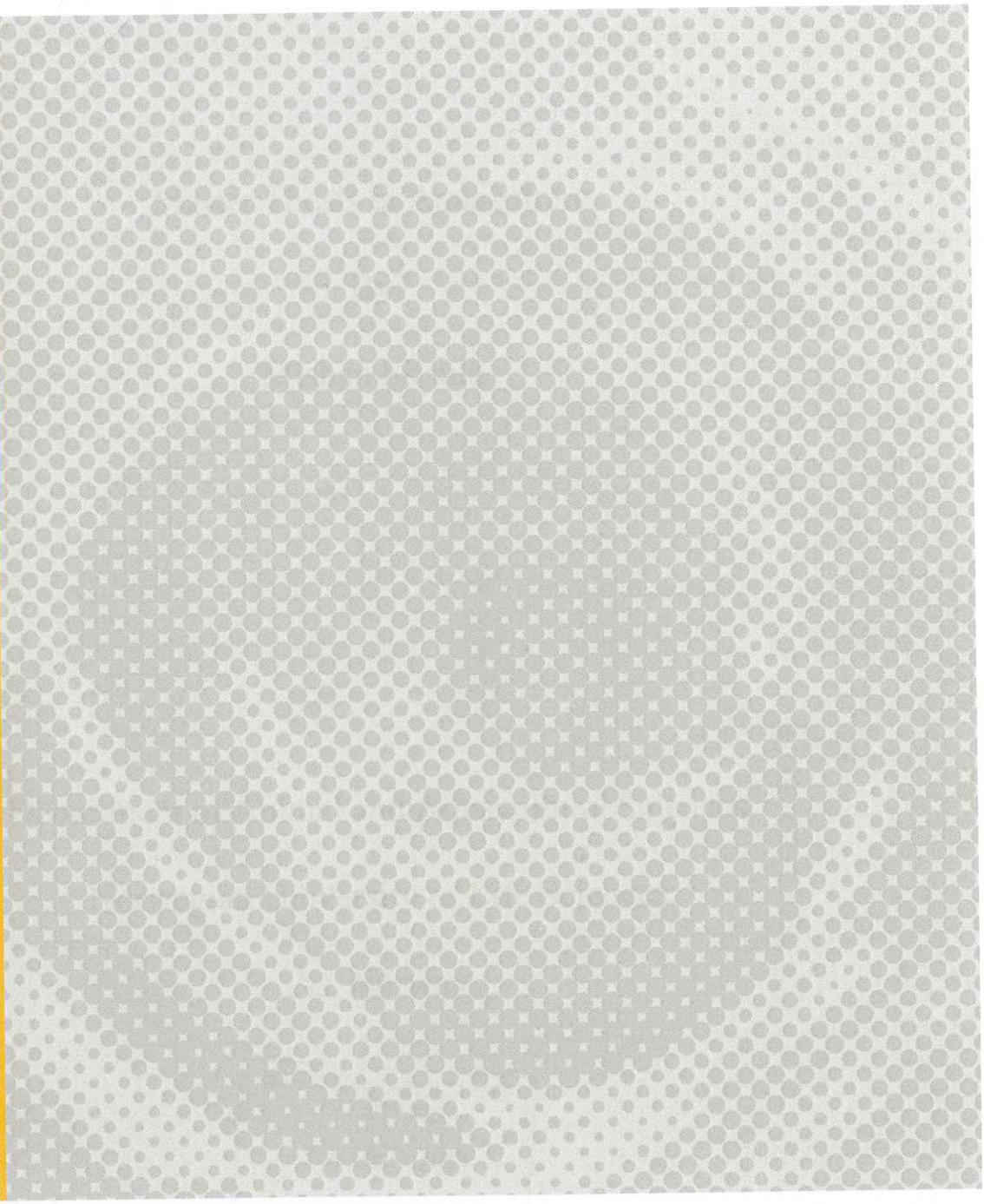
Measuring the fat content in intestine of Atlantic salmon by using computerized tomography (CT).

Mishan Sitaula



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Ås, 2013 *Mishan Sitaula*

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ABRIVATIONS

ALA=	Alpha linolenic acid
BW=	Body weight
CT=	Computer tomography
DHA=	Docosahexaenoic acid
EPA=	Eicosapentanoic acid
FM=	Fat in muscles
GW=	Gutted weight
ID=	Identity
IF=	Intestinal fat
IW=	Total intestinal weight along with fat
KF=	Condition factor
PUFA=	Polyunsaturated fatty acid
TAG=	Triacylglycerol
VSI=	Viscerosomatic index

SUMMARY

The aim of our study was to use the CT scanning as a non-destructive method to find out the amount of fat in the Atlantic salmon intestinal area. Farmed Atlantic salmon (*Salmo salar*) was subjected to computerized X-ray tomography (CT) to evaluate CT as a non-destructive and rapid method for analyzing intestinal fat percentage. The salmon that was used in this study were in size, weight and slaughter percentage representative for the commercial salmon in Norway today. The study consisted of a total of 95 Atlantic salmons with weight varying from 2,125 g to 7,369 g and lengths varying from 82 cm to 58 cm. Out of 95 fishes 25 of them were selected for scraping the intestinal fat manually. The correlation between the length and weight of the fishes is significant (R^2=0.79).

The fish were scanned at four anatomical positions (behind pectoral fin (scan 1), in front (scan 2) and behind the dorsal fin (scan 3) and, in front of anal fin (scan 4) through vent). The intestinal fat content correlated positively with CT values over all scan positions, demonstrating that CT can be used as a non-destructive method to predict the intestinal fat percentage in salmon ($p<0.001$). The highest fat content seemed to be present at the position in front of dorsal fin (scan 2) and the lowest fat content in front of anal fin (san 4). The position in front of the dorsal fin i.e. scan 2 (R^2=0.68) and the average scan (R^2=0.71) gave highest correlations value. The pixel values and the viscerosomatic index (VSI) for all scans were significantly correlated and, scan 2 gave a highest correlation value (R^2=0.65) providing the most information about the total fat content in the intestine and the slaughter yields. The scrap intestinal fat for 25 fishes and their VSI values were also significantly correlated meaning that CT scanner can be used in the production line in order to estimate the yield of fish carcass.

Our study show that the amount of intestinal fat can to a significant degree can be estimated by CT-scanning which has not been shown before. This method seems to be promising and suggesting that it can be used in breeding for salmon with less storage of fat in the viscera and consequently better slaughter percentages.

1. Introduction

Atlantic salmon is a high value fish species and the amount of fat contained in the salmon is one of the important quality criteria for consumer acceptance (Sigurgisladottir et al., 1997). Fat content in the fillet influences the quality characteristics such as gaping (Shearer., 2000) and color (Røra et al., 1998). Conventional fat measuring methods are laborious, time consuming and costly including dissection and chemical analyses (Rye., 1991). Therefore, it is important to have rapid, reliable methods of lipid measurement. In the industry and as a research tools fast, easy and non-destructive methods for analyzing traits of fish quality is becoming increasingly more important (Sigurgisladottir et al., 1997; Jørgensen and Thomassen., 2002; Kolstad et al., 2001). Computer tomography (CT) methods were developed and used to predict the body composition of Atlantic salmon in early nineties (Rye., 1991).

The computer tomographic technique uses X–rays that are collimated to provide a fan shaped beam that is passed through the body, while an array of detectors is positioned on the opposite side of the subject to detect the transmitted radiation (Ellis., 2000). This recording in the detector provides information about the relative density of a great number of small units in the cross sectional CT image (Kolstad et al., 2008). The great number of small units can be correlated to the body composition through computer programs (Rye., 1991; Jopson et al., 1995; Kolstad et al., 2004; Kolstad et al., 2008). Rye., (1991) used CT to predict carcass composition of Atlantic salmon. Kolstad et al., (2004) used CT scanning to quantify fat deposition and distribution in Atlantic halibut. Also Hancz et al., (2004) used CT scanning to measure the total body composition changes of common carp. It is not only the composition of the fish that we are able to measure through CT scanning but in addition it is also possible to see bones and deformities.

Atlantic salmon is considered as a fatty fish species with fat percentage in the range of 5.7-17.6% (Wold et al., 1996). Aursand et al., (1994) found that the highest fat content in the dorsal fat depot (38.4%), belly flap area (28.8%) and red muscle (27.2%), while white muscle contained 9.1% fat. The major stores are white muscle (35.4% of total fat depot), belly flap (13.7%), head (12.8%) and visceral fat (11.7%) (Aursand et al., 1994). The portion of the salmon fat which is deposited in the intestinal region is of use for the human purposes. Increase in visceral adipose tissue results in production losses when salmon are gutted during the time of harvest (Røra et al., 1998). Moreover, there is a limited supply of fish oil with

essential fatty acids like omega-3 fatty acids, EPA (Eicosapentanoic acid) and DHA (Docosahexaenoic acid). These fatty acids are essentials for the humans and can be obtained from the captured fishes. These captured fisheries are now fished at the maximum sustainable limit (Leaver et al., 2011) and it is important that these fatty acids taken by fish should be available for human consumption directly, or it should be in the edible part like fillet if given to farmed fish like salmon.

Breeding salmon with relatively low fat in the intestinal part will in addition result in high slaughter yield. This has an economic benefit. Deposition patter of such fat may be family dependent and can be inherited. Fillet fat shows significant heritability (Gjedrem., 1997) and has been included in salmon breeding programs for decades. Selection for these quality traits is based on family information from recordings after slaughter i.e. between family selections (Folkestad et al., 2008). A study with Atlantic salmon indicates that the level of n-3LC-PUFA, expressed as a percentage of total fatty acids in muscle is highly heritable (Leaver et al., 2011). The breeding scheme requires the compositional information from a large number of individuals and needs for the non-destructive methods in order to improve the carcass composition (Rye., 1991). So the computer tomography is the suitable method that has a high potential to use in these fields.

The main objective of the present study is to use the CT scanning as a non-destructive method to find out the amount of fat in the Atlantic salmon intestinal area. To do this we had to find the correlation between the scanned “fat” and manually scraped fat in the intestinal area, giving a model that can be used to determine the amount of fat stored around the intestine of a fish.

2. Background

Fat in the animal body is stored in adipose tissue in the form of triacylglycerol (TAG). This glycerol which is esterified with three fatty acid form densely packed water free fatty droplets. During the time of increased energy demand or fasting periods, these fatty acids stored are metabolized and released into the circulation system as an energy source. Human studied have shown that if energy intake is higher than the energy expenditure, and if a fat depot does not have a capacity to store fat from diet, an accumulation of a fat in other tissues and organs like hearts, liver and, muscles takes place (Frayn., 2000).

2.1. Synthesis of fat

Body composition seems to be connected to the form in which energy is supplied. Body fat can either originate from dietary supplied or de novo synthesized fatty acids. The pathway involving the lipogenesis includes the conversion of acetyl-coenzyme-A arising from the carbohydrates or the carbon residues remaining from amino acid deamination to fatty acyl – coenzyme-A via a series of carboxylation, condensation and reduction reactions (Wathne., 1995). Triacylglycerols are then formed by esterification of three fatty acyl molecules to glycerol-1-phosphate as shown in figure 2.1.

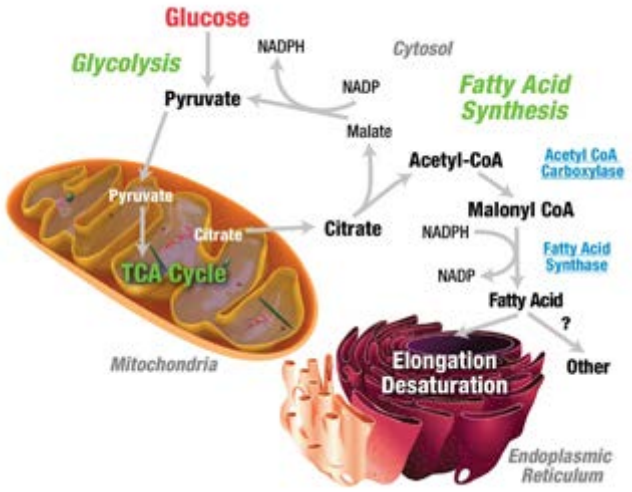


Figure.2.1. Pathways for de novo lipogenesis (Source: Herman and Khan., 2012)

2.2. Fatty acid uptake

There are not so many studies about the fatty acid uptake mechanisms into the adipocytes for fish. In case of mammals, the transport of fatty acids into adipocytes can occur through diffusion across the lipid bilayer of the plasma membrane mediated by protein (Todorcevic., 2009). Proteins those are associated are plasma membrane fatty acid binding protein, fatty acid transport protein, fatty acid translocase and caveolin-1(Potter et al., 1987; Schaffer et al., 1994; Abumrad et al., 1993; Trigatti et al., 1999; Todorcevic., 2009).

2.3. Fat deposition

Viscera, muscle and liver are the sites for lipid storage in fish (Sheridan., 1988). An increased in visceral fat index results in waste of energy and also results in decrease in dressing out percentage. In Atlantic salmon the primary sites of fat deposition are visceral adipose tissue and the connective tissue sheets called myosepta in the muscle (Nanton et al., 2007). Visceral fat are the fat depots that are found around inner organs and visceral adipose tissue is a connective tissue composed of adipocytes in major quantity along with adipose precursor cells, fibroblasts, endothelia cells, pericytes, monocytes and blood cells (Todorcevic., 2009). Growth in adipose tissue is due to increase in cell size and increase in cell number. Mature adipocytes are the largest cells of the body (Bernlohr., 2002) and they can increase their size by incorporating more TAG.

The relationship between excess lipid deposition in viscera and health of the fish is still not clear. But Tørud and Hillestad., (2004) observed the occurrence of high mortality due to poor heart health and stress in slaughter-sized salmonids. Increased fat level in different tissues and organs can lead to increase inflammation and risk for development of a diseases like type 2 diabeties, heart diseases etc. (as mentioned in Todorcevic., 2009). But it is relatively unknown whether it happens in fish also or not.

2.4. Methods of fat determination

Several methods exist for estimating the lipid content of the fish: chemical analysis, the Torry fat meter, computerized tomography (CT) and near infrared (NIR) spectrophotometry (Stien et al., 2006).

Solvent based extraction methods like ethyl acetate extraction is traditional method to determine the fat content in salmon fillets (NS 9402., 1994). These methods are reliable but time consuming and relatively costly (Folkestad et al., 2008).

NIR is the electromagnetic spectrum from 400 to 1100 nm. Predicting the fat content by Near-infrared spectroscopy is used in lab studies with accuracy of about +/- 0.8% (Wold et al., 1987). Estimation of fat content by measuring the whole fish is more challenging because skin also absorbs and, reflects electromagnetic radiation heavily in the NIR region. So it is difficult to obtain representative measurements from the interior of the fish muscle (Folkestad et al., 2008). But promising results was obtained on measuring fat content in whole and live salmon with NIR spectroscopy (Solberg et al., 2003). These all mentioned NIR were used for lab purpose not for the commercial purpose. Nuclear magnetic resonance (NMR) can also be used as the non-invasive fat determination in whole salmon (Veliyulin et al., 2006).

2.5. CT Scanning

CT utilizes the capacity of different tissues to attenuate X-rays. X-rays are a form of electromagnetic radiation with wavelengths between 0.01 and 10nm. The relation between CT values expressed in Hounsfield units (HU) and linear attenuation coefficient suggests a direct relationship between CT values to tissue type. These signals from images are collected and constructed by computer programs. Three-dimensional information about the body can be obtained by a series of contiguous cross-sections. This technique is very much sensitive and allows soft tissue such as adipose and, lean tissue to be clearly differentiated (Hounsfield., 1979). Soft tissues are expected to take CT values in range of -200 to+200HU while bone tissue and air takes CT values far outside above and below this range (Kolstad et al., 2008).

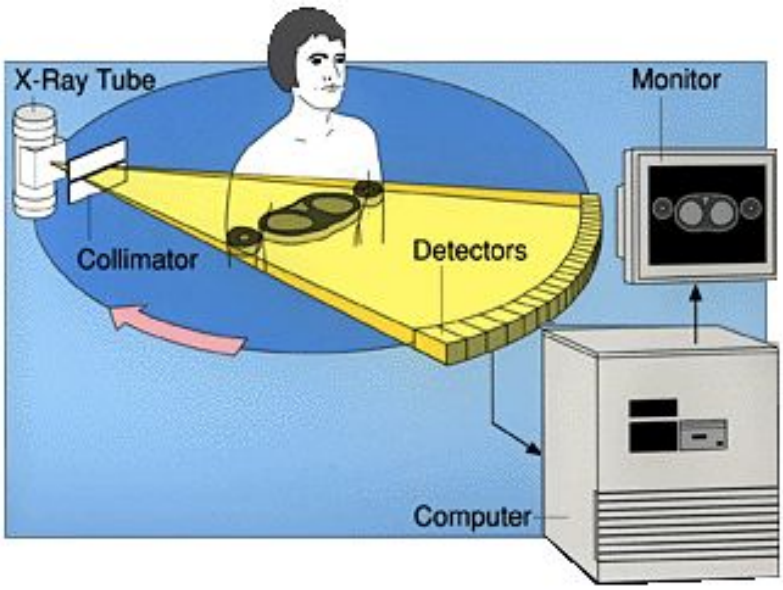


Fig.2.2. Diagrammatic illustration of CT scanning. (Source: http://www.imaginis.com/ct-scan/how-does-ct-work)

The study shows that CT scan can be used to predict dry matter percentage in Atlantic cod, and also can be used as a tool to compare dry matter percentage between groups such as males vs. females and family groups (Kolstad et al., 2008). CT scan was used by Gjerde., (1987) in a study on rainbow trout selection and found a high correlation between the observed and predicted values of water (0.88), protein (0.68) and fat (0.89) content. Rye., (1991) used CT to predict carcass composition of Atlantic salmon. Kolstad et al., (2004) used CT scanning to quantify fat deposition and distribution in Atlantic halibut. Also Hancz et al., (2004) used CT scanning to measure the total body composition changes of common carp.

2.6. Triacylglycerol synthesis and lipid droplet formation

Deposition of intracellular fat depends on the energy supplied to the organism. Excess amount of energy is stored in the form of TAG in the adipose tissue (Bernlohr et al., 2002). It is not only glycerol-3 phosphate from glycolysis taking part in producing TAG but also pyruvate from glyceroneogenesis can take part in glycerol formation (Ballard et al., 1967; Todorcevic., 2009). A Glyceroneogenesis pathway is important during the time of limited supply of glucose (Hemre et al., 2002) and occurs mostly in salmonids fish.

According to Todorcevic., (2009), adipose tissue have relatively low capacity to utilize fatty acids for energy production and mitochondrial fatty acids oxidation is still important to determine the amount of visceral adipose tissue. It was shown that the moderate amount of long chain n-3 fatty acids (as in fish oil) can reduce lipid accumulation in adipose tissue by increasing the fatty acid beta oxidation capacity while higher amount of n-3 fatty acid levels damage mitochondrial membranes which results the loss in ability to oxidize.

2.7. Intestinal fat

It is seen that level of EPA and DHA in feed can influence the amount of intestinal fat in Atlantic salmon (Todorcevic et al., 2008, Torstensen et al., 2011). In vitro experiment, it has shown that fatty acids rich in plant oils like 18:1n-9 leads to the higher lipid accumulation and low mitochondrial fatty acid beta-oxidation in fat cells of salmon compared to EPA and DHA (Todorcevic et al., 2008). Few studies of salmon is in accordance with the studies of mammals that have shown that fish oil rich in EPA and DHA can reduce the fat deposition in tissue and organs (Belzung et al., 1993; Flachs et al., 2005). Eicosapentanoic acid (20:5n-3, EPA) and docosahexanoic acid (22:6n-3, DHA) are those fatty acids to lower the triacylglycerol (TAG) accumulation in human adipocytes both in vivo and in vitro. But Todorcevic., (2009) showed the same situation in fish. Both EPA and DHA significantly lower TAG accumulation and increase fatty acid beta-oxidation in salmon adipocytes compared to oleic acid. Exact working mechanism about anti-obesity activity of EPA and DHA are still unknown but following mechanism can be involved, EPA and DHA inhibits important enzyme in lipid synthesis, increase the mitochondrial fat burning capacity and reduces the fatty acids intake in fat cells. Some studies show that it induces the fat cells death (Liu et al., 2008).

2.8. Saturated fat

Salmon feed is the main source of saturated fat. Each of the oils contains different proportion of saturated fats like fish oil (18-33%), palm oil (45-49%), chicken oil (28-30%). The content of the saturated fat in salmon feed went down over a time where a high percentage of fish oil was replaced by plant oil especially rape seed oil. Research has shown that if the percentage

of saturated fat increases compared to feed based on fish oil, the content in salmon fillet does not increase or increase very little (Torstensen et al., 2000; Bell et al., 2002). If the percentage of saturated fat in feed is reduced compared with the diet based on fish oil, the percentage in fillet is also reduced (Bell et al., 2001; Bell et al., 2004; Torstensen et al., 2004; Liland et al., 2013). Thus we can conclude that reducing the content of saturated fat in feed will have stronger influence on finished product then increasing the content of saturated fat in feed. The advantage of using saturated fat is that it can be calculated as neutral in relation to n-3/n-6 ratio and even if saturated fat has a bad rumors in connection to human nutrition. But salmon will be a small contributor in a complex diet.

Vegetable are characterized with high levels of 18-carbon fatty acids such as oleic acid (18:1 n-9) in rapeseed oil and olive oil, linoleic acid (18:2n-6) in sunflower oil and soybean oil, and alpha-linolenic acids (ALA) (18:3n-3) in linseed oil. The vegetable oils do not contain n-3 highly unsaturated fatty acids such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (22:6n-3). In contrast, fish oils are rich in n-3 higher unsaturated fatty acids. Todorcevic., (2009) showed that high dietary levels of n-3 higher unsaturated fatty acid can reduce the fat level in the visceral adipose tissue of Atlantic salmon in comparison to the fish fed vegetable oil diet. But higher oleic acid feed leads to a higher lipid accumulation around visceral area than EPA and DHA.

2.9. Omega-6

Fish oil has little omega-6 (<5%) while content of omega-6 in plant oil like soya, maize and sunflower is high. Content of the omega-6 in feed reflects strongly in a fillet (Bell et al., 2004; Liland et al., 2013). Digestion of polyunsaturated fats is better than monounsaturated and saturated fats (Johnsen et al., 2006; Sigurgisladottir et al., 1992). It is known from other breed that a higher intake of omega-6 reduces the changing of ALA (18:3n-3) to EPA and DHA because omega-3 and omega-6 competes for the same enzymes in altering to long and more unsaturated fatty acids (Emery et al., 2013). The important thing is maybe the negative properties of omega-6 fatty acids in the diet of the people. Unfortunately, this also seems to happen with omega-6 from salmon, since a diet with soybean oil-fed salmon, are high in omega-6, led to insulin resistance, fat accumulation in the liver and obesity in mice. There is a need for knowledge of the upper tolerable amount of omega-6 in feed for salmon both for

salmon feed intake, growth, health and welfare and, the interests of fillet contribution of omega-6 to a diet and the importance of the effect eating salmon will have on people's health when omega-6 contribution increases in proportion to the contribution of omega-3.

2.10. Relation between omega-3 and omega-6

Both for human and fish, it is the amount of the EPA+DHA in body that decides the health and not how much they take from food or feed. The reason for this can be many. In case of human, competition between omega 3 and omega 6 about placement in phospholipids is a central factor. In cell membrane omega-6 competes with EPA+DHA in the same positions in phospholipids. In the American people having a high level of omega-6 intake must adjust up the intake of EPA+DHA correspondingly to reach the wishing level of omega-3 level in cells and protection against the development of heart diseases (Blasbalg et al., 2011). Feed research with salmon containing constant amount of EPA+DHA but different in n-3/n-6 ratio shows that amount of EPA+DHA in fillet was constant (Liland et al., 2013). Amount of EPA+DHA in phospholipids in cell membranes was not target in the research (Liland et al., 2013).

Omega-3 and omega- 6 also competes for same enzymes systems in the syntheses of long polyunsaturated fatty acids from 18:2n-6 and 18:3n-3. In fish research with high 18:2n-6 (soybean oil) or high 18:3n-3 (linseed oil) shows that high 18:2n-6 gives increase 20:4n-6 in fillet (Liland et al., 2013) and high 18:3n-3 gives high EPA and DHA in fillet (Zheng et al., 2005). In one research with Atlantic salmon, introduction of a feed with moderate amount of 18:3n-3 led to a moderate increase in the level of DHA (Ruyter et al., 2000). High level of 18:3n-3 in feed predominantly resulted in accumulation of EPA but not in additional increasing in part of DHA. Correspondingly 22:5n-6 was not detected in fish groups feeds with increased level of 18:2n-6 even if increasing level of 20:4n-6 was observed. This low changing of carbon 22 fatty acids can depend upon the competition between 18:3n-3 and 24:5n-3 about Δ6-desaturase. Result indicates that the higher concentration of carbon-18 polyunsaturated fatty acids can inhibit altering to 22:6n-3. These indicate that it is important to take consideration to ratio between different 18 carbon fatty acids in feed to salmon for best possibility altering to DHA.

2.11. Resource accounts for EPA and DHA utilization

Leaver et al., (2011) has shown that EPA and DHA level in salmon muscle has a hereditability on h^2=0.77. In studies now going on, one investigates if salmon's capacity to produce EPA and DHA from ALA can be made better with the help of genetic selection (Berge et al., 2012). In addition in order to utilize a limited resource in the best possible way, it will be important that new knowledge about the total resource accounting throughout the life cycle of Atlantic salmon when different feeding strategies is varied. This should be studied as well in wide range.

2.12. Gene modification of fish

There is lots of genetic modification performed in the fish species and most of the modification is performed in fishes like salmon, tilapia and carp. Transgenic fishes have many positive attributes for aquaculture; increased growth, disease resistance, cold tolerance, sterility and altered metabolism to change the requirements for fish-based diets. We know little about the environmental and health impacts of genetically modified organisms.

Aqua Bounty technologies have produced a transgenic salmon modified with growth hormone from Chinook salmon. The result is a salmon with altered growth pattern. The production of the transgenic salmon is reported to be reduced to 18 months instead of normally three years. It should be noted that the production time is dependent on several factors such as family history, feed, harvest, production conditions and temperature. Today the production times for Norwegian farmed salmon at sea from 15 to 19 months achieve slaughter weight of approximately 3.5 to 4.5 kg. Nibe croaker is possible marine fish with limited ability to produce DHA. Kabey et al., (2012) have produced a transgenic Nibe croaker with a gene from Masu-salmon. This gene encodes a key enzyme (elongase) in the synthesis of long chain fatty acids. The transgenic Nibe croaker showed reduced EPA levels but increased production of DPA (22:5n-3), which is synthesized by chain extension of EPA. There is no information about the changes in DHA levels in fish. It is shown that the EPA and DHA level may be increased in transgenic zebra fish by genetically modifying the genes involved in the synthesis of long chain fatty acids; desaturase and elongase (Alimuddin et al., 2008). Level of EPA and

DHA was approximately 1.2-1.4 times higher in transgenic zebra fish compared with normal zebra fish.

Fish and mammals do not have enzymes which make it possible to convert n-6 to n-3 fatty acids. Round worm (*Caenorhabditis elegans*) has however, a gene fat-1 which has this property. Kang et al., (2004) have shown that transgenic mice with the gene from *C. elegans* can provide double bonds to an unsaturated fatty acid to convert n-6 to n-3 fatty acids. This result is a decrease in n-6 fatty acids and an increase in n-3 fatty acids. If this technology is also applied in food producing animals it will be possible to enrich animal products with n-3 fatty acids.

Genetic modification makes it possible to improve and add new features in fish, which can be of great value for the aquaculture industry. Salmon for example can be modified to maintain high capacity of EPA/DHA synthesis after transfer from fresh water to sea water, or added the gene for the enzyme capable of converting the n-6 to n-3 fatty acids. It is currently limited number of studies with transgenic salmon or trout, and much uncertainty about what impact genetic modification may have on the environment and fish health.

3. Materials and methods

3.1. Fish materials

One batch of Atlantic salmon was used in this study. Salmons were fed extruded commercial salmon feed in experimental cages at the sea water research station of AKVAFORSK at Averøy at the west coast of Norway.

3.2. Computer X-ray tomography (CT) analyses

95 whole fishes were taken for computer X- ray tomography (CT) analyses. The non-invasive examination of whole body samples was done by a SIEMENS Somatom AR HP computer X-ray tomograph (Siemens, Germany). Salmon carcass parallel cross sectional images were taken on four different positions as illustrated in the figure 3.1. Total scanning time was less than 1 min per image. The instrument settings used were: voltage 130 kV, slice thickness (i.e. scanning width) 3mm, zoom factor 2, exposer time 3 s and dosage 300 mAs. The X-ray beam goes 360^0 around the object, where a detector on the opposite side measures X-ray attenuation expressed by CT value in Hounsfield units (HU). From this CT images were created, with matrices of 512’512 elements (pixels) with defined areas. The CT values of the pixels calculated by the scanner were the weighted mean of the attenuation of X-ray for the tissues found in the volume of the pixel, and are linearly related to tissue density. The CT values from each pixel was converted into grey scale values and then displayed as an image on a monitor. Variation in grey scale values thus reflected variation in X- ray attenuation. Soft tissues are expected to take CT values in the range of -200 to +200HU, while bone tissue and air takes CT values far outside above and below this range (Kolstad et al., 2008).

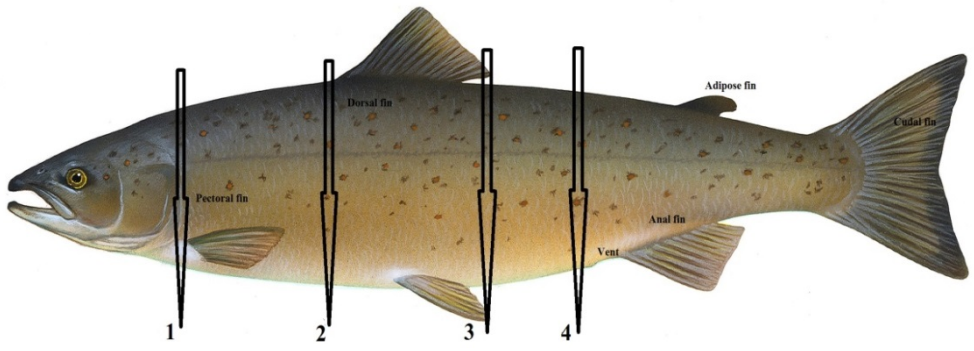


Figure.3.1. Fish diagram showing the four scan positions.

3.3. Gutting of the fishes

All the fishes after CT scanning were packed properly in the box with ice in it and transferred to Nofima. They were stored in the fridge for 2-3 days. Gutting of the fishes was performed on 5th, 6th and 7th of February 2013. Each fish was visually inspected for wounds. The weight and length of fishes were noted. After that each fish were dissected to remove the digestive track. Slaughtering was performed according to standard procedures at Nofima fish lab. The fat score of the fish were noted down. Weights of the liver and intestinal track were recorded. Out of 95 samples of intestinal tracks 25 of them were taken for further fat scraping process (figure 3.2). The fats collected from scraping around the intestinal region were collected and weights were noted. A very gentle and careful process was applied to scrape the fat from the intestinal regions. It was assumed that about 90% of fat were removed from the intestinal region.

Figure.3.2. Fat from visceral part of salmon.

3.4. CT image analysis

The CT images were analyzed further by software called MATLAB (AQUAGEN CT) to find the range of CT values for edible soft tissue excluding skin, fins and bone respectively. The image analysis software was then used to calculate mean CT value for the pixels within the defined range for soft tissue in each image. The stepwise four images (scan 1, scan 2, scan 3 and scan 4) of the same salmon are shown in the figure 3.3 below respectively. To be able to analyses for the pixels corresponding to fat in the intestinal part, each picture had to be manually selected and divided in two by drawing around the intestinal cavity on computer screen. These divided two parts give the values of fat in viscerosomatic area in one part and rest part of the body in another, as shown in figure 3.4.

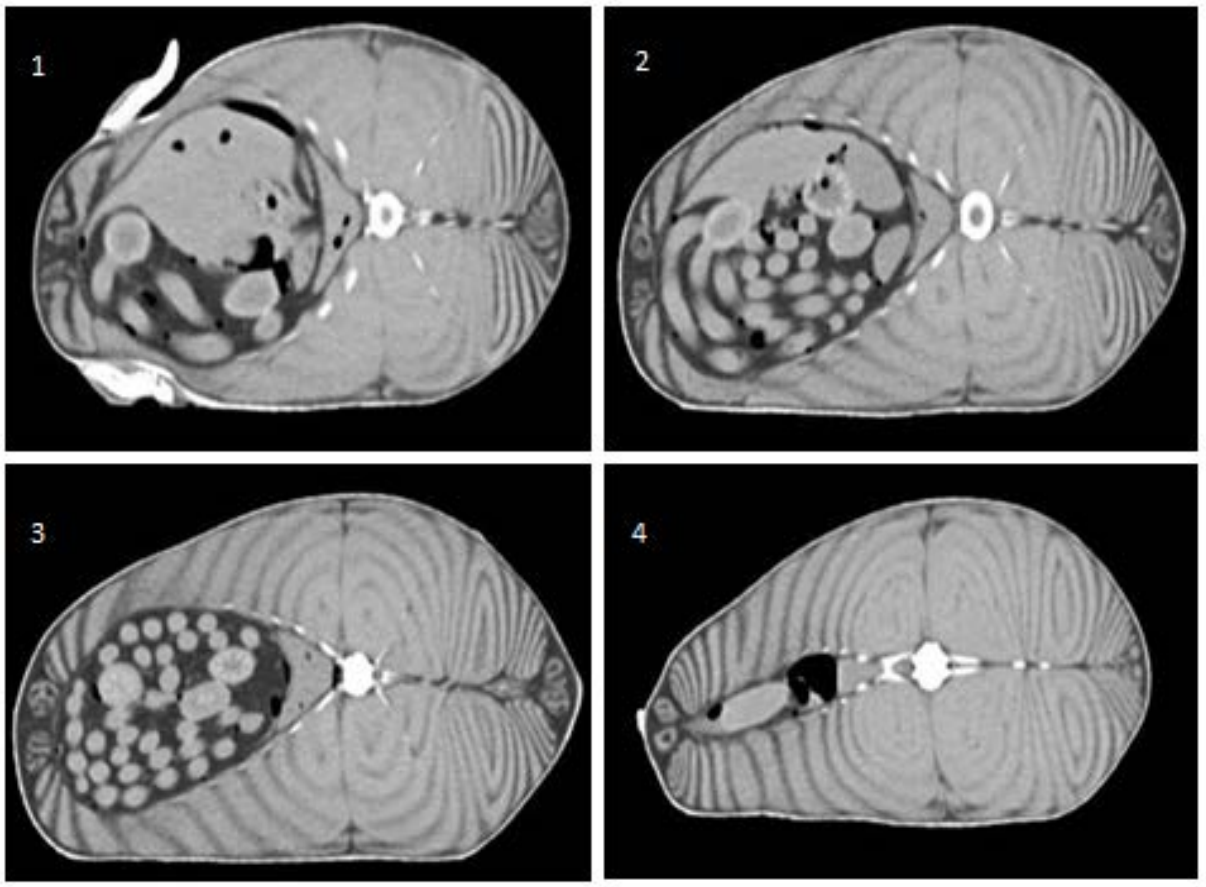


Figure.3.3. Images of each scan in position 1, 2, 3, and 4 respectively.

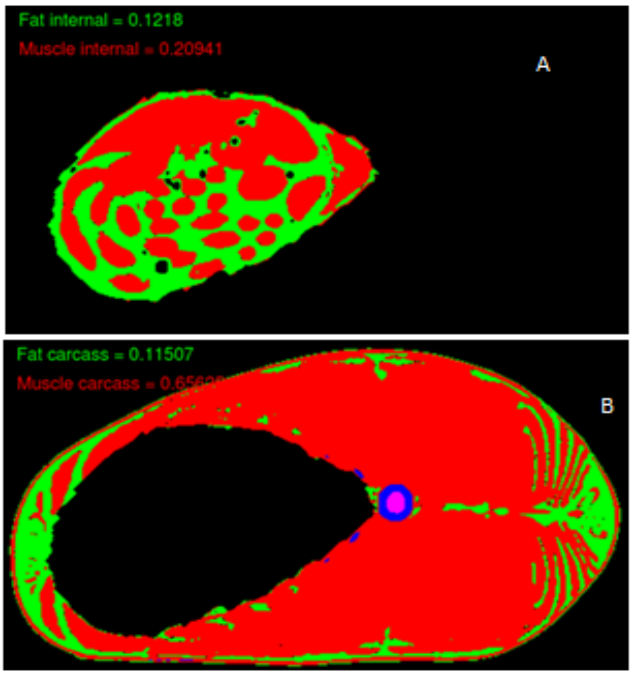


Figure.3.4. Images showing the amount of fat and muscles in carcass and intestine.

3.5. Statistics

Simple statistical analysis from excel sheet was used for the interpretation of data. All the data from the experiment were rechecked and they were within the range of standard deviation. Scatter diagram was used to predict the linear relationship and representative models between the variables.

4. Results

All the fishes taken for the experiment were of representative salmon for size and weight in commercial scale in Norway. The average weight and length of fish were calculated to be $\bar{x}$ = 4,475.7 g with standard deviation (s.d.) = 1,034.86 g and 72.19 cm with standard deviation (s.d.) = 4.99 cm respectively as shown in Table 4.1.

Table.4.1. Showing some parameters with their average values for 95 fishes.

Parameters	**Average**	**Standard deviation**
Weight	4875.7 g	1034.86 g
Gutted weight	4346.3 g	906.91 g
Length	72.19 cm	4.99 cm
Intestine with fat weight	534.4 g	140.9 g
Viscerosomatic index	10.75 %	1.18 %

To find the correlation between body length and body weight

The linear correlation between body length (cm) and body weight (g) was quite well significant (R^2=0.79) as shown in figure 4.1.

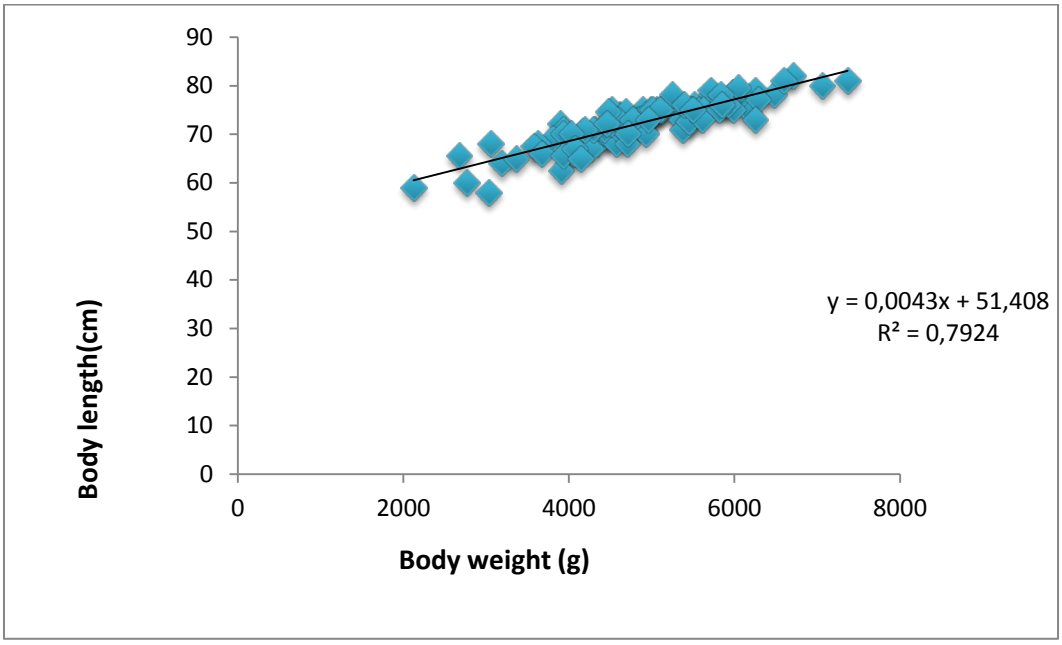


Figure.4.1. Graphical representation between body length (cm) and body weight (g).

Table.4.2. Table showing the values for 25 fishes from where visceral fat were scraped.

ID	BW(g)	GW(g)	Length(cm)	KF((g/cm3)x100)	VSI(%)	IW(g)	IFW(g)
49	4717	4198	70	1.38	11	519	253
50	4296	3855	71	1.2	10.27	441	170
51	4836	4364	73	1.24	9.76	472	78
52	4191	3793	71	1.17	9.5	398	82
53	4509	4024	72	1.21	10.76	485	197
72	5487	4861	75	1.3	11.41	626	219
73	5920	5257	76	1.35	11.2	663	267
74	5573	4941	75	1.32	11.34	632	242
75	4990	4466	75	1.18	10.5	524	212
77	5250	4649	78	1.11	11.45	601	225
78	5730	5143	78	1.21	10.24	587	121
79	5375	4702	71	1.5	12.52	673	226
80	5013	4479	75	1.19	10.65	534	228
85	5791	5073	76	1.32	12.4	708	270
86	5395	4797	76	1.23	11.08	598	281
87	5714	5100	79	1.16	10.75	614	233
88	5832	5202	78	1.23	10.8	630	231
89	5625	4920	75	1.33	12.53	705	291
90	4961	4380	73	1.28	11.71	581	287
91	5610	4966	73	1.44	11.48	644	287
92	5817	5061	75	1.38	13	756	318
93	4712	4192	70	1.37	11.04	520	168
94	5845	5253	76	1.33	10.13	592	171
95	4458	3983	72	1.19	10.66	475	167
96	5497	4900	75	1.3	10.86	597	262

(BW= Body weight in gram; GW=Gutted weight in gram; KF=Condition factor, calculated as (weight (g)/length^3 (cm)^3)x 100; VSI=Viscerosomatic index, calculated as (viscerosomatic mass/body weight)x 100%; IW=Weight of intestine along with fat (g) ; IFW=Weight of manually scraped fat (g))

The parameters like body weight, gutted weight, body length, condition factor, viscerosomatic index and fat score are presented in appendix table.

To find the correlation between pixel value of intestinal fat and scraped intestinal fat.

The variation of pixels value of the intestinal fat of 25 fishes is shown diagrammatically in the figure 4.2.

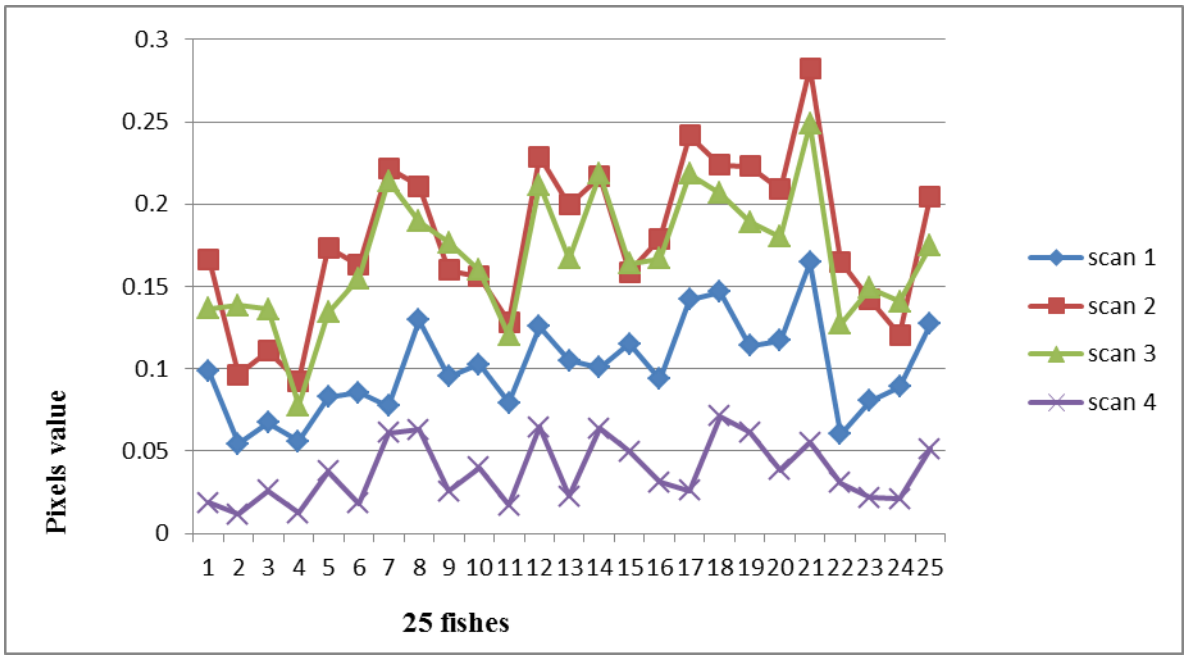


Figure.4.2. Graphical representation of volume of intestinal fat in terms of pixel values for 25 fishes.

The linear relationship between pixel values corresponding to intestinal fat from the CT scanning and amount of intestinal fat are statistically significant (R^2>0.5) except for scan 4 (R2<0.5) as shown in table 4.3. The significance of scan 4 is not so important because it lies at the end part of the fish body. The R^2 value for scan 1, 2, 3 and average are significantly correlated R^2=0.57, 0.68, 0.62 and 0.71 respectively as shown in figure 4.3 and 4.4. Out of four scans scan number 2 has a high R^2 value and also the position of the scan is in the intestinal region. So scan number 2 and average scan were chosen to predict the model of the equation. The model from these relationship obtained from the average scan relationship is best of all. The equations presented for scan 2 and average scan are as **y=1081x+25,895** and **y=1714.3x+11.553** respectively.

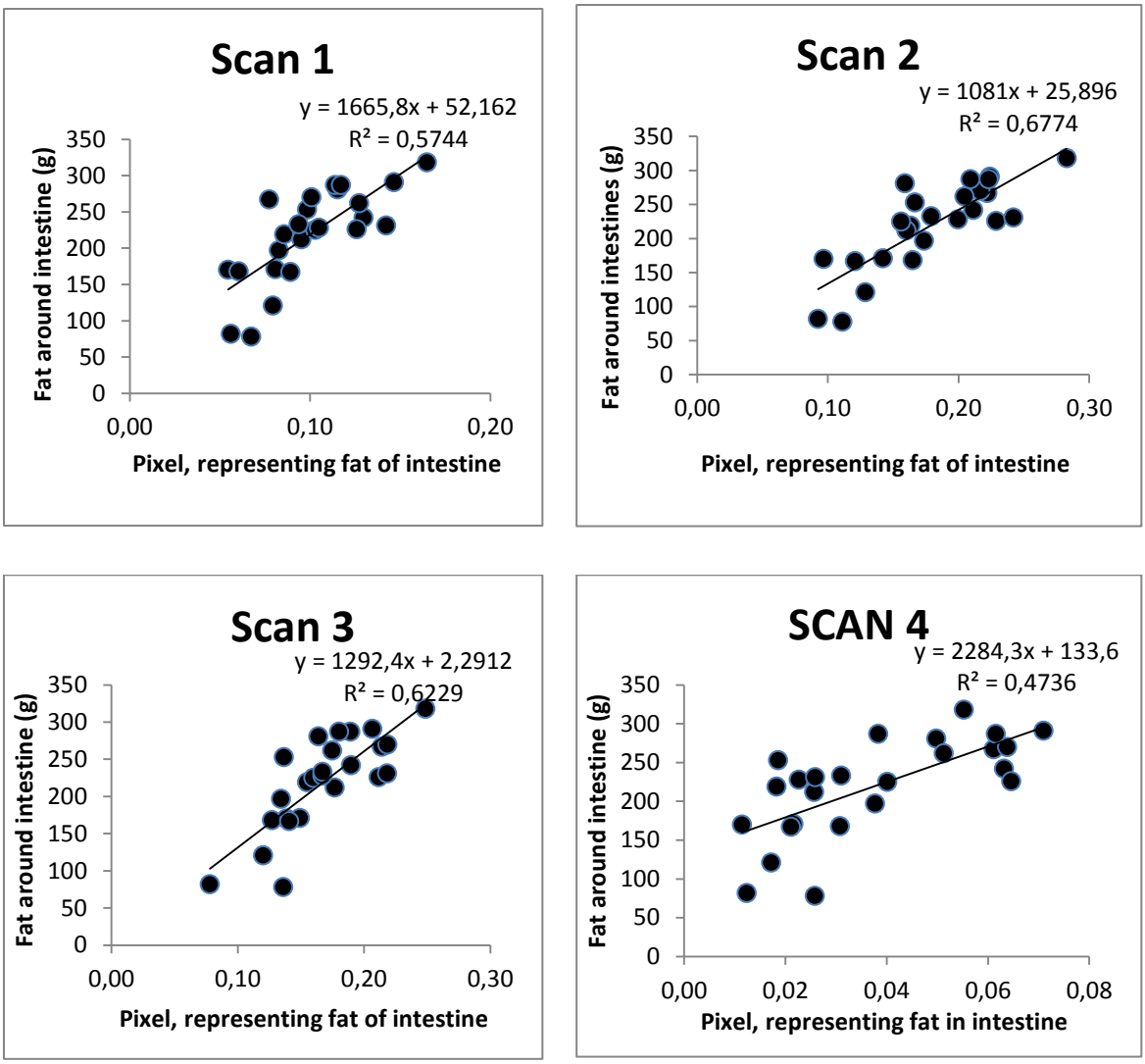


Figure.4.3. Graphical representation between fat around the intestine and CT scan value in the four different positions.

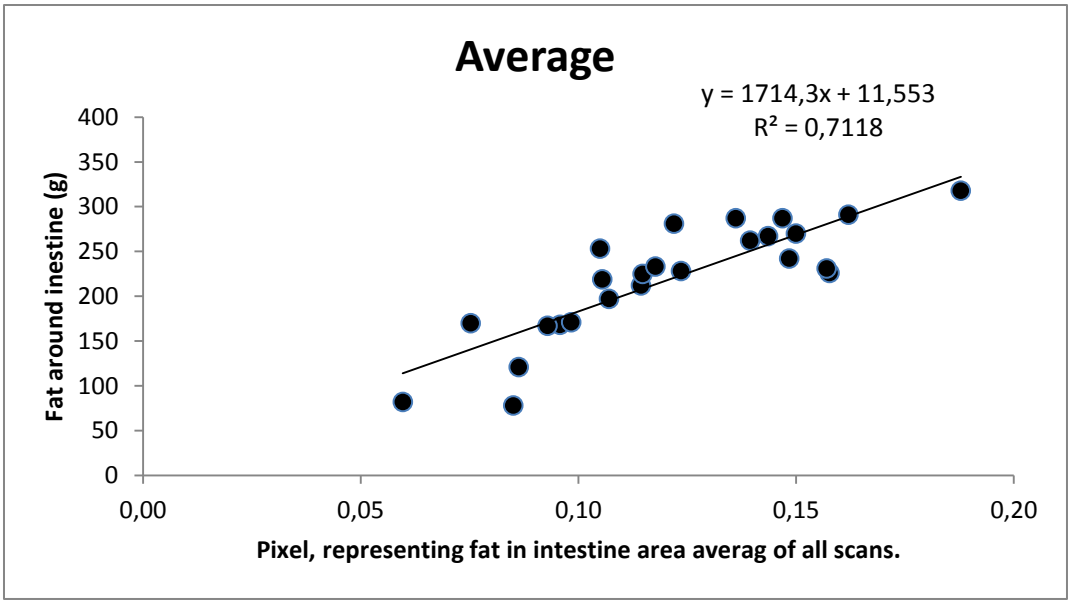


Figure.4.4. Graphical representation between fat around the intestine and average CT scan value.

Table.4.3. Table showing the R^2 value and p-value.

Scan	R2 value	p-value
1	0.57	0.000011
2	0.68	0.000004
3	0.62	0.000003
4	0.43	0.000143
Average	0.71	0.000001

To find the correlation between scraped intestinal fat and viscerosomatic index (VSI).

The linear relationship between scraped intestinal fat and viscerosomatic index (VSI) shows the significant variation (figure 4.5). The value for R^2 for scan 2 and average are given as 0.62 and 0.62 respectively.

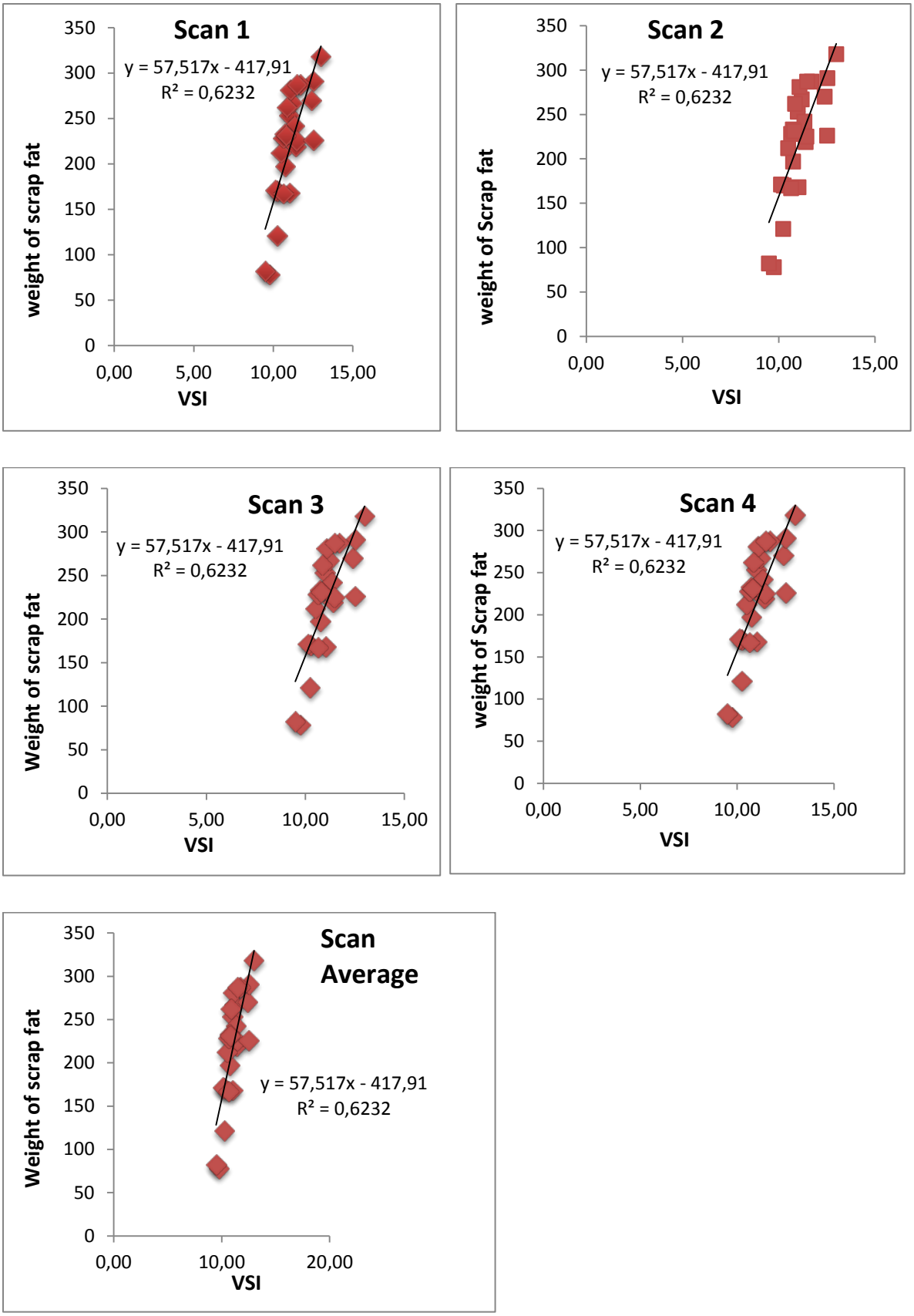
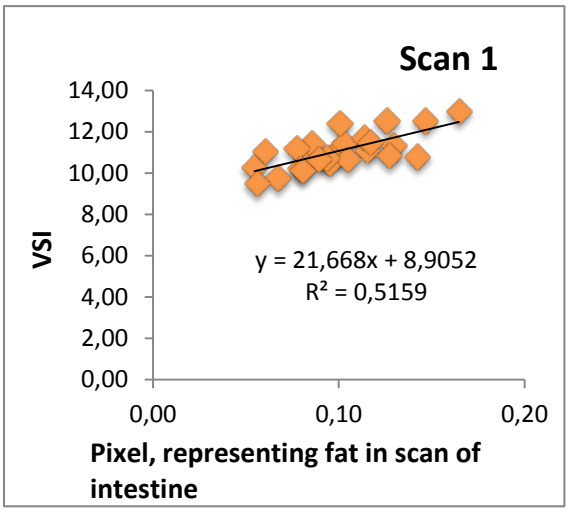
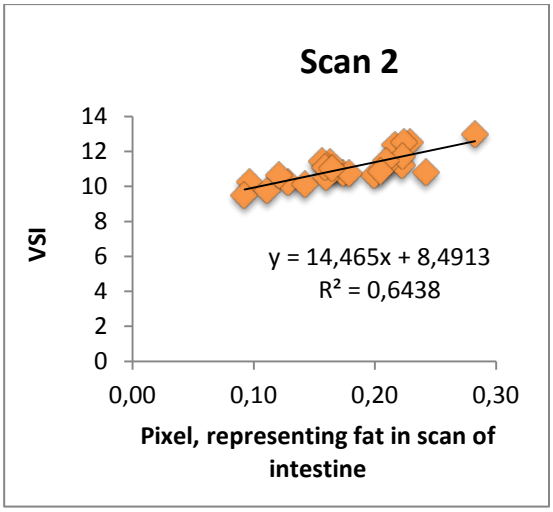


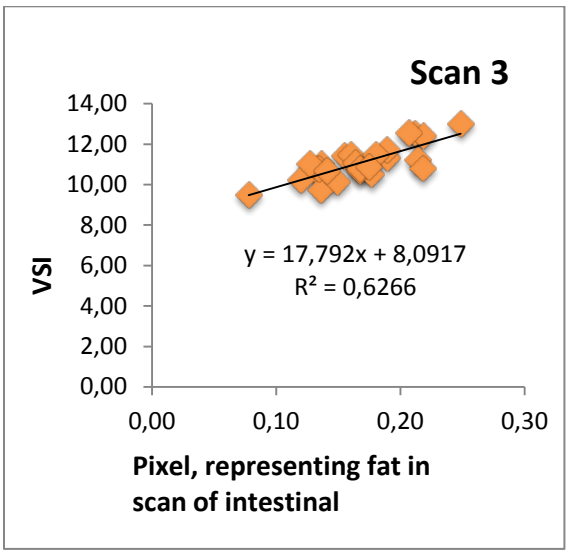
Figure.4.5. Graphical representation between a weight of scrap fat (g) and VSI (%).

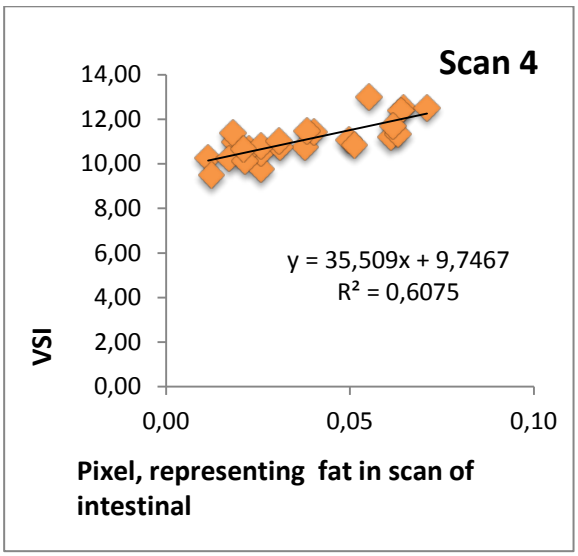
To find the correlation between the viscerosomatic index (VSI) and CT pixel value:

The linear relationship between the viscerosomatic index (VSI) and pixel value for scan 2 and, average are statistically significant (figure 4.6). The R^2 values for scan 2 and average are 0.64 and 0, 70 respectively. Figure 4.6 continues until next page.









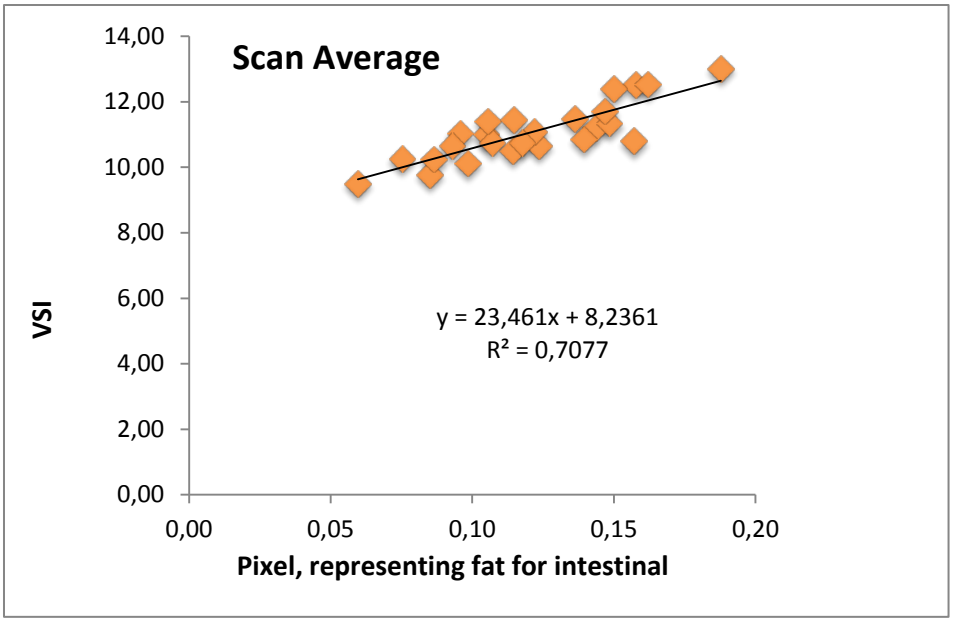


Figure.4.6. Graphical representation between VSI and CT pixel value for scan 1,2,3,4 and average.

To find the correlation between the viscerosomatic index (VSI) and calculated intestinal fat

The linear relationship between the viscerosomatic index (VSI) and calculated intestinal fat for the scan number 3 is statistically significant (R^2= 53) (figure 4.7).

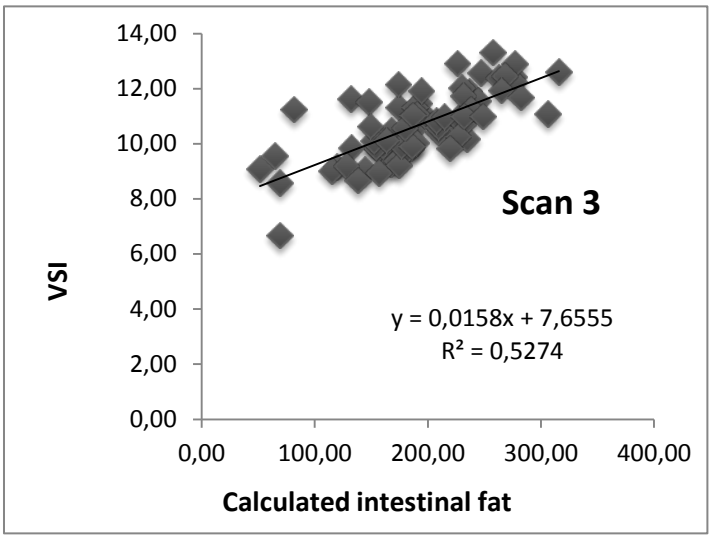


Figure.4.7. Graphical representation between VSI (%) and calculated intestinal fat (g) for scan 3.

To find the correlation between fat score and average pixel value of the intestine

The correlation between the average pixel value and fat score is shown in figure 4.8 below. The correlation was positive but was insignificant. The value of the fat score of all the fishes is shown in appendix table.

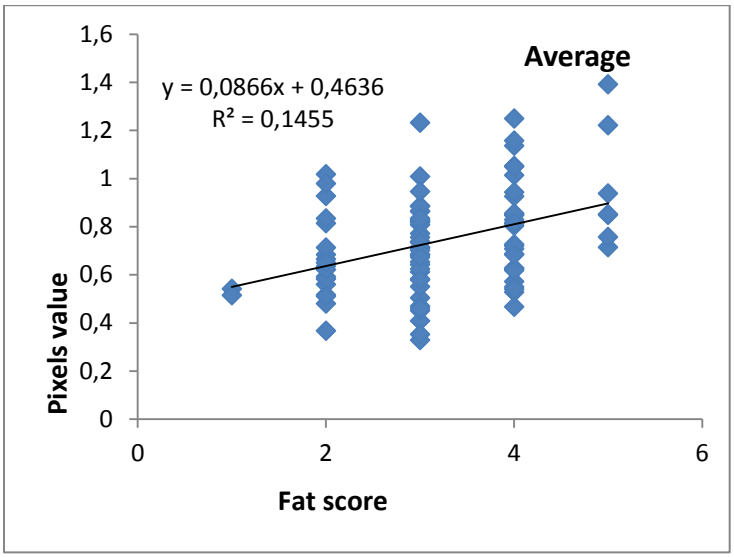


Figure.4.8. Graphical representation between pixel value of average and fat score.

5. Discussion

The salmon that was used in this study were in size, weight and slaughter percentage representative for the commercial salmon in Norway today. This should mean that the results obtained from the CT-calculations can be used to determine the fat content in intestines of slaughter-sized salmon for example breeding studies.

The correlation between the length and weight of the fishes is significant as shown in figure 4.1. Shearer., (1994) found out the importance of fish size that determines the composition of fish body. The fish size and fat deposition are closely related and are demonstrated in small rainbow trout (Reinitz and Hitzel., 1980) and, in Atlantic salmon (Rye and Gjerde., 1996). This means that there must be some relation between the body weight of the fish and fat deposition in the visceral area. But we did not find any significant correlation between body weights and scraped fat.

In salmonids the most marked variation in viscera weight is due to differences in visceral fat deposits (Gjerde and Schaeffer., 1989; Elvingson and Johansson., 1993). Viscera weight and abdominal fat deposition exhibit fairly high heritability for Atlantic salmon, the values were 0.3 (Rye and Gjerde., 1996) and 0.46 (Gjedrem., 1997). Thus, the prospects of achieving a rapid genetic gain in intestinal fat content by the selection of salmonids appear quite hopeful (Quillet et al., 2005).

While CT has been used for studying carcass composition in many fish species, to our knowledge it has not previously been used to estimate the fat content around the intestines. Visceral adipose tissue constitutes a major part of fat depots in salmonids. 11.7% of the total fat in a 2.8 kg salmon was visceral fat (Aursand et al., 1994). An increased in visceral fat index results in waste of energy and also results in decrease in dressing out percentage.

In this study we found out that the pixel value of intestinal fat obtained from the experiment correlated quite well with the intestinal fat content. The correlation was highest in the scan position number 2 (figure 4.3.) i.e. in front of the dorsal fin. The highest fat content seemed to be present at the position in front of dorsal fin and the lowest fat content in the last position i.e. position number 4 through vent and in front of the anal fin (figure 4.3.). Variables of the second scan positions seemed to be the most important regarding prediction of fat content. Fat analyzed in muscle part in position 2 also correlates better with fat in whole fish muscle than fat analyzed in the other positions (Rye., 1991). The fillet fat content in Atlantic salmon

decreases in the cranial-caudal direction as in several other salmonids species (Hardy and King., 1989; Einen et al., 1998). Study has shown that the Atlantic salmon contains in belly flap contains about three times more fat than the rest of the fillet (Aursand et al., 1994; Wathne., 1995; Einen et al., 1998). This could be easily seen also on the scans produced in this study.

CT has been used in research purpose for long time. It can be a non-destructive online tool for assessing carcass quality in the salmon industry. The sum of four scans positions provides information on fat proportion in the fish than in a single scan. It would, however be more efficient if one scan position could provide sufficiently accurate information. Our findings that the appropriate scan position which explains a significant part of variation in salmon intestinal fat also is the preferred scan for muscle fat determination indicates that the same scan position can be used for both purposes, reducing the time when a high number of fish has to be analyzed. Carcass evaluation can then be done in less than 5 min, including scanning, file transferring and image handling through CT scanning (Jopson et al., 1995).

According to the figure 4.6, CT pixel values and the viscerosomatic index (VSI) for all scans were significantly correlated. From figure 4.5, we can see that scrap intestinal fat for 25 fishes and their VSI values also were significantly correlated. This means that CT scanner can be used in the production line in order to estimate the yield of fish carcass. Kolstad et al., (2008) concluded that CT can be used as a non-destructive carcass evaluation method in research and for industrial process. While investment cost can be the most heavy point on it. Also the correlation between the pixel value and VSI for scan position 2 is higher in comparison to others (figure 4.6.) and so scan position 2 provides the most information about the total fat content in the intestine and the slaughter yields.

6. Conclusion

The results from the present study show that the amount of intestinal fat can to a significant degree can be estimated by CT-scanning. To the best of our knowledge, this has not been shown before. A further confirmation of our models by using another set of salmon should be done, but we think that our results are promising and suggesting that this method could be a good help for example in breeding for salmon with less storage of fat in the viscera and consequently better slaughter percentages.

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APPENDIX

Appendix table showing body weight, gutted weight, body length, condition factor, viscerosomatic index and fat score of all 95 fishes. (Table continues until last page. Each parameter are explained below the last table)

ID	BW	GW	LENGTH	KF(g/cm3)x100	VSI	FATSCORE
1	2677	2434	65.5	0.95	9.08	2
2	2125	1922	59	1.03	9.55	2
3	2765	2512	60	1.28	9.15	3
4	3897	3637	72	1.04	6.67	3
5	3935	3598	71	1.10	8.56	3
6	3803	3361	69	1.16	11.62	4
7	3034	2718	58	1.56	10.42	5
8	3054	2778	68	0.97	9.04	2
9	3623	3216	68	1.15	11.23	4
10	6278	5647	78	1.32	10.05	4
11	5979	5302	78.5	1.24	11.32	3
12	6479	5674	78	1.37	12.42	5
13	3577	3246	67.5	1.16	9.25	4
14	3187	2900	64	1.22	9.01	3
15	3367	3014	65	1.23	10.48	5
16	3672	3226	66	1.28	12.15	4
17	3936	3550	70	1.15	9.81	2
18	7369	6440	81	1.39	12.61	3
19	7062	6276	80	1.38	11.13	4
20	6713	5915	82	1.22	11.89	3
21	6140	5460	75.5	1.43	11.07	4
22	6086	5354	78	1.28	12.03	3
23	6251	5530	79	1.27	11.53	3
24	5998	5264	78	1.26	12.24	3
25	5989	5400	75	1.42	9.83	3
26	6048	5417	79.5	1.20	10.43	3
27	6599	5927	81	1.24	10.18	4
28	6290	5700	77	1.38	9.38	3
29	4342	3796	68	1.38	12.57	5
30	4456	4021	70	1.30	9.76	3

31	4486	3993	69.5	1.34	10.99	5
32	4169	3756	70.5	1.19	9.91	3
33	4459	3946	72	1.19	11.50	4
34	4311	3886	71	1.20	9.86	3
35	4060	3650	66	1.41	10.10	4
36	4572	4048	68	1.45	11.46	3
37	4616	4020	74	1.14	12.91	4
38	4429	3955	71	1.24	10.70	3
39	4915	4359	72	1.32	11.31	1
40	4162	3745	69.5	1.24	10.02	3
41	3907	3423	62.5	1.60	12.39	5
42	4516	3978	75	1.07	11.91	4
43	3935	3573	65.5	1.40	9.20	3
44	4893	4381	75	1.16	10.46	2
45	4265	3845	67	1.42	9.85	4
46	4645	4100	70	1.35	11.73	4
47	4890	4349	73	1.26	11.06	4
48	4569	4116	73	1.17	9.91	3
49	4717	4198	70	1.38	11.00	2
50	4296	3855	71	1.20	10.27	4
51	4836	4364	73	1.24	9.76	2
54	4028	3629	70	1.17	9.91	4
55	4915	4379	72	1.32	10.91	3
56	4703	4205	68	1.50	10.59	4
57	4684	4189	74.5	1.13	10.57	2
58	4930	4294	70	1.44	12.90	4
59	4587	4177	73	1.18	8.94	3
60	4769	4253	73	1.23	10.82	3
61	4476	4065	74.5	1.08	9.18	2
62	4073	3720	67	1.35	8.67	2
63	4145	3691	65	1.51	10.95	4
64	5331	4667	74	1.32	12.46	4
65	5125	4599	75	1.21	10.26	3
66	5474	4936	73	1.41	9.83	2
67	5836	5140	77.5	1.25	11.93	5

68	5357	4755	73	1.38	11.24	4
77	5730	5143	78	1.21	10.24	1
78	5375	4702	71	1.50	12.52	3
79	5013	4479	75	1.19	10.65	3
80	5454	4902	74.5	1.32	10.12	3
81	5452	4853	72.5	1.43	10.99	4
82	5094	4532	75	1.21	11.03	2
83	6251	5521	73	1.61	11.68	4
84	5791	5073	76	1.32	12.40	3
85	5395	4797	76	1.23	11.08	4
86	5714	5100	79	1.16	10.75	3
87	5832	5202	78	1.23	10.80	4
88	5625	4920	75	1.33	12.53	4
89	4961	4380	73	1.28	11.71	3
90	5610	4966	73	1.44	11.48	3
91	5817	5061	75	1.38	13.00	3
92	4712	4192	70	1.37	11.04	2
93	5845	5253	76	1.33	10.13	3
94	4458	3983	72	1.19	10.66	2
95	5497	4900	75	1.30	10.86	3

(BW= Body weight in gram; GW=Gutted weight in gram; KF=Condition factor, calculated as (weight (g)/length^3 (cm)^3)x 100; VSI=Viscerosomatic index, calculated as (viscerosomatic mass/body weight)x 100%; fat score was scored manually)