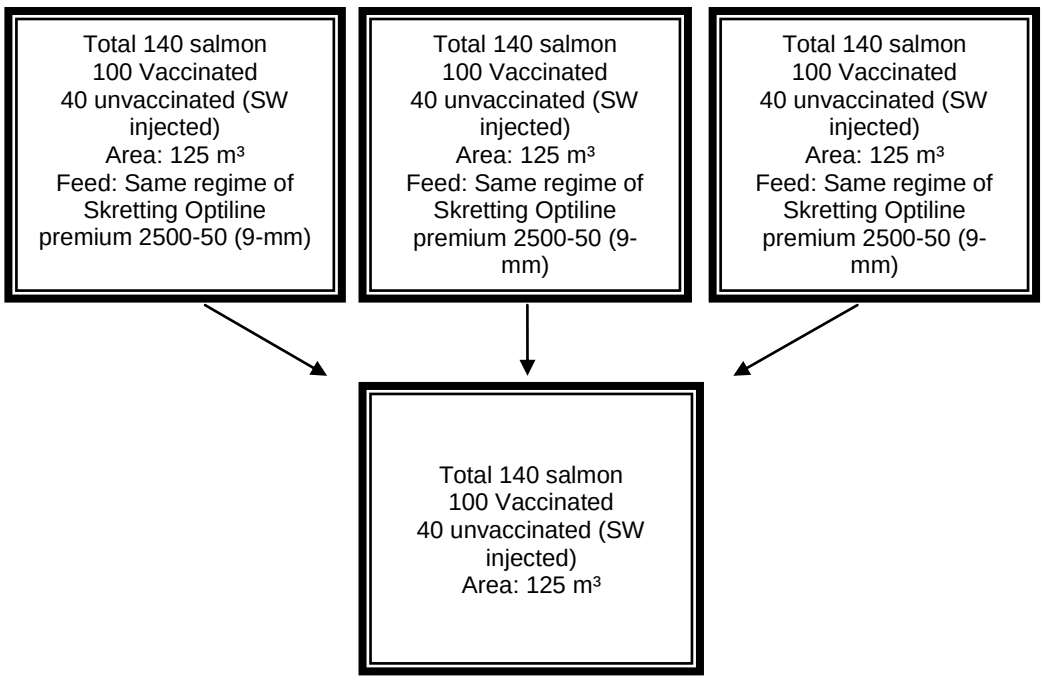


Fig. 3.1. The experiment design where the block designs expressing the net pens area, in the same row net pens were used from June to August and after August the rest salmon were transferred into one net pen which used until 19th September 2014.

3.2. Vaccine

All salmon were vaccinated by hand (vaccinated) or injected with saltwater (1 % NaCl) (unvaccinated) using a 6- component injection vaccine from MSD Animal Health *(Norvax Minova 6)*; 0.1 ml dose, mineral oil adjuvance, and protection against Furunculosis, Vibriosis, Cold water vibriosis, Winter ulcers and Infectious Pancreas Necrosis (IPN). The minimum body weight of the salmon at vaccination was 35 g. Starvation time before vaccination was 3 days. After injection, the vaccinated and unvaccinated (SW- injected) salmon were mixed and transferred back to their respective tanks. The quality of the vaccination was controlled on April 4th 2013 by MSD Animal Health (Marina, 2014).

3. 3. Feed

A commercial feed from *Skretting Optiline Premium 2500- 50 (9 mm)* was used from 5th June to 19th August 2014. The diet was made from 25 kg bag feed, 600 ml water and top coating with 250 ml of rapeseed oil. The process of the diet was: At first the feed was coated with water and then the feed was spread on a tray for 24 hours to be dry and ready for top coating with rapeseed oil. The feed was top coated with oil and the feed was spread again on a tray for 24 hours before start feeding the salmon. Same amount of diet was delivered each time in the three net pens.

The feeding was done every day in the following time schedule:

06: 00 – 06: 45 11: 10 – 11: 55 15: 00 – 15: 45 18: 30 – 19: 15

Feed conversation ratio (FCR) was calculated by the following formula:

FCR = Feed intake (g) / Wet weight gain (g)

3.4. Water quality

3.4.1. Temperature (°C)

Throughout June to August, 2014; the mean water temperature (water depth up to 3 m) was 13.5 °C. The maximum temperature (16.4 °C) was recorded on August 09th and 18th; on the contrary, the minimum temperature (10.3 °C) was recorded on July 2nd (Fig 3.2).

3. 4.2. Dissolved Oxygen (DO, mg / L)

The highest DO (9.9 mg / L) was recorded on 3rd July and the lowest DO (8.9 mg / L) was recorded on 19th August, 2014. The mean DO during the period (June to August 2014) was 9.7 (Fig. 3.3).

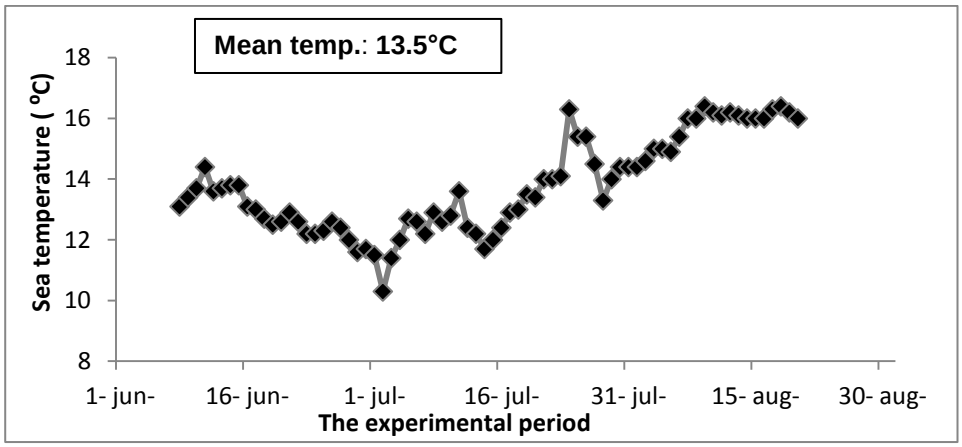


Fig. 3.2. The sea temperature (°C) (up to 3 m) during the experiment (from June to August 2014).

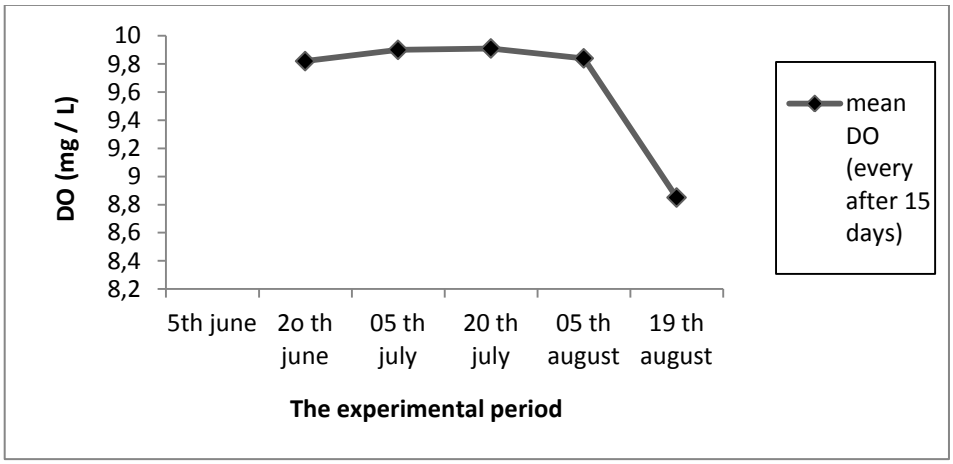


Fig. 3.3. The mean DO (mg / L) in sea- water during the experiment (from June to August 2014).

3.5. Crowding stress

During June, August and September 2014 samplings, some salmon either taken from net pens and immediately taken to slaughter house and slaughtered to investigate the quality conditions, theses salmon are termed as ‘’standard’’ or some salmon when lifted from net pens were kept in a net for few minutes with overcrowded condition and then they taken to slaughter house to investigate the quality parameters, these are termed as ‘’crowded’’. The

objective of being salmon in crowded to see if there happen any changes in fish quality parameters by stress.

3.6. Salmon sampling

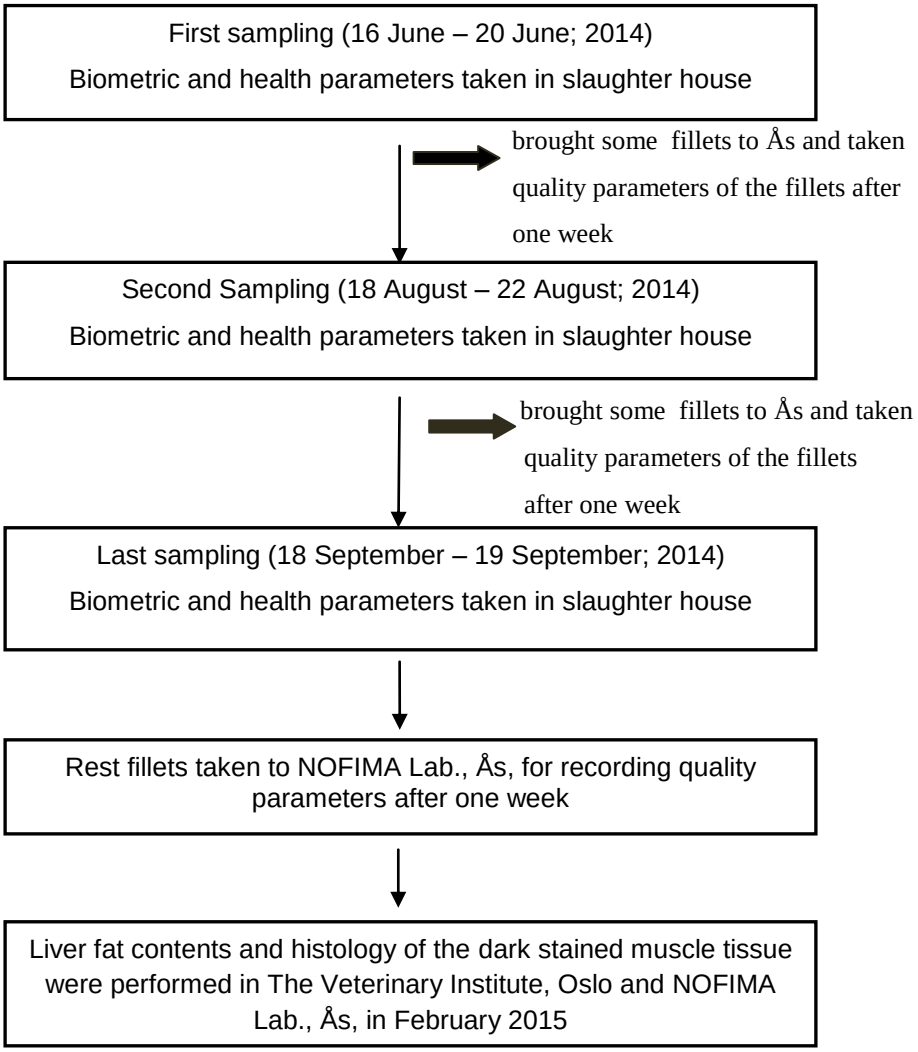


Fig. 3.4. The field and laboratory works plan throughout the experiment

The sampled salmon from each net pen were brought to the slaughter room. Then salmon were killed with a hit to the head and bled in sea- water for 15 minutes after cutting the gill arches. Thereafter, the measurement started with round body weight, gutted weight and fork length. Then salmon were gutted, cleaned and registered several quality parameters. 212 salmon were collected for recording some biometric and health traits scores in the slaughter house immediately such as fish length and weight, adhesion, cataract, liver colour, visceral and heart fat, melanin in organs / belly. Then immediately filleted by hand by a fish expert. The time from slaughtering until filleting was less than one hour. Then some fillets were packed in sealed plastic bags, preserved on ice, and transported to fish laboratory at

Nofima, Ås, for fillet quality analysis six days after slaughter. 96 left- sided salmon fillets in total were used for recording fillet quality parameters in the lab such as fillet colour score, fillet pigment (mg / kg), fillet pH measurement, fillet gaping "A'' score, fillet texture measurement, melanin in fillet score, spots characteristics scores.

3.7. Biometric Traits

3.7.1. Growth

At the slaughter room, the weights of the sampled salmon were measured by using an electric balance and lengths were measured by a centigrade scale. Condition Factor (CF), Thermal Growth Co- efficient (TGC) and Specific Growth Rate (SGR) were measured by the following formulas:

Condition Factor: $CF = W\ (g) \times (\text{fork length, cm})^{-3} \times 100$

Weight Gain: $WG = W_1\ (g) - W_0\ (g)$

Thermal Growth Co- efficient: $TGC = [(\sqrt[3]{W_1}) - (\sqrt[3]{W_0})] \times (\text{days} \times {}^{\circ}C)^{-1} \times 1000$

Specific Growth Rate: $SGR = 100 \times (\ln W_1 - \ln W_0) / t$

Where, W: The body weight of the sampled salmon in grams

W_0: The initial mean body weight of salmon in grams

W_1: The final mean body weight of salmon in grams

t = Time (days) between W_1 and W_0

3.7.2. Slaughter and fillet yield (%)

The slaughter and fillet yield were calculated by the following formulas:

Slaughter yield = Gutted weight (g) / Body weight (g)

Fillet yield = 2 × Fillet weight (g) / Body weight (g)

3.7.3. Liver and heart weight (g)

The liver and heart weight were taken by using an electric balance. Registration of heart weights took place with removing hearts bulbous and atrium.

3.7.4. Hepato- Somatic Index (HSI) **(%)**

The HSI (%) was calculated by using the following formula:

HSI = Liver weight (g) / Body weight (g) × 100

3.7.5. Cardio- Somatic Index (CSI) (%)

The CSI (%) was calculated by using the following formula:

$$CSI = \text{Heart weight (g)} / \text{Body weight (g)} \times 100$$

3.8. Fillet quality

3.8.1. Fillet color score

The fillet color visual evaluation was done by using a *Salmo*Colour Fan™ (DSM) which had a ranging from 20 to 34; where 20 was the palest color and 34 was the most intense color.

3.8.2. Fillet pigment (mg / kg) and fat (%)

The color images of the weighted frozen fillets were captured by the equipment PhotoFish AS. This modernized image system consists a closed box with standardized light and color conditions, a digital camera and a computer for the image and software for analyses. The results presented color as total amount of pigment (mg / kg), while the fat in percentage of the whole tissue.

3.8.3. Fillet pH measurement

The pH was measured of the dorsal part of the fillet with a pH meter 330i SET (Wissenschaftlich - Technische Werkstatten Gmbh & Co.KG, WTW, Weilheim, Germany) with a pH muscle electrode (Schott pH- electrode, Blueline 21 pH, WTW, Weilheim, Germany). The electrodes were for obtaining consistency in the results, kept clean and frequently calibrated in buffers during the measurements.

3.8.4. Fillet gaping score

The fillets gaping "A'' scores (Andersen's Test) were recorded in the Fish Lab of Nofima, Ås. The fillet gaping "A'' (Fig. 3.6.b) registration was performed by using a scale ranged from 0 - 5, where score 0 represented no gaping and score 5 represented the maximal gaping score (Andersen et. al.1994).

3.8.5. Fillet texture analysis

The texture analyses of the fillets were done by using a Texture Analyzer TA- XT2 (Stable Micro System, Surrey, England). A flat- ended cylinder (Ø 12.5 mm) was pressed into the fillet at 1mm s-1 until it reached 90% of the fillet height. It was pressed on the dorsal muscle of the fillet and on the Norwegian Quality Cut (NQC) (anterior and posterior to the

dorsal fin). The parameter (total work) used from the time - force graphs, was the total area under the graphs (N*s) (Fig 3.6.c).

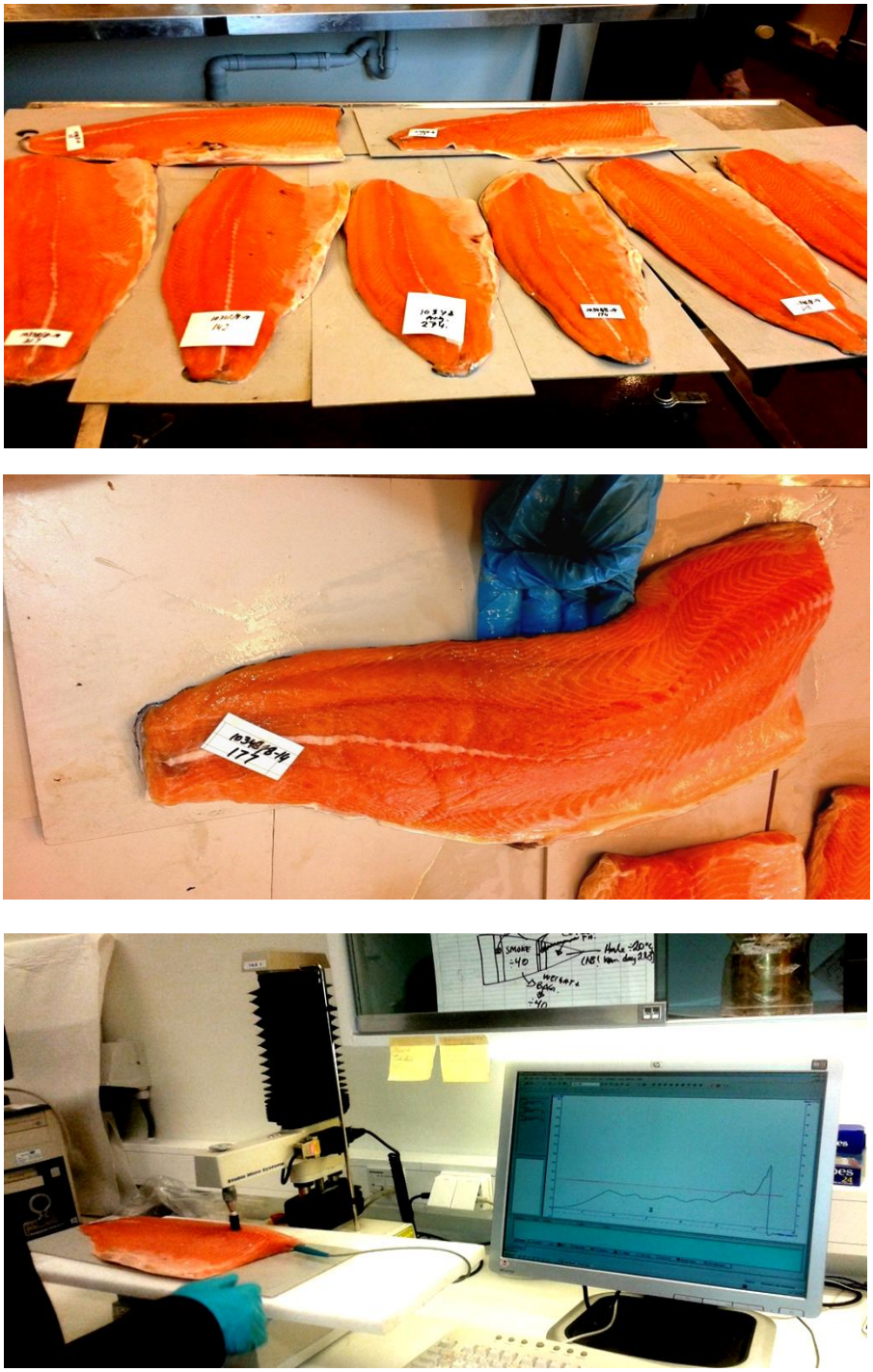


Fig. 3.5. The figures according to consecutive orders a) The thawed fillets kept on a table for weight measurement at Fish Lab of NOFIMA Ås b) The gaping was recorded according to the standard scale ranging from 0 - 5 c) The Texture measurement was done by a Texture Analyzer TA- XT2 which expressed electronically as time-force graphs, was the total area under the graphs (N*s).

3.9. Fish health

3.9.1. Adhesion score

The organ adhesions were classified according to a standardized scoring system by using a scale which ranged from 0 to 6. (Midtlyng et al., 1996).

3.9.2. Cataract score

The cataracts of the eyes were measured by using a scale which ranged from 1 to 3 (Mørkøre et. al., 2013).

3.9.3. Liver color score

The liver color was evaluated according to scale from 1 – 5 (Mørkøre et al. 2013) where score 1 was light, 2 was light- brown, 3 was brown, 4 was dark- brown and 5 was dark.

3.9.4. Visceral and heart fat score

The visceral and heart fat scores were measured by using a scale which ranged from 0 - 5 (Mørkøre et. al., 2013).

3.9.5. Blood plasma chemicals

The blood plasma chemicals were analyzed according to standard technique. The blood sample collection was as: 3 salmon from each group of standard and stressed salmon → 3 pooled blood samples used from each group of standard and stressed salmon.

3.9.6. Liver fat (%) analysis

8 vaccinated salmon liver samples and 8 unvaccinated salmon liver samples from each net pen were collected for analyzing the fat contents (Appendix).

2 g homogenized liver sample from the eight vaccinated or unvaccinated salmon from each net pen transferred into an Erlenmeyer flask separately and the following steps were performed in the hood to extract the fat contents according to the Folch extraction principles:

1. Added 6 ml 0.9 % NaCl
2. Added 50 ml chloroform:methanol (2 : 1)
3. Homogenized for 60 seconds with a homogenizator (with knife) (Fig. 3.7.a)
4. Added 6 ml 0.9 % NaCl (the solution separated into phases)
5. Homogenized for 5 seconds with a homogenizator (with knife)

a) The lower phase was chloroform : methanol : water in the ratio (86 : 14 : 1) and contains almost all lipids
b) The upper phase was chloroform : methanol : water in the ratio (3 : 48 : 47) and contains mostly water soluble components

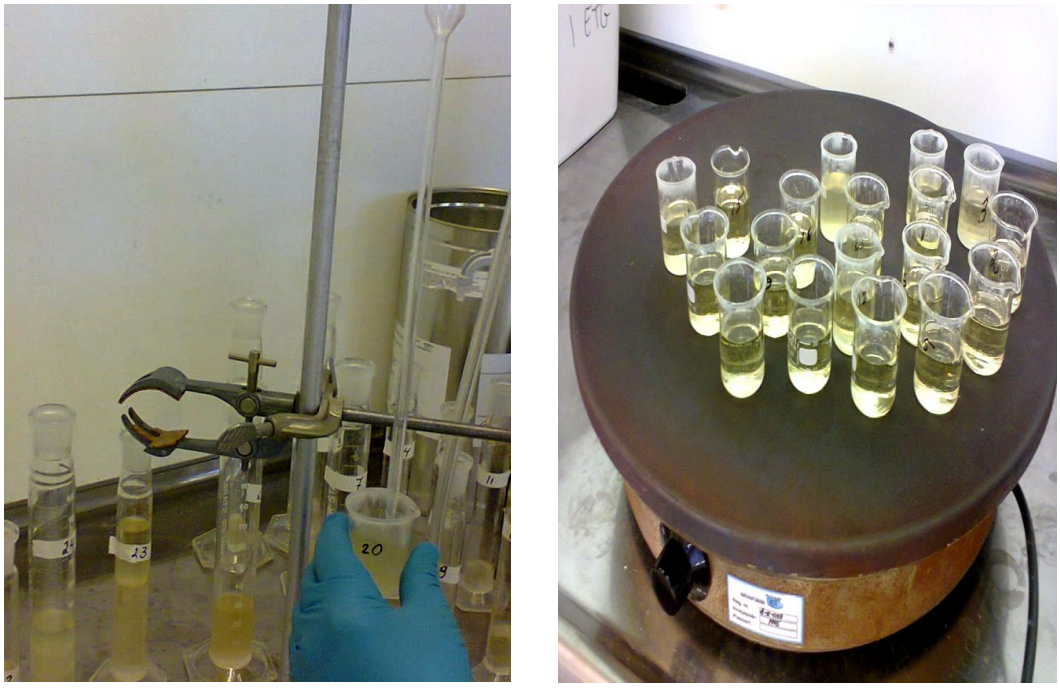
6. Filtered the homogenate through a cotton filter inside at funnel into a flask or graded cylinder.
7. Caped the flasks and kept in freezer for one hour.
8. Removed the upper water / methanol phase and any protein. Pipetted the lower chloroform phase (20 ml) to a new weighted 25 ml beaker. (Fig. 3.7.b)

Calculation of fat %

By the use of 100 ml chloroform/methanol:

$$\% \text{ fat} = \frac{\text{g fat} \times 100}{\frac{I \times U}{37.5}}$$

Here,
g fat = evaporated sample in beaker
I = weighted of the sample in g
U = Pipetted chloroform extract (20 ml) in ml beaker
37.5 = Total volume of solvent (33.3 ml ×100/89) = 37.5 ml
(Chloroform in extract solution = 50 × 2/3 = 33.3 ml)



a b

Fig. 3.6. a) Methanol, chloroform and NaCl were homozenized for 60 seconds b) Chloroform contained 25 ml beakers were kept on a oven to evaporate chloroform and turn in to extract lipids

3.9.7. Melanin parameters

3.9.7.1. Fillet spots characteristics

The characteristics of spots in fillet (melanin spot, blood spot and scar spot) scorings were recorded as 1 was present and 0 was absent.

3.9.7.2. Melanin in Fillets score

The fillets were scored visually for melanin deposit type by using a normal standard scale ranged from 0 - 8.

3.9.7.3. Melanin in abdominal wall / organs score

The melanin deposit amount in the abdominal organs and abdominal wall of the salmon were recorded by using the Visual Analogue Scale (VAS) which ranged from 0 - 3. (Taksdal et al. 2012).

The scale was used as follows:

0 = no melanin;

1 = pin points / small spots;

2 = considerable amount of melanin;

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

3.9.7.4. Melanin in tissues / cells

The dark stained muscle tissues of the fillet were taken from Averøy to The Veterinary Institute, Oslo and the histology was performed by the following standard procedure:

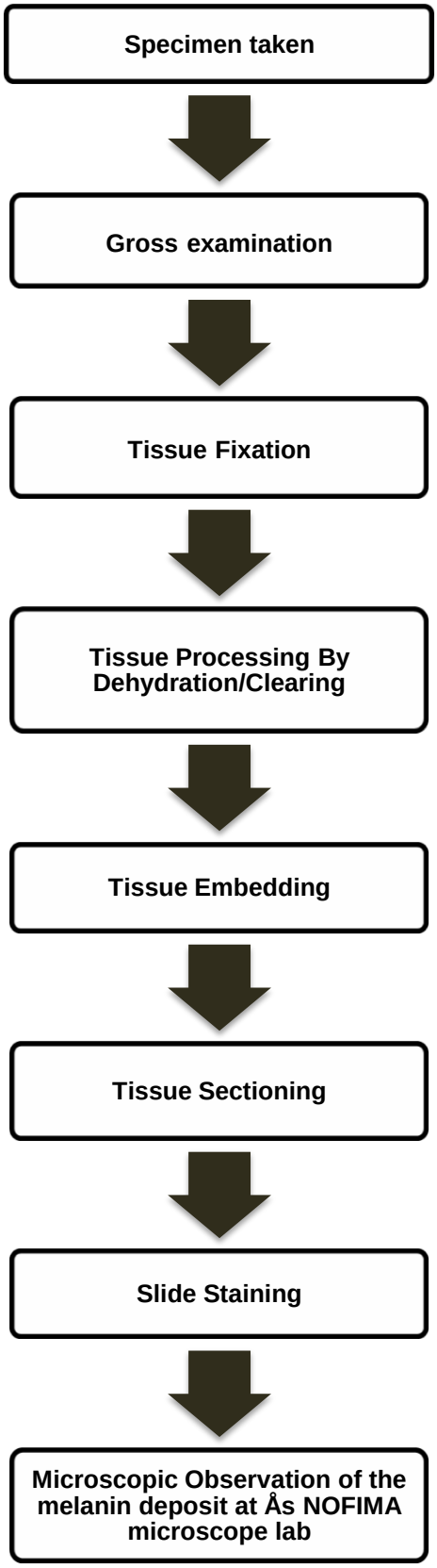


Fig. 3.7. Histology steps of the dark stained muscle tissue of salmon in the experiment.

3.10. Statistical analysis

Statistical analysis was performed by the Statistical Analyses System 9.4 (SAS Institute Inc. Cary, NC, U.S.A.). The software is a collection of statistical models that can establish the differences between group means and to study correlation among the variables, where the user is able to determine the model of preference.

In the statistical model for each parameter, vaccine and slaughter were major variables. As, there were imbalanced gender and body weight distribution across the treatments, so gender and body weight were used as covariate in the statistical model. Statistical analysis revealed differences in the results of all parameters between the vaccinated and SW- injected salmon and stressed and standard salmon.

Microsoft Excel 2013 used for the graphical presentation of results of some parameters. Growth difference in significantly (P value) was calculated by T - test and other results in the biometric, quality and health parameters were analyzed using the Analysis of Variance (ANOVA) in SAS. The results will be presented as Least Square Means with Standard Error (LSMeans ± SE). Sample numbers used for analysis of the result in each parameter. Pearson's correlation coefficient was used to investigate dependence between the variables and the level of significance was set at 5 % ($P < 0.05$).

3.11. Histological analysis

Tissues / cells were observed by using a modern light microscope. Computer system recorded the representative images of the sectioned tissue slides for later investigation of the melanocyte distribution, shape, macrophage, empty cells.

4. RESULTS

Results are presented in five sections. The first section includes the production parameters (growth, feed conversion ratio, maturity and mortality). The second section includes the biometric traits, the third section includes the fillet quality and the fourth section includes the fish health. The last section includes the histology of the dark stained muscle tissue. The results are presented as Least Square Means ± Standard Error (LSMeans ± SE).

4.1. Production parameters

The mean body weight of the salmon was 2548 ± 2 g in June and 3713 ± 7 g at the end of growth trial in August (Table 4.1).

There was no significant difference in the mean body weight (P = 0.22) between the vaccinated and SW- injected salmon at the end of growth trial in August (Table 4.1).

The mean Thermal Growth Co- efficient (TGC) of all salmon was 2.11± 0.11, throughout the growth period (June to August). There was no significant difference (P = 0.59) in the overall mean TGC between the vaccinated and SW- injected salmon in the experiment (Table 4.1).

There was no significant difference in the mean Specific Growth Ratio (SGR) (%) (P = 0.48) between the vaccinated and SW- injected salmon in the experimental growth period (Table 4.1).

The mean FCR of the salmon (from June to August) in the net pens F2, F5 and F10 were 1.32, 1.26 and 1.26 respectively. The overall mean FCR among the three net pens was 1.28 ± 0.02.

The mean maturity (%) of the vaccinated salmon in the three net pens F2, F5 and F10 were 8.1, 4.1 and 12 respectively in August. The mean maturity (%) among the three net pens was 8.1± 2.28.

The mean mortality (%) throughout the experimental period among the three net pens was 0.48 ± 0.20. The mean mortality rate among the vaccinated salmon was 0.33% and in the SW- injected salmon was 0.83%. So, there was no significant difference (P = 0.20) in the mortality (%) between the vaccinated and SW- injected salmon.

Table 4.1. Growth performance between the vaccinated and SW- injected salmon from 5th June to 19th August 2014. Results are presented as LSMeans ± SE

Growth parameters	Overall mean ± SE		P- value
	Vaccinated salmon	SW- injected salmon	
Initial body weight (g)	*2463 ± 8*	*2636 ± 3*	*< 0.05*
Final body weight (g)	*3634 ± 9*	*3793 ± 5*	*0.22*
Body weight gain (g)	*1171 ± 1*	*1157 ± 3*	*0.90*
Specific Growth Ratio (%)	*0.6 ± 0.04*	*0.5 ± 0.01*	*0.48*
Thermal Growth Co-efficient	*2.1 ± 0.15*	*1.9 ± 0.05*	*0.59*

Here, SE is the standard error, P value is the level of significance ($P < 0.05$) which indicate significant difference in mean value of each parameter between the vaccinated and SW- injected salmon.

4.2. Biometric traits:

The mean Condition Factor (CF) of the collected salmon was 1.2 ± 0.07. The mean CF of the vaccinated salmon was significantly higher (P = 0.0014) compared with the CF of the SW- injected salmon (Table 4.2). There was a significant effect of gender of salmon on CF (P = 0.0008) (Fig. 4.1). In case of stress effect on CF, the lowest mean CF was found (1.1 ± 0.03) in the SW- injected standard slaughtered salmon from September sampling (Table 4.3).

The mean slaughter yield (%) of the sampled salmon was 89.7 ± 1.4. There was no significant difference in the slaughter yield by the effect of vaccine (P = 0.3108) and gender (P = 0.3559) (Table 4.2). The highest mean slaughter yield (%) was in the SW- injected standard slaughtered salmon (90.4 ± 0.5) from September sampling, on contrary the lowest slaughter yield (%) was in the SW- injected crowded slaughtered salmon from August sampling (88.9 ± 0.5) (Table 4.3).

The mean fillet yield (%) of the sampled salmon was 62.5 ± 1.8. The mean fillet yield (%) of the vaccinated salmon was significantly higher (P = 0.0227) compared with the mean fillet yield (%) of the SW- injected salmon (Table 4.2). The mean fillet yield (%) of the female salmon was significantly higher (P < 0.0001) compared with the mean fillet yield (%) of the male salmon (Fig. 4.2).

The mean Hepato- Somatic Index (HSI) (%) of the collected salmon was 0.9 ± 0.07. There was no significant difference in the mean HSI (%) between the vaccinated and SW- injected salmon (P = 0.1402) (Table 4.2). But the mean HSI (%) from the sampled female salmon was significantly higher (P = 0.0336) than the male salmon (Table 4.2). The lowest mean HSI (%) was found (0.85 ± 0.03) both in the vaccinated crowded slaughtered salmon and SW- injected crowded slaughtered salmon from August sampling (Table 4.3).

Table 4.2. Biometric traits of the vaccinated and SW- injected salmon. Results are presented as LSMeans ± SE

Para-Meters	Vaccinated LS Mean ± SE	SW Injected LS Mean ± SE	P value				Model P Value
			Vaccine	Slaughter	Body Weight	Sex	
Condition Factor	*1.9* [a] ± 0.008	*1.5* [b] ± 0.009	*0.0014*	< 0.0001	< 0.0001	0.0008	< 0.0001
Slaughter Yield (%)	*89.8* [a] ± 0.2	*89.6* [a] ± 0.2	*0.3108*	0.2582	0.2147	0.3559	0.1094
Fillet Yield (%)	*62.7* [a] ± 0.2	*62.1* [b] ± 0.2	*0.0227*	0.2823	< 0.0001	< 0.0001	< 0.0001
Hepato - Somatic Index (%)	*0 .9* [a] ± 0.008	*0.9* [a] ± 0.009	*0.1402*	< 0.0001	0.1931	0.0336	< 0.0001
Cardio - Somatic Index (%)	*0.1* [a] ± 0.001	*0.1* [a] ± 0.001	*0.2268*	0.0078	0.0001	0.0451	< 0.0001

Model results from four- way analyses of variance (ANOVA) where, SE is the standard error and P value is the level of significance. Different superscripts within the same row indicate significant difference ($P < 0.05$) in a parameter between the vaccinated and SW-injected salmon.

Table 4.3. Biometric traits of the standard and stressed slaughtered salmon. Results are presented as LSMeans ± SE

Parameters	Vaccinated salmon				SW- injected salmon			
	Stan ± SE	**Crow ± SE**	**Stan_B ± SE**	**Crow_B ± SE**	**Stan ± SE**	**Crow ± SE**	**Stan_B ± SE**	**Crow_B ± SE**
Condition Factor	1.2 ^{ab} ± 0.01	1.3 ^{a} ± 0.03	1.1^{b} ± 0.02	1.2 ^{ab} ± 0.03	1.2 ^{ab} ± 0.01	1.2 ^{ab} ± 0.03	1.1^{b} ± 0.03	1.1^{b} ± 0.02
Slaughter Yield (%)	89.6 ^{a} ± 0.2	89.7 ^{a} ± 0.5	90.1^{a} ± 0.3	90.3 ^{a} ± 0.4	89.6 ^{a} ± 0.2	88.9 ^{b} ± 0.5	90.4 ^{a} ± 0.5	89.3 ^{b} ± 0.4
Fillet Yield (%)	62.8 ^{a} ± 0.2	63.3 ^{a} ± 0.6	62.6 ^{a} ± 0.4	62.5 ^{ab} ± 0.6	62.4 ^{ab} ± 0.2	62.3 ^{ab} ± 0.6	62.2 ^{ab} ± 0.6	61.1 ^{b} ± 0.5
Hepato - Somatic Index (%)	0.9 ^{ab} ± 0.01	0.85 ^{b} ± 0.03	1.0 ^{a} ± 0.02	1.0 ^{a} ± 0.03	0.9 ^{ab} ± 0.01	0.85 ^{b} ± 0.03	0.95 ^{ab} ± 0.03	1.0 ^{a} ± 0.02
Cardio - Somatic Index (%)	0.1 ^{a} ± 0.00	0.1 ^{a} ± 0.00	0.1 ^{a} ± 0.00	0.1 ^{a} ± 0.00	0.1 ^{a} ± 0.00	0.1 ^{a} ± 0.00	0.1 ^{a} ± 0.00	0.1 ^{a} ± 0.00

Here, SE is the standard error, Stan = standard salmon from August, Crow = stressed salmon from August, Stand_B = Standard salmon from September and Crow_B = Stressed salmon from September sampling of 2014. Different superscripts within the same row indicate significant difference ($P < 0.05$) in a parameter between different standard and stressed slaughtered salmon.

Fig. 4.1. The Condition Factor (CF) (LS Mean ± SE) of the sampled male and female salmon in the experiment. Later A inside the column bar expresses that the mean CF of the female salmon was significantly higher (P = 0.0008) than the mean CF of the male salmon.

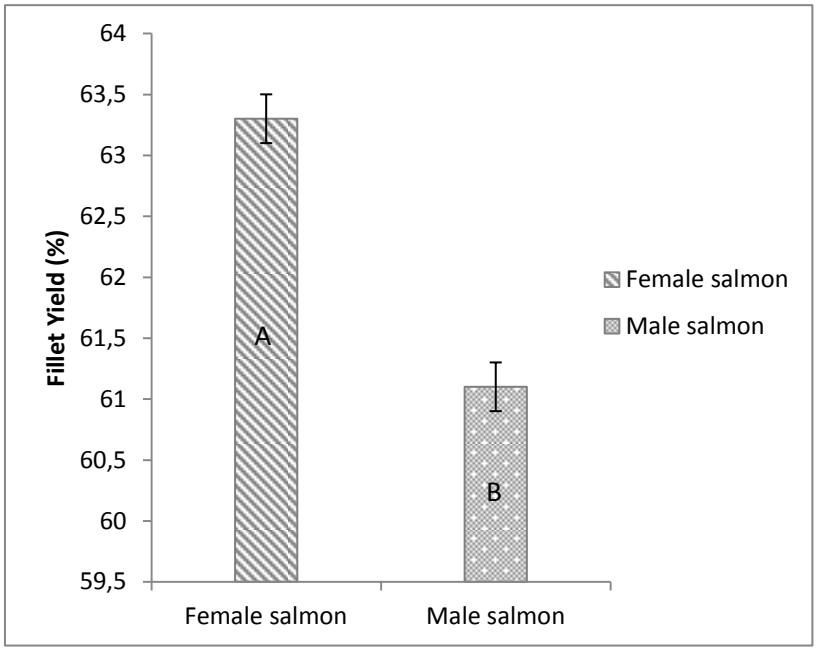


Fig. 4.2. The Fillet yield (%) (LS Mean ± SE) of the sampled male and female salmon in the experiment. Later A inside the column bar expresses that the mean Fillet yield of the female salmon was significantly higher (P < 0.0001) than the mean Fillet yield of the male salmon.

4.3. Fillet quality

The mean score for fillet gaping of the collected salmon was 0.4 ± 0.6. There had no significant difference (P = 0.1442) in the 'gaping mean score' between the vaccinated and SW- injected salmon (Table 4.4). There was numerical difference in the gaping mean score between the SW- injected standard slaughtered salmon from August sampling (0.7 ± 0.09) and the vaccinated standard slaughtered salmon from September sampling (0.01 ± 0.13) (Table 4.5).

The mean score for fillet color (%), pigment measure (mg / kg) and pH of the collected salmon were 26.2 ± 0.8, 6.3 ± 0.9 and 6.2 ± 0.08 respectively. There had no significant difference in the fillet color mean score (P = 0.4087), pigment measure (P = 0.2972) and pH (P = 0.2607) between the vaccinated and SW- injected salmon (Table 4.4).

The mean fillet fat (%) of the sampled salmon was 16.7 ± 1.2. There was significant difference in the mean fillet fat (%) (P = 0.0248) between the vaccinated and SW- injected salmon (Table 4.4).

The mean measured fillet firmness (N*s) in the dorsal part and NQC (Norwegian Quality Cut) of the sampled salmon were 11.7 ± 2.1 and 9.2 ± 1.2 respectively. There was significant difference in the flesh firmness in the NQC (P = 0.0006) between the vaccinated and SW- injected salmon (Table 4.4). There was also significant difference (P < 0.05) in the NQC texture between the SW- injected standard slaughtered salmon from September sampling (10.3 ± 0.4) and the vaccinated standard slaughtered salmon from August sampling (8.6 ± 0.2) (Table 4.5).

Table 4.4. Fillet quality parameters of the vaccinated and SW- injected salmon. Results are presented as LSMeans ± SE

Parameters	Vaccinated LS Mean ± SE	SW Injected LS Mean ± SE	P value				Model P Value
			Vaccine	Slaughter	Body Weight	Sex	
Fillet pH	*6.2* [a] ± 0.01	*6.2* [a] ± 0.01	*0.2607*	< 0.0001	0.0594	0.7828	< 0.0001
Fillet gaping (score)	*0.3* [a] ± 0.07	*0.4* [a] ± 0.08	*0.1442*	0.0004	0.8954	0.4851	0.0009
Fillet colour (%)	*26.3* [a] ± 0.2	*26.4* [a] ± 0.2	*0.4087*	0.2379	0.4605	0.8740	0.6548
Fillet pigment (mg / kg)	*6.3* [a] ± 0.2	*6.5* [a] ± 0.2	*0.2972*	0.2750	0.4357	0.8743	0.6162
Fillet fat (%)	*17.1* [a] ± 0.2	*16.5* [b] ± 0.2	*0.0248*	0.1650	0.0752	0.7506	0.0449
Fillet dorsal texture (N*s)	*11.5* [a] ± 0.4	*11.8* [a] ± 0.4	*0.5812*	0.6247	0.1525	0.3282	0.3847
Fillet NQC texture (N*s)	*8.6* [b] ± 0.2	*9.6* [a] ± 0.2	*0.0006*	0.8420	0.0002	0.6277	0.0008

Model results from four- way analyses of variance (ANOVA) where SE is the standard error and P value is the level of significance.

Different superscripts within the same row indicate significant difference ($P < 0.05$) between the vaccinated and SW- injected salmon.

Table 4.5. Fillet quality parameters of the standard and stressed slaughtered salmon. Results are presented as LSMeans ± SE

Parameters	Vaccinated salmon				SW- injected salmon			
	Stan ± SE	**Crow ± SE**	**Stan_B ± SE**	**Crow_B ± SE**	**Stan ± SE**	**Crow ± SE**	**Stan_B ± SE**	**Crow_B ± SE**
Fillet pH	6.2 ^{a} ± 0.02	6.1 ^{a} ± 0.03	6.2 ^{a} ±0.02	6.3 ^{a} ± 0.03	6.2 ^{a} ± 0.02	6.2 ^{a} ± 0.03	6.3 ^{a} ± 0.03	6.3 ^{a} ± 0.03
Fillet gaping (score)	0.5 ^{ab} ± 0.09	0.7 ^{a} ± 0.20	0.01^{b} ± 0.13	0.05 ^{ab} ± 0.19	0.7 ^{a} ± 0.09	0.3 ^{ab} ± 0.25	0.1 ^{ab} ± 0.21	0.4 ^{ab} ± 0.18
Fillet colour (%)	26.1^{b} ± 0.2	26.3^{b} ± 0.3	26.0 ^{b} ± 0.2	26.4 ^{ab} ± 0.3	26.2 ^{b} ± 0.1	27.0 ^{a} ± 0.3	26.4 ^{b} ± 0.2	26.5 ^{ab} ± 0.3
Fillet pigment(mg / kg)	6.2 ^{ab} ± 0.2	6.3 ^{ab} ± 0.3	6.1 ^{b} ± 0.2	6.4^{ab} ± 0.3	6.4^{ab} ± 0.2	6.7^{a} ± 0.3	6.5^{ab} ± 0.2	6.6^{a} ± 0.3
Fillet fat (%)	16.8 ^{ab} ± 0.2	17.4 ^{ab} ± 0.4	16.8 ^{ab} ± 0.3	18.0^{a} ± 0.3	16.3^{b} ± 0.2	16.6 ^{ab} ± 0.4	16.7 ^{ab} ± 0.3	17.4 ^{ab} ± 0.4
Fillet dorsal texture (N*s)	11.5^{ab} ± 0.3	11.6^{ab} ± 0.7	11.4 ^{ab} ± 0.5	12.3^{a} ± 0. 6	11.6^{ab} ± 0.4	12.1 ^{ab} ± 0.7	12.2 ^{ab} ± 0.6	11.2^{b} ± 0.5
Fillet NQC texture (N*s)	8.6 ^{d} ± 0.2	8.7 ^{c} ± 0.4	9.3 ^{bc} ± 0.3	9.2 ^{bc} ± 0.3	9.6^{b} ± 0.2	9.5^{ab} ± 0.4	10.3^{a} ± 0.4	9.8^{ab} ± 0.4

Here, SE is the standard error, Stan = standard salmon from August, Crow = stressed salmon from August, Stand_B = Standard salmon from September and Crow_B = Stressed salmon from September sampling of 2014. Different superscripts within the same row indicate significant differences ($P < 0.05$) between different standard and stressed slaughtered salmon.

4.4. Fish health

4.4.1. Organ health

The mean score for adhesion of the sampled salmon was 0.48 ± 0.65. The adhesion mean score of the vaccinated salmon was significantly higher ($P < 0.0001$) compared with the mean adhesion score of the SW- injected salmon (Table 4.6). The adhesion mean score in the vaccinated standard slaughtered salmon from August sampling (1.1 ± 0.07) was numerically higher than the adhesion mean score of all other standard and stressed slaughtered salmon (Table 4.7).

The mean score for cataracts (the sum of cataract in left eye and right eye) of all sampled salmon was 0.6 ± 0.83. The cataract mean score in the vaccinated crowded slaughtered salmon from August sampling (1.6 ± 0.28) was numerically higher than all other standard and stressed slaughtered salmon (Table 4.7).

The mean score for liver color of the sampled salmon was 3.5 ± 0.70. The liver colour mean score was highest (4.1 ± 0.24) in the vaccinated crowded slaughtered salmon from September sampling and the lowest (3.2 ± 0.24) was in the vaccinated crowded slaughtered salmon from August sampling (Table 4.7).

The visceral and heart fat mean score of the sampled salmon were 3.3 ± 0.56 and 0.2 ± 0.36 respectively. There was significant difference in the visceral fat mean score ($P = 0.0073$) but was not in the heart fat mean score ($P = 0.8769$) between the vaccinated and SW-injected salmon (Table 4.6). The visceral fat mean score in the female salmon was numerically and significantly ($P = 0.0010$) higher compared with the visceral fat mean score in the male salmon (Fig. 4.3). The highest visceral (3.8 ± 0.18) and heart fat mean score (0.4 ± 0.13) was in the vaccinated crowded slaughtered salmon and the SW- injected standard slaughtered salmon respectively from September sampling (Table 4.7).

Table 4.6. Organ health parameters of the vaccinated and SW-injected salmon. Results are presented as LSMeans ± SE

Parameters	Vaccinated LS Mean ± SE	SW Injected LS Mean ± SE	P value				Model P Value
			Vaccine	Slaughter	Body Weight	Sex	
Adhesion (score)	*0.7* [a] ± 0.07	*-0.1* [b] ± 0.08	< 0.0001	0.0005	0.1587	0.4086	< 0.0001
Cataract (score) (sum of cataract in left and right side eyes)	*0.8* [a] ± 0.09	*0.7* [a] ± 0.10	*0.4863*	< 0.0001	0.9647	0.5873	0.0002
Liver color (score)	*3.6* [a] ± 0.07	*3.5* [a] *± 0.08*	*0.7460*	0.0008	0.9247	0.0713	0.0016
Fat in viscera (score)	*3.7* [a] ± 0.06	*3.2* [b] ± 0.06	*0.0073*	< 0.0001	0.0321	0.0010	< 0.0001
Fat in heart (score)	*0.2* [a] ± 0.04	*0.2* [a] ± 0.04	*0.8769*	0.9390	0.3814	0.0456	0.5896

Model results from four- way analyses of variance (ANOVA) where SE is the standard error and P value is the level of significance.

Different superscripts within the same row indicate significant difference ($P < 0.05$) between the vaccinated and SW- injected salmon.

Table 4.7. Organ health parameters of the standard and stressed slaughtered salmon. Results are presented as LSMeans ± SE

Parameters	Vaccinated salmon				SW-injected salmon			
	Stan ± SE	**Crow ± SE**	**Stan_B ± SE**	**Crow_B ± SE**	**Stan ± SE**	**Crow ± SE**	**Stan_B ± SE**	**Crow_B ± SE**
Adhesion (score)	1.1^{a} ± 0.07	0.6 ^{ab} ± 0.22	0.3 ^{ab} ± 0.13	0.9 ^{a} ± 0.21	0.02^{b} ± 0.08	-0.003 ^{d} ± 0.22	-0.002 ^{d} ± 0.23	-0.02 ^{c} ± 0.19
Cataract (score) (sum of cataract in left and right side eyes)	0.4 ^{c} ± 0.09	*1.6*^{a} ± 0.28	0.6 ^{c} ± 0.16	0.8 ^{b} ± 0.27	0.4 ^{c} ± 0.10	1.3 ^{ab} ± 0.28	0.4 ^{c} ± 0.32	0.8 ^{b} ± 0.25
Liver color (score)	3.5 ^{ab} ± 0.08	3.2 ^{b} ± 0.24	3.7 ^{ab} ± 0.14	4.1^{a} ± 0.24	3.4 ^{b} ± 0.08	3.3 ^{b} ± 0.24	3.9 ^{ab} ± 0.25	3.8 ^{ab} ± 0.22
Fat in viscera (score)	3.2 ^{ab} ± 0.06	3.1^{ab} ± 0.19	3.7 ^{a} ± 0.11	3.8 ^{a} ± 0.18	2.9 ^{b} ± 0.06	2.7^{b} ± 0.19	3.6 ^{a} ± 0.20	3.8 ^{a} ± 0.17
Fat in heart (score)	0.1^{a} ± 0.04	0.2^{a} ± 0.12	0.1^{a} ± 0.07	0.2^{a} ± 0.12	0.2 ^{a} ± 0.04	0.04 ^{b} ± 0.12	0.4 ^{a} ± 0.13	0.1^{a} ± 0.11

Here, SE is the standard error, Stan = standard salmon from August, Crow = stressed salmon from August, Stand_B = Standard salmon from September and Crow_B = Stressed salmon from September sampling of 2014. Different superscripts within the same row indicate significant differences ($P < 0.05$) between different standard and stressed slaughtered salmon.

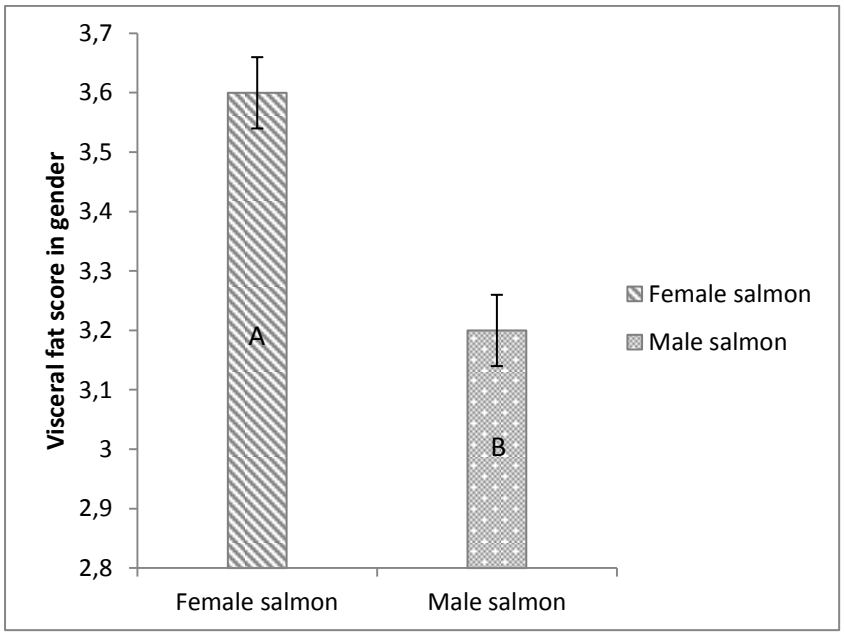


Fig. 4.3. The visceral fat score (LSMean ± SE) of the sampled male and female salmon in the experiment. Later A inside the column bar expresses that the visceral fat mean score of the female salmon was significantly higher (P = 0.0010) than the visceral fat mean score of the male salmon.

The liver fat (%) of the sampled vaccinated and SW- injected salmon from August month were 5.56 ± 0.3 and 5.40 ± 0.3 respectively. There was no significant difference (P = 0.7202) in the liver fat (%) mean values between the vaccinated and SW- injected salmon from August sampling (Table 4.8).

Table 4.8. Fat (%) (LSMean ± SE) in the sampled liver between the vaccinated and SW-injected salmon from August sampling 2014

Salmon	Liver fat (%) LSMean ± SE	P value
Vaccinated	*5.56 ± 0.3*	0.7202
SW- injected	*5.40 ± 0.3*	

Results from one- way analyses of variance (ANOVA) where SE is the standard error and P value is the level of significance (P < 0.05) which indicate significant difference between the vaccinated and SW- injected salmon.

4.4.2. Blood plasma chemicals

The mean model values of the sampled salmon for liver function enzymes albumine (ALB), alanine aminotransferase (ALT), asparate aminotransferase (AST) and lactate dehydrogenase (LD) were 23.2 ± 1.32 gm / L, 4.5 ± 0.71 (µkat / L), 417.5 ± 58.07 (IU / L) and 503.3 ± 202.09 (U / L) respectively. There had numerical difference in ALT mean value between the vaccinated standard (5.3 ± 0.3) and the vaccinated stressed slaughtered salmon (3.6 ± 0.3) (Table 4.9).

There had numerical difference in the mean value of chloride (Cl), sodium (Na) and glucose (Glu) between the vaccinated standard and vaccinated stressed slaughtered salmon (Table 4.9).

There had also numerical difference in cortisol between the standard and stressed slaughtered salmon. The highest mean cortisol (nmol / L) was measured in the vaccinated stressed slaughtered salmon (720 ± 104.2) and the lowest was measured in the vaccinated standard slaughtered salmon (260.3 ± 133.7) (Table 4.9).

Table 4.9. Blood plasma chemical parameters of the standard and stressed slaughtered salmon from September sampling.

Results are presented as LSMeans ± SE

Parameters	Vaccinated salmon		SW- injected salmon	
	Standard LSMean ± SE	Stress LSMean ± SE	Standard LSMean ± SE	Stress LSMean ± SE
Albumine (gm / L)	23.6 ^{ab} ± 0.6	23.0 ^{ab} ± 1.0	24.3 ^{a} ± 0.3	21.6 ^{b} ± 0.8
Alanine aminotransferase (µkat / L)	3.6 ^{b} ± 0.3	5.3 ^{a} ± 0.3	4.0 ^{ab} ± 0.5	5.3 ^{a} ± 0.3
Aspartate aminotransferase (IU / L)	426.6 ^{a} ± 36.3	411.0 ^{ab} ± 24.9	425.6 ^{ab} ± 28.2	406.6 ^{b} ± 41.9
Calcium (mmol / L)	3.2 ^{b} ± 0.03	3.6 ^{a} ± 0.17	3.3 ^{ab} ± 0.03	3.5 ^{ab} ± 0.03
Creatinine kinase (U / L)	7468.3 ^{a} ± 1018.8	6877.6 ^{ab} ± 166.7	6789.3 ^{ab} ± 779.2	5432.6 ^{b} ± 782.8
Chloride (mmol / L)	139.0 ^{b} ± 0.5	149.3 ^{a} ± 1.2	140.0 ^{b}± 2.0	149.6 ^{a} ± 3.5
Cortisol (nmol / L)	260.3 ^{c} ± 133.7	720.0 ^{a} ± 104.2	333.3 ^{b} ± 97.2	679.6 ^{ab} ±110.7
Globuline (g / L)	23.6 ^{ab} ± 0.3	22.6 ^{ab} ± 0.8	24.0 ^{a} ± 00	21.0 ^{b} ± 1.00
Glucose (mmol / L)	5.43 ^{b} ± 0.4	8.76 ^{a} ± 0.3	5.80 ^{ab} ± 0.4	8.26 ^{ab} ± 0.9
Creatinine (µmol / L)	14.0 ^{b} ± 0.5	17.3 ^{a} ± 1.4	15.3 ^{ab} ± 0.3	17.6 ^{a} ± 0.8
Lactate dehydrogenase (U / L)	311.6 ^{b} ± 50.9	612.0 ^{ab} ± 115.8	673.3 ^{a} ± 187.5	416.3 ^{ab} ± 57.2
Sodium (mmol / L)	169.6 ^{b} ± 0.6	181.3 ^{a} ± 0.3	171.6 ^{ab} ± 1.8	181.6 ^{a} ± 3.5
Total biliorubin (mg / dL)	2.3 ^{a} ± 0.33	1.0 ^{b} ± 00	2.0 ^{a} ± 00	1.0 ^{b} ± 00
Total protein (g / L)	47.3 ^{ab} ± 0.8	45.6 ^{ab} ± 1.8	48.3 ^{a} ± 0.3	42.6 ^{b} ± 1.7
Urea (mmol / L)	1.2 ^{b} ± 0.05	1.4 ^{ab} ± 0.05	1.4 ^{ab} ± 0.05	1.5 ^{a} ± 0.03
Inorganic P (g / L)	5.9 ^{b} ± 0.3	6.9 ^{a} ± 0.3	5.8 ^{b} ± 0.1	6.5 ^{ab} ± 0.3

Here SE is the standard error. Different superscripts within the same row indicate significant difference ($P < 0.05$) between different standard and stressed slaughtered salmon.

4.4.3. Melanin parameter

The melanin spot mean score in fillet of the collected salmon was 0.1 ± 0.33. There was no significant difference (P = 0.2792) in the melanin spot mean score in fillet between the vaccinated and SW- injected salmon (Table 4.10). The SW- injected crowded slaughtered salmon from September sampling (0.3 ± 0.10) contained numerically higher mean melanin spot in fillet than any other slaughtered standard and stressed salmon (Table 4.11).

The melanin in abdominal wall and organs mean score of the sampled salmon were 1.4 ± 0.72 and 0.5 ± 0.61 respectively. The melanin mean score of abdominal wall and organs were significantly higher (P = 0.0169 and P < 0.0001 respectively) in the vaccinated salmon compared with the SW- injected salmon (Table 4.10). There was significant effect of gender in the mean melanin score in abdominal wall (P = 0.0113) but not in organs (P = 0.4753) (Table 4.10). The highest mean melanin score in abdominal wall (1.8 ± 0.23) and organ (0.8 ± 0.19) was recorded in the vaccinated crowded slaughtered salmon from September sampling (Table 4.11).

The mean melanin black spot, blood spot and scar spot of the collected salmon were 0.07 ± 0.25, 0.01 ± 0.11 and 0.06 ± 0.24 respectively. There was numerically much difference in the melanin black spot mean score between the SW- injected standard salmon from September sampling (0.2 ± 0.09) and the SW- injected crowded salmon from August sampling (- 0.01 ± 0.08) (Table 4.11).

Table 4.10. Melanin parameters in the vaccinated and SW- injected salmon. Results are presented as LSMeans ± SE

Parameters	Vaccinated LS Mean ± SE	SW Injected LS Mean ± SE	P value				Model P value
			Vaccine	Slaughter	Body Weight	Sex	
Melanin in fillet (score)	*0.09* [a] ± 0.04	*0.1* [a] ± 0.04	*0.2792*	0.2939	0.0273	0.9518	0.0700
Melanin in abdominal wall (score)	*1.6* [a] ± 0.08	*1.1* [b] ± 0.09	*0.0169*	0.3279	0.0998	0.0113	0.0435
Melanin in organs (score)	*0.9* [a] ± 0.06	*0.2* [b] ± 0.07	< 0.0001	0.4017	0.0201	0.4753	< 0.0001
Melanin black spot (score)	*0.05* [a] ± 0.03	*0.1* [a] ± 0.03	*0.3282*	0.4953	0.3175	0.4819	0.4997
Blood spot (score)	*0.01* [a] *0.02 ± 0.01*	*0.02* [a] ± 0.01	*0.3473*	0.6484	0.2174	0.8418	0.7132
Scar spot (score)	*0.07* [a] ± 0.03	*0.08* [a] ± 0.03	*0.8947*	0.2289	0.0103	0.1204	0.0224

Model results from four- way analyses of variance (ANOVA) where SE is the standard error and P value is the level of significance.

Different superscripts within the same row indicate significant differences ($P < 0.05$) between the vaccinated and SW- injected salmon.

Table 4.11. Melanin parameters in the standard and stressed slaughtered salmon. Results are presented as LSMeans ± SE

Parameters	Vaccinated salmon				SW- injected salmon			
	Stan ± SE	**Crow ± SE**	**Stan_B ± SE**	**Crow_B ± SE**	**Stan ± SE**	**Crow ± SE**	**Stan_B ± SE**	**Crow_B ± SE**
Melanin in fillet (score)	*0.09*^{a} ± 0.04	0.04 ^{a_b} ± 0.11	0.2 ^{a} ± 0.06	0.05 ^{b} ± 0.11	0.1 ^{a} ± 0.04	-0.02 ^{c} ± 0.11	0.2 ^{a} ± 0.12	0.3 ^{a} ± 0.10
Melanin in abdominal wall (score)	1.6 ^{a_b} ± 0.08	1.3 ^{a_b} ± 0.25	1.4 ^{a_b} ± 0.14	1.8 ^{a} ± 0.23	1.3 ^{a_b} ± 0.08	1.7 ^{a} ± 0.24	1.1^{b} ± 0.25	1.2 ^{b} ± 0.22
Melanin in organ (score)	0.8 ^{a} ± 0.07	0.5 ^{a_b} ± 0.21	0.5 ^{a_b} ± 0.12	0.8 ^{a} ± 0.19	0.2^{b} ± 0.07	0.5 ^{a_b} ± 0.21	0.5 ^{a_b} ± 0.22	0.1 ^{b} ± 0.18
Melanin black spot (score)	0.05 ^{a_b} ± 0.03	0.01 ^{b} ± 0.08	0.1 ^{a} ± 0.05	0.1 ^{a} ± 0.08	0.1 ^{a} ± 0.03	-0.01 ^{c} ± 0.08	0.2 ^{a} ± 0.09	0.1 ^{a} ± 0.07
Blood spot (score)	-0.002 ^{c} ± 0.01	-0.01^{b} ± 0.04	0.04 ^{a} ± 0.02	0.01 ^{a} ± 0.04	0.03 ^{a} ± 0.01	0.004 ^{a_b} ± 0.04	0.003 ^{a_b} ± 0.04	0.001 ^{a_b} ± 0.04
Scar spot (score)	0.05 ^{a_b} ± 0.03	0.03 ^{b} ± 0.08	0.1 ^{a} ± 0.05	0.1 ^{a} ± 0.07	0.05 ^{a_b} ± 0.03	-0.01 ^{c} ± 0.08	0.1 ^{a} ± 0.08	0.2 ^{a} ± 0.07

Here SE is the standard error, Stan = standard salmon from August, Crow = stressed salmon from August, Stand_B = Standard salmon from September and Crow_B = Stressed salmon from September sampling of 2014. Different superscripts within the same row indicate significant differences ($P < 0.05$) between different standard and stressed slaughtered salmon.

4.5. Histology of the dark stained muscle tissue

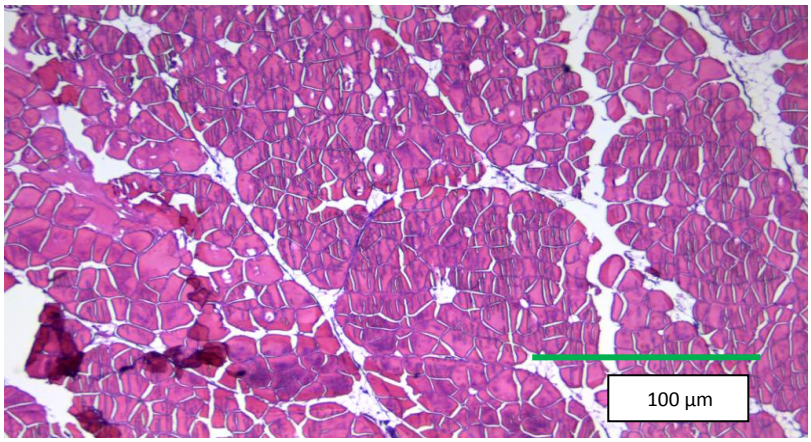
Although during microscopic observation of the melanin in laboratory were taken more tissue sample slides but for better understanding has provided the comparative report between a vaccinated and SW- injected salmon muscle tissue slide.

In the SW-injected salmon flesh tissue slide

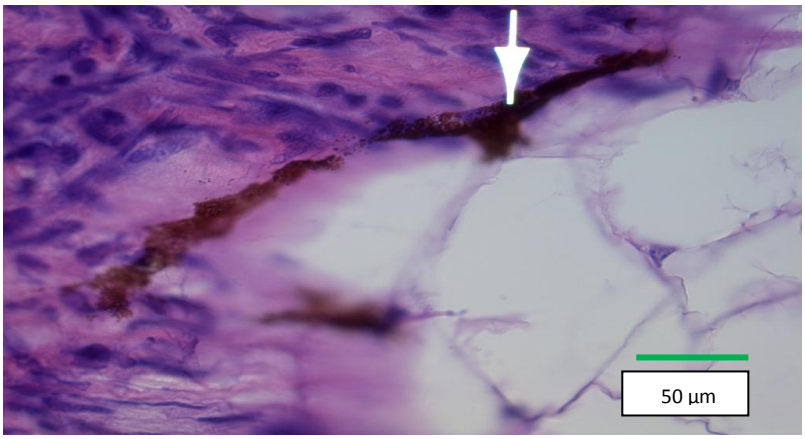
It was observed much connective tissue in the slide (Fig 4.4.a). Melanomacrophages were observed which created like a long dense pigmentation with surroundings much macrophage and empty / damaged cells (Fig 4.4.b; arrowhead expressed as pigmentation). At another location of the tissues it was observed a straight and long melanin spot with surroundings many connective tissues (Fig 4.4.c). Some cells were oval shaped and some were dendritic shaped (Fig 4.4.c).

In the vaccinated salmon flesh tissue slide

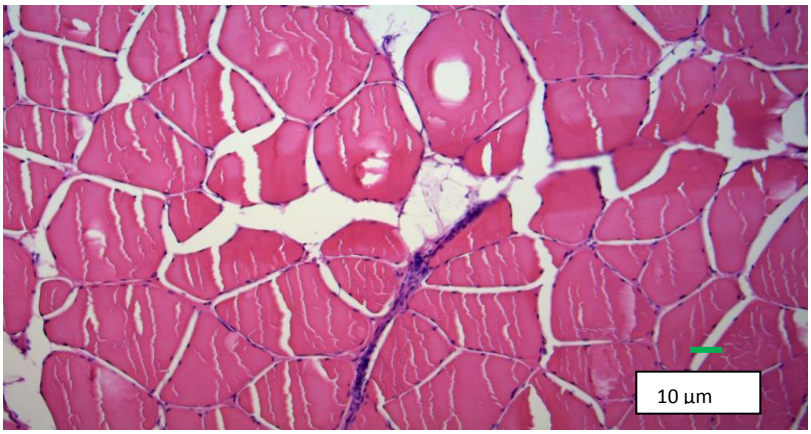
It was observed that one empty vacuole encircled with melanomacrophages and much leukocytes, fibrosis and fatty infiltrates in other parts in the tissue sample (Fig 4.5.i). At another location in the view of the tissue sample, it was observed that some melanomacrophages with much macrophages and few damaged cells (Fig 4.5.ii & 4.5.iii).



a

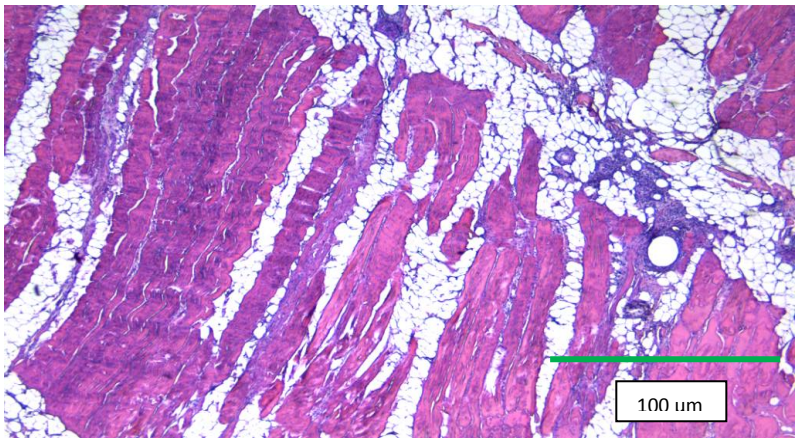


b

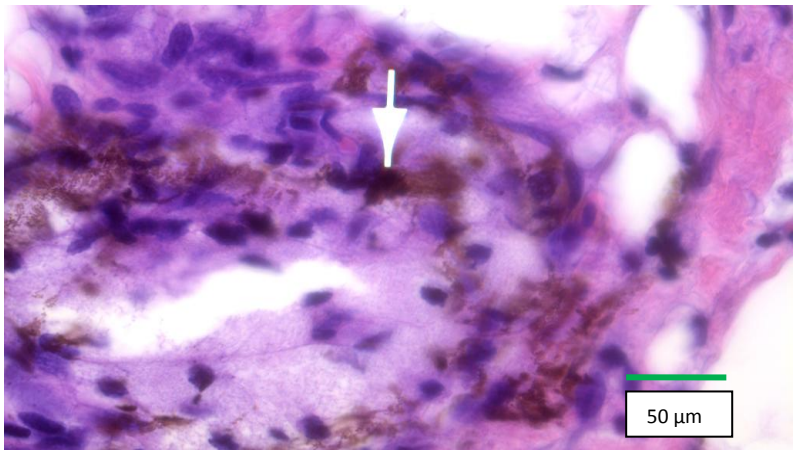


c

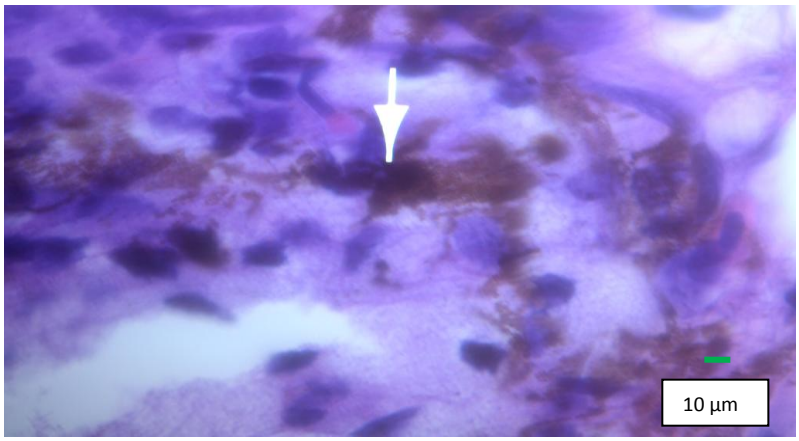
Fig. 4.4. Histological investigation of a dark stained muscle tissue from a SW- injected salmon where it shows different sides view of the tissue which was stained by Hematoxylin & Eosin and objects magnification for pictures a, b, as 2.5 ×, 63 × respectively at one location of the tissue and picture c as 10 × at another location of the tissue. Scale bar (µm) are showed on the images. Arrowhead showing the pigmentation in the tissue.



i



ii



iii

Fig. 4.5. Histological investigation of a dark stained muscle tissue from a vaccinated salmon which was stained by Hematoxylin & Eosin and objects magnification for i, ii & iii picture as 2.5 ×, 63 × and 100 × respectively in the tissue slide. Scale bar (µm) are showed on the images. Arrowhead expressing the melanocytes.

5. DISCUSSION

5.1. Growth

There was no significant difference (paired t- test, P = 0.90) in the final growth increment (LSMean ± SE) between the vaccinated (1171 ± 1) and SW- injected salmon (1157 ± 3) (Table 4.1). There was an exceptional report of fish growth in the experiment as some unvaccinated salmon showed less growth increment. It was due to may be for feed utilization imbalance, early maturity and stress on the salmon. This finding is coinciding with the findings of Ackerman et. al. (2000) and Pylkko et. al. (2000) but opposed with Rønsholdt and McLean (1999) and Melingen (2001). The contradictory results reported most likely descent from use of different vaccine formulations and use of different protocols to evaluate the effect on feed intake and growth of fish after vaccination. The present growth rate also agreed with the finding of (Forsberg 1995; Boeuf and Le Bail 1999). They stated that seasonal variation in growth is a characteristic present in immature salmon as growth is dependent on water temperature and day length. But, Melingen (2001) found in their experiment (68 weeks after vaccination) that vaccinated salmon had a considerably shorter body and lower weight than unvaccinated fish.

5.2. Maturity

The finding about early sexual maturity from the present experiment is agreed with the findings of Fleming (1998); Aunsmo et al. (2008); Taranger et al. (2010) and Fjelldal et. al. (2012). Fjelldal et. al. (2012) reported from their investigation that vaccination increased the incidence of immature fish. Fraser et. al. (2012) conducted an experiment on triplody 1 (+) smolts and found that the only two unvaccinated matured male triploids at the time of slaughter. In 0 (+) smolts, vaccination had no effect on the levels of maturation.

5.3. Mortality

The hypothesis and result of mortality of the present study coinciding with the findings of Guri Eggset et. al. (1999) and Remen et. al. (2012). They conducted an experiment on the effect of vaccination on Atlantic salmon at different times in relation to the smoltification process. Three groups of fish were vaccinated with an oil-adjuvanted vaccine, protective against *Aeromonas salmonicida* and *Vibrio salmonicida*: one group was vaccinated during smoltification, the other group close to smoltification, and the third group several weeks before smoltification. They found that the mortalities of the vaccinated groups were significantly lower (paired t test, $P < 0.05$) than those of the unvaccinated control groups.

On the other hand, Remen et. al. (2012) reported that full- feeding Atlantic salmon held in seawater at 16°C and given fluctuating oxygen levels from 90 to 70% showed reduced appetite, fluctuating from 90 to 60% also initiated acute anaerobic metabolism and increased skin lesions; fluctuations from 90 to 50% additionally initiated acute stress responses, reduced feed conversion and growth and fluctuations from 90 to 40% additionally caused impaired osmoregulation and mortalities.

5.4. Biometric traits

5.4.1. Condition factor (CF)

The vaccinated salmon (1.9 ± 0.008) had significantly (P = 0.0014) higher mean CF (LSMeans ± SE) compared with the SW- injected salmon (1.5 ± 0.009) (Table 4.2). The result is agreed with the finding of (Ackerman *et al.*, 2000), Midtlyng & Lilllehaug (1998).

5.4.2. Fillet yield

The female salmon had numerically and significantly (P < 0.0001) higher mean fillet yield (63.3 %) compared with the male salmon (61.2 %) (Fig. 4.2). The result from the present study is contradicted with the findings of Fraser et. al. (2012) and Aunsmo *et al.* (2008). Fraser et. al. (2012) reported from their experiment that at slaughter time, the male salmon were approximately 8 – 11.5% heavier than the female salmon depending on smolt regime. Aunsmo *et al.*, (2008) also reported that the male salmon was on average 1.2 kg heavier than female salmon at slaughter in approx. 6 kg fish. The present findings from the stress effects on fillet yield are agreed with the findings of Huss (1995).

5.4.3. HSI (%)

Although there was no significant difference in the mean HSI (%) (± SE) between the sampled vaccinated and SW- injected salmon but the mean HSI (%) (± SE) from the sampled female salmon was significantly higher (P = 0.0336) than the male salmon (Table 4.2). The present experiment result in liver weight is also agreed with the result of Bayne & Gerwick (2001), Poppe et. al. (2014).

5.5. Fillet quality traits

5.5.1. Gaping

The crowding stress affected in the gaping scores in salmon in the experiment. There was numerically the highest gaping mean score in the vaccinated crowded slaughtered salmon from August sampling (0.7 ± 0.20) and the lowest was in the vaccinated standard slaughtered salmon from September sampling (0.01 ± 0.13) (Table 4.5). Michie (2001), Suzuki (1981), Love (1974), Mørkøre & Rørvik (2001) also found in their study that rough handling, stress can create more gaping in fillets of salmons. So their findings about gaping in salmon fillets are agreed with the finding of the present study.

5.5.2. Fillet pH

This parameter result from the present study is coinciding with the findings of Bjerkeng et al. (1997), Periago et. al. (2005) and Rørvik et al. (2010). The present study finding about pH measurement in stressed salmon was more improved than findings of Iwamoto et. al. (1987) and Robb (2001). Iwamoto et. al. (1987) reported from their study that there was no significant difference in the final post-slaughter pH of stressed and unstressed fish of the same species, despite differences immediately postmortem (Robb, 2001).

5.5.3. Fillet firmness

The finding of the stress effect on the texture of flesh of salmon from the present study is suited with the finding of Hyldig & Nielsen (2001), Hatae et al. (1990), Sigholt et. al. (1997). Sigholt et. al. (1997) found that the handling stress had a significant influence ($P < 0.001$) on the firmness of salmon fillet and the texture of the stressed fish was softer during storage, which is detrimental especially when slicing smoked salmon.

5.5.4. Fillet fat

The mean fillet fat (%) (± SE) in the sampled vaccinated salmon (17.1 ± 0.2) was numerically higher than the SW- injected salmon (16.5 ± 0.2) (Table 4.4). The fillet fat (%) result between the vaccinated and unvaccinated salmon is coinciding with the finding of Aursand et. al. (1994) and Rasmussen (2001).

5.6. Organ health

5.6.1. Adhesion

The adhesion mean score (± SE) in the vaccinated salmon (0.7 ± 0.07) was numerically and significantly ($P < 0.0001$) higher compared with the SW- injected salmon (-0.1 ± 0.08) (Table 4.6). The present experiment finding is matching with the findings of Midtlyng and Lillehaug (1998), Gatica et. al. (2008), Berg et al. (2006), and Vågsholm and Djupvik (1999). Midtlyng and Lillehaug (1998) found in their study that none or only minor adhesions were observed in the unvaccinated fish and the most severe lesions were observed in vaccination fish. Berg et al. (2006) who vaccinated groups of salmon parr at different times of the year and found that fish vaccinated early, at a small fish size and high temperature developed more intra-abdominal lesions than fish vaccinated later on larger fish size and lower temperature. Berg et. al. (2007) found that small Atlantic salmon parr develop more intra-abdominal lesions than big parr, when they are i.e. vaccinated with the same volume of oil-adjuvant vaccine. In contrast to the present study result, Vågsholm and Djupvik (1999) found an increased risk for abdominal lesions with increasing smolt weight in a cohort study.

5.6.2. Catarcat

The cataract in both eyes mean score (± SE) (0.8 ± 0.09) in the vaccinated salmon was numerically higher but not significantly ($P = 0.4863$) than the unvaccinated salmon (0.7 ± 0.10) (Table 4.6). This finding is agreed with the findings of Berg et al. (2007) and Grini et al. (2011).

5.6.3. The visceral fat

The visceral fat mean score (± SE) was numerically and significantly higher ($P = 0.0073$) from the sampled vaccinated salmon (3.7 ± 0.06) than the sampled SW- injected salmon (3.2 ± 0.06) (Table 4.6). This result is coinciding with the findings of Midtlyng et. al. (1996); Grigorakis et. al. (2002); Berg et. al. (2006); Berg et. al. (2007).

5.6.4. Fat contents in liver

The result of effect of vaccine on liver fat content from the present experiment is coinciding with findings of Hara and Radin (1978); Einen et al. (1999). Suzuki et. al. (2010) stated that fatty livers are frequently associated with metabolic disturbances which may be due to

numerous factors and lead to insulin resistance, oxidative stress, mitochondrial dysfunction, cytokine / adipokine interplay, and apoptosis.

5.7. Blood plasma chemicals

Alanin aminotransferase, cortisol, chloride, glucose, sodium, and inorganic P contents were increased in the stressed salmon than the standard salmon from September sampling (Table 4.9). Although, lactate dehydrogenase (LSMean ± SE) was increased in the vaccinated stressed slaughtered salmon (612 ± 115.8 U / L) group rather than the vaccinated standard slaughtered salmon group (311.6 ± 50.9 U / L), but in the unvaccinated stressed slaughtered salmon group (416.3 ± 57.2 U / L) contained lower than the unvaccinated standard slaughtered salmon group (673.3 ± 187.5 U / L) (Table 4.9).

The finding from the present experiment is agreed with the findings of Morales et al. (2005); Sumpter (1997); Ellis et. al. (2002); Edwin et. al. (2006). Campbell (2004) stated that some plasma chemicals may be useful tools to evaluate the health and stress condition of fish. Because stress has been reported to elevate plasma cortisol (Haukenes et. al. 2008) and glucose levels (David et. al. 2005). Many researchers consider as a "rule of thumb" that fishes undergoing stressful situations exhibit plasmatic increases of cortisol and glucose (Balm et. al. 1989, Barcellos et. al. 1999). In experiments of acute stress, the cortisol response is rapid but regularly becomes weak or disappears some hours after the exposure to stress (Davis Jr. & McEntire 2006). On the other hand as previously stated, stress hormones such as catecholamines, cortisol and others may be influenced by internal or external conditions in the history of the fish (anoxia, pollution, nutritive stress, physical stress) (Reid et. al. 1998). Sugar levels increase during stress, however some authors reported a weak rise of glucose (Davis Jr. & McEntire 2006), others found no change (Rotllant & Tort 1997, Jentoft et al. 2005), and even a decrease (Wood et. al. 1990). Primary stress responses trigger the sequential secondary response (e.g. increase in plasma glucose, lactate and hematocrit and decrease in chloride, sodium and potassium) in teleosts (Mommsen et al. 1999; Barton 2002). Bianca (2009) found that plasmatic levels of cortisol were increased quickly after exposure to acute stress and the standard conditions are restored in few hours. Barton (2002) stated that blood corticosteroid levels as an indicators of stress because the extreme sensitivity of the Hypothalamo–pituitary Interrenal (HPI) axis. These results are agreed with the finding of Pickering et al. (1982) who proved that

stress might increase secretion of catecholamine which initially suppressed insulin secretion and subsequently increasing plasma levels of glucose. Barnhart (1969) reported that creatinine levels in serum were correlated to age in rainbow trout. Sandnes et al. (1987) reported from their study that total protein, albumin and the total protein / albumin ration did not show any significant seasonal variations. Barton et. al. (1986) found an increase of plasma potassium in juvenile chinook salmon (*Oncorhynchus tshawytscha*) after multiple acute stressors. McDonald and Milligan (1992) reported that plasma potassium rises in teleosts after exercise strenuous enough to result in intracellular acidosis, which causes an outward leak of potassium from cells. Gatica M. C. et. al. (2010) found from their study that after crowding, the blood monovalent ion Cl- increased over 10 %. There was no significant difference in cortisol concentration between anaesthetized and crowded fish. Changes seen in the levels of blood Na+, Cl- and osmolality in the crowded fish were consistent with this mechanism and the high levels of cortisol found in this study.

5.8. Melanin parameters

5.8.1. Melanin in Fillets

The hypothesis that vaccine can create much melanin in salmon was not perfect all times in the present study. There had no significant difference (P = 0.2792) in the melanin in fillet mean score between the sampled vaccinated and unvaccinated salmon (Table 4.10). But there had significant differences in the parameter between some standard and stressed slaughtered salmon (Table 4.11). The result is agreed with the findings of Mørkøre (2012) and Koppang et al. (2005). 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. According to Koppang et al. (2005) 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 this coloration changes.

5.8.2. Melanin in abdominal wall and organs

There were significantly higher melanin in abdominal wall mean score (P = 0.0169) and organs mean score (P < 0.0001) from the sampled vaccinated salmon than the SW- injected salmon (Table 4.10). The result is coinciding with the findings of Koppang et. al. (2005; 2010); Arciuli M. et. al. (2012). Koppang et al. (2010) stated that melanization of the abdominal wall is linked to vaccination. Koppang et. al. (2005) observed that abnormal pigmentation of organs 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.

5.8.3. Melanin in tissues

It was investigated the features of melanin deposition in the vaccinated and unvaccinated salmon flesh in the experiment. There was much melanocyte in leukocytes both in the vaccinated and unvaccinated salmon (Fig 4. 4 & Fig. 4. 5). The melanocyte was created in the unvaccinated salmon muscle tissue may be due to feed contents, pathological effects or environmental effects. There was a large vacuole in cells surrounding with melanocytes, fibrosis and fatty infiltrates in the vaccinated salmon flesh tissue sample (Fig 4.5.i). The effect of vaccine on melanin deposit in tissues are coinciding with the finding of Agius & Roberts 2003; Scalia et. al. 1990; Sichel et. al. 1987. Koppang et.al. (2005) from histological investigation revealed granulomatous inflammation containing varying numbers of melano-macrophages. Vacuoles, either empty or containing heterogeneous material, were frequently seen. Sichel et. al. (1997) and Agius & Roberts (2003) found form histological analysis, a granulomatous inflammation in the affected tissue with different shaped melanin containing cells, interpreted as melanomacrophages, a specialized type of leukocytes found in ectothermic vertebrates.

6. CONCLUSION

The present study demonstrated variation in some biometric, fillet quality and organ health parameters of the vaccinated and unvaccinated salmon.

There was no significant difference of the mean final body weight ($P = 0.90$) between the vaccinated and SW- injected salmon.

The vaccinated salmon showed significantly higher mean CF ($P = 0.0014$) and fillet yield ($P = 0.0227$) compared with the unvaccinated salmon.

There were significant higher values in the mean adhesion score ($P < 0.0001$), fat in viscera ($P = 0.0073$) and fat in fillet score ($P = 0.0248$) in the vaccinated salmon compared with the unvaccinated salmon.

There was significant softer texture ($P = 0.0006$) in the posterior part of the fillet of the vaccinated salmon.

Melanin spots were found both in the vaccinated and unvaccinated salmon.

The findings from the present experiment can be economically important as the vaccine improved condition factor and fillet yield of salmon. It is recommended from the findings that the vaccine can improve the condition factor, fillet yield, fat contents in liver and fat contents in fillet flesh in salmon aquaculture.

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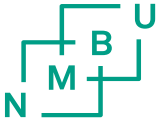
8.APPENDIX

Liver fat (%) in the vaccinated and unvaccinated (SW- injected) salmon from August sampling were measured in laboratory

Net pens	Salmon	Obser-vation No.	Sample (g)	Glass (g)	Glass +lipid (g)	Fat (%)	Average Fat (%)	CV (%)
F2	**SW- injected**	1	1.99	20.37	20.44	5.54	*5.7*	3.6
		2	1.99	20.13	20.19	5.82		
	Vaccinated	3	1.93	19.20	19.26	5.71	*5.6*	1.9
		4	1.95	18.77	18.83	5.55		
F5	**SW- injected**	5	1.99	21.13	21.18	4.60	*4.9*	8.8
		6	1.94	19.57	19.63	5.22		
	Vaccinated	7	1.90	20.56	20.63	6.09	*6.1*	0.3
		8	1.95	19.73	19.79	6.12		
F10	**SW- injected**	9	1.92	20.14	20.19	5.75	*5.6*	3.8
		10	1.92	20.71	20.76	5.45		
	Vaccinated	11	1.97	20.52	20.57	5.02	*5.0*	0.2
		12	1.98	19.47	19.53	5.01		

Results as mean of parallel samples, in % fat of weighed sample (one decimal and CV in %).

Variation between parallels shows good if coefficient of variation (CV) < 3.5 %.



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