M.Sc. 2012

EFFECT OF DIETARY FAT LEVEL AND EXERCISE ON GROWTH, FEED UTILIZATION, NUTRIENT DIGESTIBILITY AND FAT DEPOSITION IN ATLANTIC SALMON (*SALMO SALAR L.*)

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MASTER THESIS 30 CREDITS 2011

NORWEGIAN UNIVERSITY OF LIFE SCIENCES MDCCCLIX

NORWEGIAN UNIVERSITY OF LIFE SCIENCES (UMB)

Effect of dietary fat level and exercise on growth, feed utilization, nutrient digestibility and fat deposition in Atlantic salmon (*Salmo salar* L.)

Master thesis

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16/05/2011

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- Co-supervisor: Dr. Bente Ruyter

Abstract

The aim of the present study was to investigate the effects of dietary fat level and exercise on growth, feed utilization, nutrient digestibility and fat deposition in farmed Atlantic salmon (*Salmo salar* L.). Atlantic salmon (1409 ± 43.3 g) were reared for 97 days in net pens in sea water with water temperatures ranging from 8.9 to 5.5°C and natural photoperiod. The experiment was designed as a 2x2 factorial design with water current (high and low) and fat level (high and low) as the main factors. The feeds were formulated with a mixture of fish oil and rapeseed oil (50:50) to obtain low fat (250 g kg^{-1}) and high fat (350 g kg^{-1}) diets. Fish were individually marked with PIT-tag and distributed into total 12 net pens (100 fish/pen). The results showed that high fat diets had higher diameter and lower Doris durability than the low fat diets. High fat diet also showed a significantly higher digestibility of dry matter, fat and energy. No significant effect of diet was observed on body composition or fat deposition in liver, heart, visceral fat and white muscle. The numerical values showed a higher fat content in organs from fish fed high fat diets. High water current showed improved weight gain and growth rate as well as feed utilization. No differences were observed in organo-somatic index (OSI), proximate chemical composition of the body or fat deposition in organs and white muscle. Fat digestibility was lower in salmon from high current. No other differences in nutrient digestibility were noted. Growth tended to be highest in fish fed high fat diet and kept at high current. These results suggest that training in combination with high fat diet improve productivity at the fish farm.

Key words: Atlantic salmon; exercise; high fat diet; growth; digestibility; feed utilization; fat deposition

Acknowledgments

I would like to express my deep gratitude to my supervisors, Dr. Mette Sørensen and Dr. Bente Ruyter for their guidance, valuable advices and discussion throughout this thesis work at Nofima Marine Research Center and Department of Animal and Aquacultural Sciences.

I would like to acknowledge to Inger Øien Kristiansen and Målfrid Tofteberg Bjerke for their technical advice in laboratory; and also staffs in UMB, Nofima Marine, and Tromsø Aquaculture Research Station for their help and support.

Thanks to my family, my fiancé and his family, also my friends for supporting me achieve this goal today.

Giao Quynh Nguyen Luyen

Ås, 15th May 2011

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ADC	Apparent nutrient digestibility
BL^{-1}	Body length per second
CF	Condition factor
CMS	Cardiomyopathy syndrome
CSI	Cardio-somatic index
DM	Dry matter
FCR	Feed conversion ratio
FO	Fish oil
HSI	Hepato-somatic index
HUFA	Highly unsaturated fatty acid
NQC	Norwegian quality cut
OSI	Organo-somatic index
PIT	Passive integrated transponder
PUFA	Polyunsaturated fatty acids
S.E.M	Standard error of the mean
SGR	Specific growth rate
TGC	Thermal growth coefficient
VFI	Viscero-fat somatic index
VO	Vegetable oil
WG	Weight gain

1. Introduction

1.1 Feed for Atlantic salmon

Feed is supposed to promote an efficient growth at minimal cost. Feed composition need to be carefully designed in order to meet nutrient requirements to a growing fish. Body composition of the fish is changing when the animal is growing. Shearer, *et al.* (1994) reported that the protein content of Atlantic salmon fed commercial diets increased slightly, whereas fat content gained considerably with growth and increased fish size over the life cycle. The increasing fat deposition in the growing salmon indicated a need for more energy in the feed for body weight gain. Thus, chemical composition in feed for salmon is changing throughout the life stage (Table 1). An increasing energy demand is met by use of more oil in the diet. At present, the fat level in commercial diets for large Salmon may be up to 40% (Sørensen, *et al.*, 2011). High fat content has a protein-sparing effect because dietary protein will be used for muscle growth and not as a source of energy (Bendiksen, *et al.*, 2003b). Carnivorous fish such as salmonids have a limited ability to digest and utilize starch as an energy source because the metabolic capacity is adapted to utilize protein and fat for energy (Lovell, 2002). Fats have become a major supply of energy in the salmon diet because it historically had the lower cost per energy unit.

Table 1. Example of nutrient and energy composition of commercial Atlantic feed (Biomar, 2011)

Feed type	Fat %	Protein%	Gross energy (MJ/kg)
Start feed-smolt	15-22	58-48	21.0-22.4
Growing feed	28-32	36-40	23.4-25.2
Super growing feed	38-42	36-40	25.5-27.3

Cold water fish, such as salmonids, have a specific requirement for essential fatty acids (n-3 HUFA) in the diet to meet nutritional requirement and maintain the membrane functions at low water temperature. This is mainly because marine fish have a limited ability to convert 18:3 n-3 and 18:2 n-6, relative to vegetable oil sources, to 20:5 n-3 and 22:6 n-3 (Sargent, *et al.*, 2002). Traditionally, fish oils of marine origin are often used in salmonids culture to provide sufficient n-3 highly unsaturated fatty acids (HUFA). In particular, fish oil from capelin and herring are rich sources of n-3 fatty acids and therefore are excellent fat sources for salmonids. However, a steady increase in aquaculture production increases the use of global fish oil, therefore putting more pressure on the marine capture fisheries and sustainable supply of fish oil in the long term (Tacon,Metian, 2008). Vegetable oils (VO) are used as alternative sources to partly replace fish oil (FO) because of the high cost of marine oils. Studies have shown that vegetable oils can replace up to 50-60% of FO in diets for Atlantic salmon without negative effects on growth, feed efficiency, and fish health during seawater culture phase (Rosenlund, *et al.*, 2001; Bell, *et al.*, 2003; Karalazos, *et al.*, 2007; Wilson, *et al.*, 2007; Bell, *et al.*, 2010). In particular, rapeseed oil has shown positive effects in studies with Atlantic salmon post-smolts fed high energy diets (Karalazos, *et al.*, 2007; Karalazos, *et al.*, 2011).

A concern with high fat diet is that salmon tend to deposit excess fat in tissues as a response to increased dietary fat level. Research has shown that Atlantic salmon (2.5 kg) fed a high (38 - 47%) dietary fat compared to a low (31%) dietary fat deposited higher muscle fat (Hemre,Sandnes, 1999). Furthermore, fatty acid composition in the tissues also reflects the fatty acid profile of fat sources used in the feed. Changes in fatty acid profile consequently change the fat composition in red and white muscle (Torstensen, *et al.*, 2000; Torstensen, *et al.*, 2004; Torstensen, *et al.*, 2005), in egg and fry (Rennie, *et al.*, 2005), in liver, belly flap and intestine (Torstensen, *et al.*, 2000; Ruyter, *et al.*, 2006; Jordal, *et al.*, 2007). Also fatty acid composition in the diet may affect fish health which was reported by Seierstad *et al.* (2005), who observed that there was significantly correlation between body weight, fish length and heart weight to cardiac pathology in farmed Atlantic salmon when 50/50% mixture of fish oil and rapeseed oil used in feed. The authors suggested that the higher content of n-6 polyunsaturated fatty acids (PUFA) may have unintended negative effects on heart, resulting in histopathology. Consequently, cardiac lesion may increase mortality in seawater phase for farmed salmon (Wagner, *et al.*, 2004). These researches suggested that both level and source of fats should be considered in order to avoid unintended negative effects. From a consumer perspective it is also important to ensure a high content of health-promoting 20:5 n-3 and 22:6 n-3 (Sargent, *et al.*, 2002).

1.2 Exercise affecting fish health

Research in human nutrition have reported that intake of a high caloric diet, combined with less physical training led to overweight/obesity and higher cardiovascular disease risk (Schrauwen,Westerterp, 2000; Palaniappan, *et al.*, 2002; Goris,Westerterp, 2008; Ramel, *et al.*, 2009; Zhang, *et al.*, 2010). However, regular physical activity in combination with a low caloric diet is reported to reduce obesity and risk of heart disease (Clifton, 2008; Woolf, *et al.*, 2008; Tjonna, *et al.*, 2009). According to Hjeltnes, *et al.* (2009), high mortality has become one of the main challenges of salmon industry during grow-out phase in sea water. Mortality was caused by high disease outbreaks caused by viral and bacterial diseases and ecto-parasites. A salmon already infected with bacterial or viral diseases (or weakened for other reasons) are also more susceptible for infections with for example sea lice. Also, Shehzad (2009) reported that at least 42.2% of fish from 2700 farmed salmon from 291 different families in Norway suffered from heart inflammation (epicarditis), under the examination of Nofima and Norwegian School of Veterinary Science in 2008. It can be questioned if there is a relationship between the lifestyle of the fish and diseases which could influence production quality and losses of the fish farms.

Exercise of fish is related to swimming activity of fish, and is strongly correlated to water current velocity. At present, the major goal for commercial Atlantic salmon farming is how to achieve the highest performance at the lower cost in respect to growth performance, fish health and economic targets (Hardy,Barrows, 2002). Salmon are reared in net pens with high density until they get optimum marketable-size. Consequently, the limited space for swimming activity under present farming conditions compared to wild salmon, as well as the use of low protein/high fat diets, may cause unintended negative effects. The excess fat deposition found in white adipose tissues of fish fed high energy diets, may affect fish health leading to inflammation in salmonids (Todorcevic, *et al.*, 2010). Previous research has shown that exercise may enhance fat metabolism (Davison, 1997). Forster and Ogata (1996) reported that juvenile Red sea bream under sustained training had lower fat content in whole body compared to un-trained fish. Previous studies have also shown that moderate exercise training led to improved growth and food conversion efficiency in many cultured fish species (reviewed by Davison, 1997), which reflected positive appetite response to swimming activity in fish (Yogata,Oku, 2000; Bugeon, *et al.*, 2003; Ibarz, *et al.*, 2011). There are also research showing that long-term training in fish enhanced muscle structure with stronger connective tissues and firmer texture giving better flesh quality (Bugeon, *et al.*, 2003). The positive effect of exercise on reproduction in adult Sockeye salmon showed that non-exercised females had delayed maturation, lower rates of egg deposition and survival compared to exercised females (Patterson, *et al.*, 2004). Sustained exercise at low speed (0.5 BL s^{-1}) increased oxygen consumption, cardiac output, and blood oxygen content in Chinook salmon (Gallaugher, *et al.*, 2001), indicating beneficial effects on cardiovascular system (Farrell, 2002) and therefore enhanced fish health.

1.3 Physical quality of extruded diets

Extrusion technology is used for production of commercial high energy diets for salmonids. Ingredients are transformed into a dough by use of high temperature (120-140^{o}C) and high pressure (20-30 bars), and shaped to pellets that can be fed to fish. During the process, dietary starch is gelatinized aiding binding and expansion as the pellets leave the die under pressure reduction. This process allows improved control with pellet bulk density due to formation of air pockets in the structure. Degree of expansion is used to adjust oil absorption capacity and buoyancy control (Hardy,Barrows, 2002) to meet dietary energy requirement for salmon species. Moreover, gelatinization of starch during the process leads to improved digestibility. Changes in processing conditions and ingredient compositions affect physical quality of feed. Because research have shown that physical quality of feed may interfere with feed intake (Aas, *et al.*, 2011), physical quality of pellets should be monitored in feeding experiments with fish.

As fish performance is an indicator of fish health, it is important to gain more knowledge about the interaction between fish nutrition, rearing condition and fish health. To achieve this target, the main objective of the current experiment was to investigate the effects of high fat and low fat in the diet, and exercise on growth, feed utilization, digestibility of main nutrients and fat deposition in farmed Atlantic salmon (*Salmo salar* L.) in seawater. The physical quality of extruded diets was also investigated.

The experiment was carried out under the Nofima Marine counterpart project in cooperation with The Fishery and Aquaculture Industry Research Fund (FHF) (2010-2011) termed 'The Robust Fish Priority program'.

2. Materials and methods

2.1 Experimental diets

Two experimental diets containing low (250 g kg^{-1}) and high (350 g kg^{-1}) fat were produced at Nofima Ingredients, Bergen, Norway. The first batch of both diets was produced in 13th July 2010 and the second batch in 15th September 2010. The oil source was a mixture of fish oil and rapeseed oil (50:50). The same main ingredients were used for both batches of feed production. The main ingredients and chemical compositions of the two experimental diets are shown in Table 2.

Table 2. Main ingredient composition (g kg^{-1}) and calculated chemical composition (g kg^{-1} DM) of the experimental diets

Ingredients (g kg^{-1})	Low fat	High fat
Fish meal[1]	343.6	291.5
Fish oil[2]	100.0	150.0
Rapeseed oil[3]	100.0	150.0
Wheat[4]	100.0	100.0
Soy protein concentrate[5]	118.5	100.0
Pea protein concentrate[6]	118.5	100.0
Wheat gluten[7]	71.5	60.6
L-Lysine HC[8]	7.0	7.0
DL-methionine[9]	5.0	5.0
Vitamin premix[10]	20.0	20.0
Mineral premix[11]	5.2	5.2
Mono sodiumphosphate[12]	10.0	10.0
Yttrium oxide Y_2O_3[13]	0.1	0.1
Chemical composition (g kg-1 DM)		
Dry matter	920.0	920.0
Crude protein	489.0	414.0
Crude fat	254.0	354.0
Carbohydrate	173.0	159.0
Ash	84.0	73.0

[1] Norse-LT-94, Norsildmel, Norway

[2] NorSalmOil, Norsildmel, Norway

[10]Vitamin premix, per kg finished product: vitamin A, 2500 IU (Rovimix A-500); vitamin C, 1.0 g Rovimix Stay-C (Ascorbate phosphate 35%); vitamin D3, 2400 IU; a-tocopherol 0.2 g; thiamin-Cl, 0.01 g; riboflavin 0.02 g; pyridoxine-Cl 0.01 g; Ca-panthotenate 0.04 g; niacin 15 g; folic acid 5.0 mg; vitamin B12, 0.02 mg; biotin 1.0 mg; myo-inositol, 0.4 g; vitamin K2, 0.04 g

[11]Mineral premix, per kg feed: Mn, 35.0 mg, ($MnSO_4$); Zn, 90 mg (Zn_2SO_4); Cu, 12 mg ($CuSO_4$); I, 2 mg (KI); Se, 0.2 mg (Na_2SeO_3); Cl, 1.25 g (KCl)

[13]Used as inner marker

2.2 Feed processing

Feed production was carried out by Nofima Ingredients, Bergen. The macro-ingredients were ground in a hammer mill (HM 21.115, Wuppertal, Germany) using a 1 mm screen; then manually weighed and premixed in Wolfking mixer (300 l, Slagelse, Denmark). The mixture of micro-ingredients (vitamins, minerals and carotenoids) and fish meal was premixed in a Bjørn Varimixer prior to adding to the Wolfking mixer. The mixing time of all ingredients was 30 minutes before the feed mash was entered into an atmospheric double differential cylinder (DDC), where it was added steam and conditioned for approximately 3 minutes. The experimental diets were extruded by a co-rotating twin-screw extruder (Wenger), using two different dies. Low fat diets were produced using a 8 mm die and the high fat diets were produced using a 7.2 mm die. Two die inserts were used for both high and low fat feeds and the same setup was used for the productions in June and September, respectively. The extruder parameters are provided in Table 3. The drying process was 50 minutes in a carousel dryer (GMBH) at 60-80 °C for cooling down extruded pellets about 50 °C prior to coating process. The Dinnissen vacuum coater (BY, Land) allows coating extra fat onto extruded pellets (10kg/batch, at 0.2 bar). The finished feeds were cooled down to ambient temperature, then sieved and packed in 25kg/bag.

2.3 Experimental fish and tagging

The experiment was performed at Tromsø Aquaculture Research Station (Tromsø, Norway) in seawater (33±1‰). Atlantic salmon (*Salmo salar* L.) (1409 ± 43.3 g), from SalmoBreed stock with a natural sex ratio and post-vaccination, were reared in net pens from 10th August 2010 to 17th November 2010. Fish were anesthetized and individually PIT-tagged before distributing randomly into total 12 net pens (100 fish/pen). The fish were kept under natural photoperiod.

High mortality occurred in the beginning of the experiment (13th August 2010) due to handling stress, such as injection of PIT-tag and distribution of fish into pens.

Cardiomyopathy syndrome (CMS) was diagnosed in the fish population before the experiment started. After distribution of fish to the pens, the fish were infected by the ectoparasites (*Caligus elongates*). Because of the mortality, pens were supplemented with new fish (19th August 2010). The total numbers of fish were 105 fish/pen. The fish were also treated with chemical agent to reduce sea lice and given sliced feed (Slice®vet, Skretting, Stavanger, Norway) in the period 20th – 26th August 2010.

Table 3. Extruder parameters during the feed production

	First production[a]		Second production[b]	
Process parameters	Low fat	High fat	Low fat	High fat
Feeding rate, kg h^{-1}	150	150	150	150
Water conditioner, kg h^{-1}	9	9	12,6	9,6
Steam conditioner, kg h^{-1}	11,4	11	14,5	12-13
Die pressure, Bar	9-10	8,1-8,4	6,6	7,4-8,4
Max barrel temperature, °C	110**	117**	112**	120**
Die temperature, °C	69***	61***	62***	64***
Torque, %	28	24-25	26-27	26-27
RPM	400	400	460	453

[a] Produced in 13th July 2010; Feeding from 13th August – 28th October 2010

[b] Produced in 15th September 2010; Feeding from 29th October 2010 – Termination of the experiment 17th November

**Temperature measured in zone 4, the temperature is expected to be up to 140 ° C

***Temperature measured in the cooling zone before the die. The extrudate was expected to have a temperature up to 120 ° C before cooling

2.4 Experimental net pen design

The net pens were arrangements and pen numbers given the different treatments are shown in Fig. 1 and Fig. 3. The high and low current treatments were designed by use of different mesh size in the nets on two sides of the pen in the current direction. The net pen constructed for low current treatment was designed by use of smaller mesh size and extra addition of a net for shielding. In this regard, the size of net pen was 5.1 x 5.1 x 6 m used 10 mm mesh size knot to knot; shielded by 5.5 x 5.5 m netting size with 10 mm mesh size knot to knot. The nets for shielding were applied on two sides of net pens exposed directly to water current. For high water current treatment, the size of net pen was 5.2 x 5.2 x 7 m used 25 mm mesh size knot to knot. All net pens were also equipped with 1.2 m height of jumping nets to prevent fish escapes. The water speeds at high and low water current treatments were recorded for two of the pens (Fig. 2). To collect uneaten feed, a lift-up system (China-hat ø 75 cm with 3" hose, LiftUP Akva AS Ltd. Eikelandsosen, Norway) was installed in all net pens.

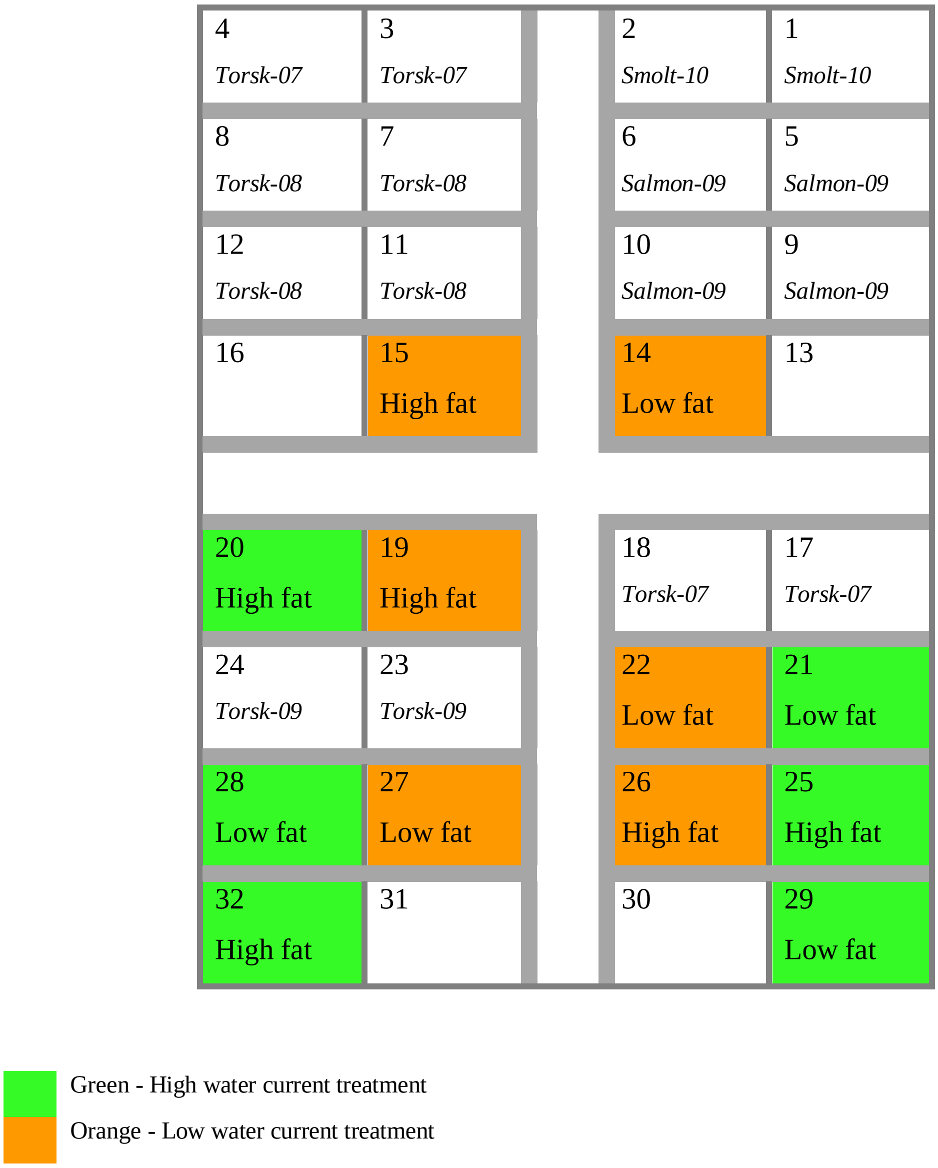

Fig. 1. Net pen arrangement and the pen numbers giving the treatments

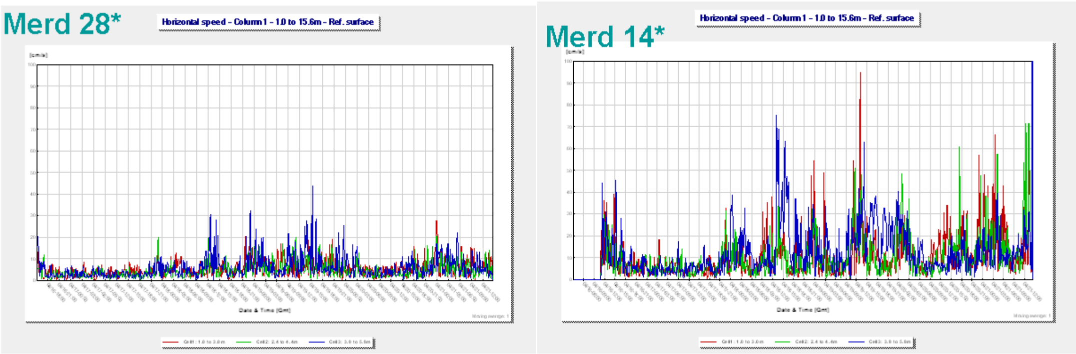

Fig. 2. The water speeds at high (net pen 28) and low water current treatments (net pen 14)

Horizontal water speed (measured by Aanderaa DCS 4100R, AADI, Bergen, Norway) at 1 - 6 meter depth inside an unexposed (left panel) and exposed (right panel) net pens at Tromsø Aquaculture Research Station in the period 16th – 21th April 2010.

Scaling at y-axis goes from 0 to 100 cm/s. Different depths are indicated with different colour codes. In the unexposed pen (ex. pen 14), water speed was low and stable being below 10 cm/s for more than 90% of the time. In the exposed pen (ex. pen 28), water speed was considerably higher and also more variable, being below 10 cm/s for only 65% of the time and with daily peaks at about 50 cm/s.

2.5 Experimental design

The experiment was designed as a 2x2 factorial design with water current (high and low) and fat content (high and low) as the main factors, resulting in 4 different treatment groups in triplicate. The pen numbers for the treatments and net pen arrangement were shown in Fig. 1 and 3.

HFHC			HFLC			LFHC			LFLC		
20	25	32	15	19	26	21	28	29	14	22	27

Fig. 3. The pen numbers for the treatments

HFHC: high fat, high current

HFLC: high fat, low current

LFHC: low fat, high current

LFLC: low fat, low current

2.6 Fish feeding

The feeding experiment was carried out from 13th August 2010 - 17th November 2010 lasted for 97 days, in which the fish were given the experimental diets for 90 days and slice feed for 6 days within the period 20th – 26th August 2010. The feeding regime was combined between automatic feeding with respect to 100% of feed intake and hand feeding in excess of 10%. The fish of each treatment were fed 4 meals/day at the fixed time following natural photoperiod, which are given in Fig. 4. During the period of the experiment, the sea water varied from 8.9 to 5.5°C given an average temperature by 7.19°C and total day degrees by 697.5°C.

However, the hand feeding was performed to replace the automatic feeder for feeding from Monday to Friday (6th September 2010 – 12th November 2010) because the automatic feeder did not work satisfactorily resulted in low quantity of distributed feed. Fish were fed 2 meals/day following appetite feeding at 9:00-10:00 am and 12:00-13:00 pm. For feeding at the weekends, the automatic feeders were used following the regime (Fig. 4).

The collection of uneaten feed was carried out by using lift-up system but the data were decided not to use in further analyses in order to calculation of the feed intake and feed recovery, which was explained due to too low biomass per net pen caused an accurate collection.

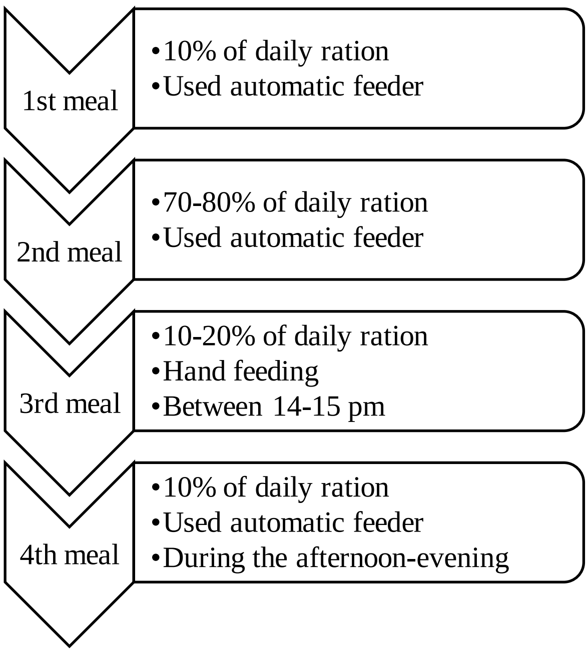

Fig. 4. Experimental feeding regime following natural photoperiod

2.7 Weighing and final sampling

For chemical composition of the experimental diets, feed samples of 2 kg were taken from diets of two feed productions, stored at − 20°C prior to analyses.

All fish were counted, individually weighed and measured length in the start and end of the experiment under anaesthesia (100 mg/l MS-222). Tricaine Methanesulfonate (MS-222) at level 100 mg/l was used for the anesthesia of fish during sampling procedure.

To estimate whole body proximate composition from the holding start population in the beginning, 10 fish (n=10) were randomly collected, anaesthetized, killed with a blow to the head, and frozen at – 20 °C prior to obtain 2 replicated samples (5 fish/sample).

At the end of the experiment, total 10 fish (n=10) were sampled in each treatment group; 5 fish for organ analysis and 5 fish for whole body composition. All fish were anaesthetized, killed with a blow to the head prior to sampling. Faeces were collected from these fish (n=10) by stripping according to Austreng (1978), which were then frozen and stored at – 20 °C until freeze drying for further analyses. For analyses of organs and growth, 5 fish (n=5) were measured length, body weight, and internal organ weights (heart, liver, visceral fat). White muscle samples (4x3x2cm) without skin were taken from the left side of the Norwegian quality cut (NQC) (Fig. 5). The organs and white muscle samples were rapidly frozen in liquid nitrogen, stored at – 80 °C for later analyses of crude fat.

To determine whole body proximate composition, 5 another fish (n=5) were taken. The fish were minced and homogenised together and stored at – 20 °C for chemical analyses. All the samples taken from each pen were represented for the mean values obtained for each treatment condition.

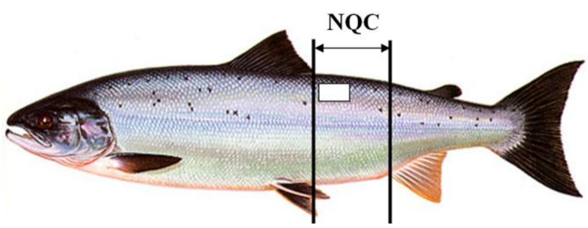

Fig. 5. White muscle sampling position for chemical composition of fat taken from the left side of the NQC (white area).

2.8 Recording and calculations

Daily feed intake and uneaten feed were recorded during experimental period. Dead fish were removed daily and mortality was also recorded to data. The following equations were used to analyze treatment effects (Karalazos, *et al.*, 2007; Karalazos, *et al.*, 2011):

Feed conversion ratio	$FCR = (\text{feed intake, g}) \times (\text{wet weight gain, g})^{-1}$
Specific growth rate	$SGR\ (\% / \text{day}) = 100 \times [\ln W_1 - \ln W_0] \times (\text{days})^{-1}$
Thermal growth coefficient	$TGC \times 1000 = [(W_1)^{⅓} - (W_0)^{⅓}] \times (\text{days} \times {}^{o}C)^{-1} \times 1000$
Condition factor	$CF = W\ (g) \times (\text{fork length, cm})^{-3} \times 100$
Weight gain	$WG = W_1\ (g) - W_0\ (g)$
Hepato-somatic index	$HSI\ (\%) = 100 \times (\text{liver weight, g}) \times W\ (g)^{-1}$
Viscero-fat somatic index	$VFI\ (\%) = 100 \times (\text{viscera weight, g}) \times W\ (g)^{-1}$
Cardio-somatic index	$CSI\ (\%) = 100 \times (\text{heart weight, g}) \times W\ (g)^{-1}$
Apparent digestibility coefficient	$ADC\ (\%) = 100 \times [1 - (F \times D^{-1}) \times (Di \times Fi^{-1})]$

Where W is the weight of the sampled fish in grams; W_0 and W_1 are the initial and the final fish mean weights in grams; F and D are the concentration of the nutrient in the faeces and diet; Di and Fi are the concentration of the inner marker (Y_2O_3) in the diet and faeces.

2.9 Chemical analysis

Chemical analyses of feed, faeces and whole body composition were carried out at Nofima Marine, Sunndalsøra, Norway, following:

- Dry matter was determined by thermal drying ground samples (0.2g) to constant weight in a drying oven (Termaks TS 8000, Termaks, Bergen, Norge) at 105^{o}C for 24 h.
- Crude protein was analyzed using the Kjeldahl-N method (Tecator Kjeltec-Auto 2300 analyzer Unit, Foss Analytical AB, Höganäs, Sweden) (N × 6.25).
- Crude fat was determined gravimetrically after pre-extraction with petroleum ether (60 ml, 15 min, at 13^{o}C) as the solvent, hydrolysis with hydrochloric acid (4M HCl, 30 min, at 100^{o}C), and final-extraction with petroleum ether (60 ml, 45 min, at

135^{o}C); using 2055 Soxtec Avanti Manual System and Soxtec System 1047 Hydrolyzing System (Foss Analytical AB, Höganäs, Sweden).

- Ash was determined by combusting ground samples (0.2g) on a hot plate (OBH Nordica single model 6525, OBH Nordica, Spånga, Sweden), following complete combustion (at 550^{o}C, 24 h) to constant weight in Carbolite Eurotherm (Carbolite Furnaces, Sheffild, Great Britain).
- Gross energy was determined by using Parr 6300 Oxygen Bomb Calorimeter (Parr, Moline, IL, USA).
- Total starch was determined by using the Kit Megazyme Total starch AA/AMG metode 6/95.
- Concentrations of inner marker (Y_2O_3) in the faeces and diets were determined by a spectrophotometric method after acid digestion; using an ICP-OES Optima 5300 DV (Perkin Elmer, USA) at Eurofins Ås, Norway.
- The total fats in liver, heart, visceral fat, and white muscle were analyzed by extracting in chloroform:methanol, 2:1 using the method described by Folch *et al.* (1957).

2.10 Physical quality of the feed

2.10.1 Doris test

Pellet durability for coated feed was measured in a Doris tester (Akvasmart, AKVA group, Bryne, Norway) with two replicates. Approximately 350 g of sifted samples (8 mm sieve) were pneumatically conveyed in the Doris tester. Pellets were subjected to high air speed and attrition between the pellet surface and side wall of the system, causing dust and fractures. After testing time, the samples were collected at the collector and sieved to remove dust particles and broken pellets. The sieving procedure was performed using Retsch sieving equipment (Retsch GmbH Haan, Germany) with two sieve sizes (8 mm − 1st sieve, 5.6 mm – 2nd sieve) and a collector sieve, run for 30 seconds at 1.5 mm amplitude. The pellet fraction on the 1st sieve was weighed and represented to unbroken pellets. The feed particles remaining on the 2nd sieve was referred to broken pellets, whereas the collector sieve for dust. The Doris durability was calculated as the percentage of pellets remaining on the 1st sieve and represented in the average of two replicates.

2.10.2 Hardness test

Hardness (strength at rupture) was measured by using a Texture-Analyser (TA-XT2®, Model 1000 R; SMS Stable Micro Systems, Blackdown Rural Industries, Surrey, UK) fitted with a 25 kg load cell, as described by Sørensen, *et al*. (2010). Each pellet was ruptured under compressing by a cylinder probe (d=20 mm), set up at a speed of 2 mm/s to achieve 60% compression. The diameter of the pellets and the force/time curve were automatically recorded and calculated for the peak force by the computer software (Texture Expert for Windows, version 1.15, Stable Micro Systems). 30 pellets were measured for each diet and represented in the average.

2.10.3 Statistical analysis

Data were analyzed for statistical differences using PROC glm procedure of SAS 9.1 software (SAS Institute Inc., Cary, NC, USA) with $P < 0.05$ as the level of significance. All data were presented as means ± standard error means (S.E.M) (n=3). Two-way ANOVA was performed to determine the main effects of diet, water current and their interaction on growth performance, nutrient digestibility, whole body and tissue compositions.

3. Results

3.1 Chemical composition and physical quality of the experimental diets

The analyzed proximate composition of the two experimental diets is shown in Table 4. As expected, the high fat diet contained more fat (328.7 g kg^{-1}) and less protein (425.6 g kg^{-1}) compared to the low fat diet (257.1 g fat kg^{-1} and 484.4 protein kg^{-1}, respectively).

The physical quality of the diets from both feed productions (Fig. 6) showed that the high fat diets had higher diameter and lower hardness compared to the low fat diets. The DORIS durability ranged between 80 – 90%, with a low variation between the diets, except for the high fat diet from the second production that showed a lower value compared to the other diets.

Table 4. Analyzed composition of main nutrients (g kg^{-1} DM) of the experimental diets[1]

	Low fat	High fat
Dry matter	909.7	934.3
In DM kg feed^{-1}		
Fat	257.1	328.7
Protein	484.4	425.6
Ash	81.8	70.2
Starch	88.7	85.6
Gross energy (kJ g^{-1})	26.3	25.6

[1]Second feed production produced 15th September 2010

Physical quality of the experimental diets

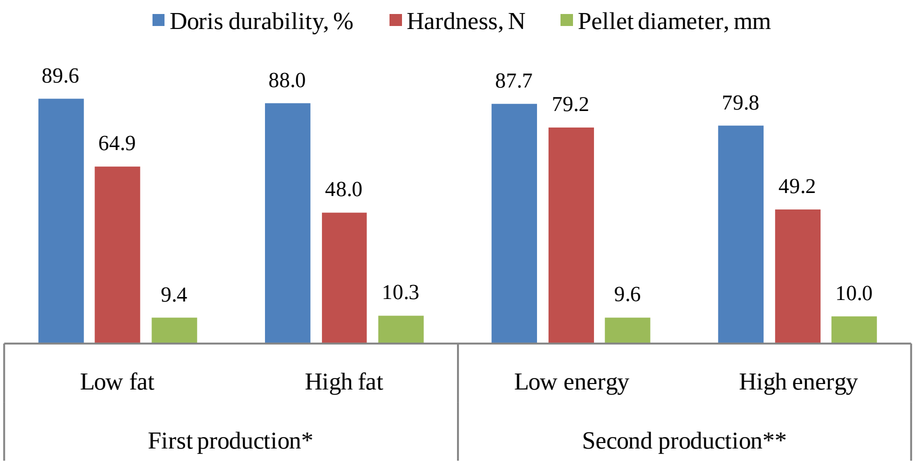

Fig. 6. Physical quality of experimental diets

*Produced 13th July 2010

**Produced 15th September 2010

3.2 Growth performance and feed utilization

The effects of diet and water current on growth performance and feed utilization are shown in Table 5. High mortality of fish was noted in all treatments. Though no significant differences were observed related to treatments, the highest mortality was observed for the salmon fed high fat diet and for fish kept at low water current.

Fat levels in diet did not affect growth performance. Water current significantly affected FCR ($P < 0.05$). FCR ranging from 2.03 – 2.73 was higher than expected. Lowest FCR was observed for fish kept at high current. Water current also tended to affect weight gain ($P=0.080$), SGR ($P=0.077$) and TGC ($P=0.077$). Best growth performance was observed for fish kept at high current. The interaction diet and water current tended to affect weight gain ($P = 0.083$), SGR ($P = 0.077$) and TGC ($P = 0.078$) (Fig. 7). Highest growth performance was shown for fish fed high fat diet and kept at high current (HFHC) (Fig. 7).

No significant effect of diet or water current was observed on CF and OSI (Table 6) among the groups, the CF ranged from 1.35 – 1.36%, the HSI from 1.34 – 1.40%, the VFI from 2.21 – 2.43% and CSI was approximately 0.11%. The interaction diet and water current tended to affect CSI ($P = 0.077$). The CSI was highest for the fish fed high fat diet kept at low current (HFLC) (Fig. 8). The raw data is presented in annex 1 and 2.

Table 5. The main effects of diet and water current on growth performance and feed utilization, mean ± S.E.M (n=3)

	Diet			Current		
	Low fat	High fat	P-value	Low	High	P-value
Start weight, g	1413.6±0.7	1414.3±0.7	0.470	1414.0±0.7	1413.8±0.7	0.858
End weight, g	2486.1±115.8	2596.6±115.8	0.519	2377.3±115.8	2705.3±115.8	0.080
Weight gain, g	1072.6±115.8	1182.2±115.8	0.522	963.3±115.8	1291.5±115.8	0.080
Mortality, %	50.1±2.8	56.2±2.8	0.155	55.9±2.8	50.38±2.8	0.197
FCR	2.35±0.19	2.42±0.19	0.811	2.73±0.19	2.03±0.19	0.032*
SGR	0.59±0.05	0.62±0.05	0.668	0.54±0.05	0.68±0.05	0.077
TGC	3.33±0.29	3.55±0.29	0.613	3.02±0.29	3.85±0.29	0.077

S.E.M − standard error of the mean; FCR − feed conversion ratio; SGR − specific growth rate; TGC − thermal growth coefficient

*Significant different ($P < 0.05$)

Table 6. The main effects of diet and water current on condition factor (CF) and organo-somatic index (OSI) of Atlantic salmon, mean ± S.E.M (n=3)

	Diet			Current		
	Low fat	High fat	P-value	Low	High	P-value
CF	1.36±0.02	1.36±0.02	0.99	1.35±0.02	1.36±0.02	0.70
CSI, %	0.11±0.00	0.11±0.00	0.43	0.11±0.00	0.11±0.00	0.43
HSI, %	1.40±0.03	1.34±0.03	0.14	1.36±0.03	1.37±0.03	0.79
VFI, %	2.21±0.11	2.43±0.11	0.22	2.40±0.11	2.24±0.11	0.34

CF − Condition factor; CSI − Cardio-somatic index; HSI − Hepato-somatic index; VFI − Viscero-fat somatic index

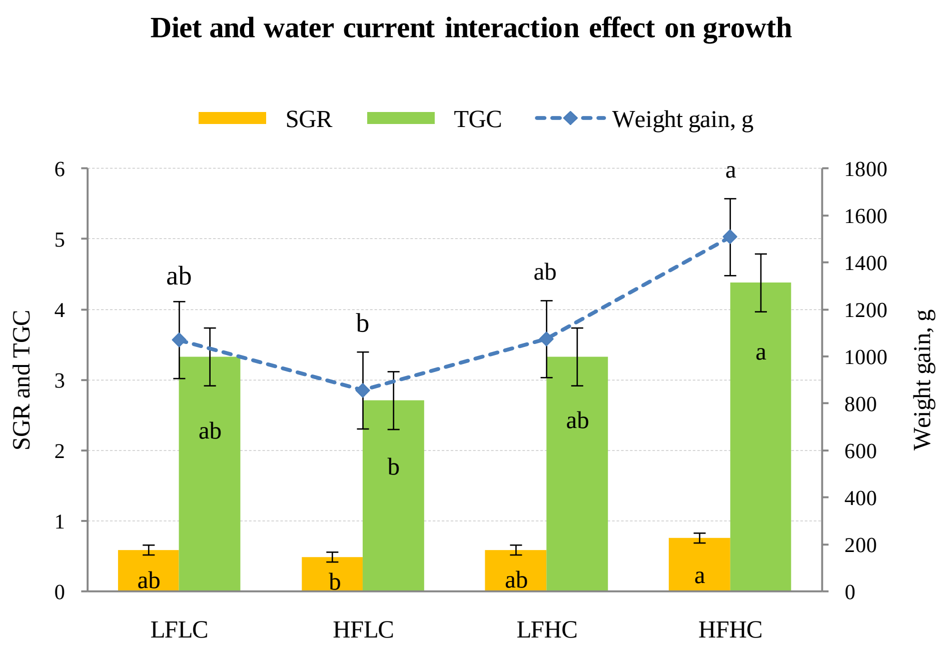

Fig. 7. The effects of diet and water current interaction on weight gain, SGR and TGC, mean ± S.E.M (n=3)

HFHC − high fat, high current; HFLC − high fat, low current; LFHC − low fat, high current; LFLC − low fat, low current

[a,b] Different superscripts indicate differences (0.05< P < 0.1) among treatment means

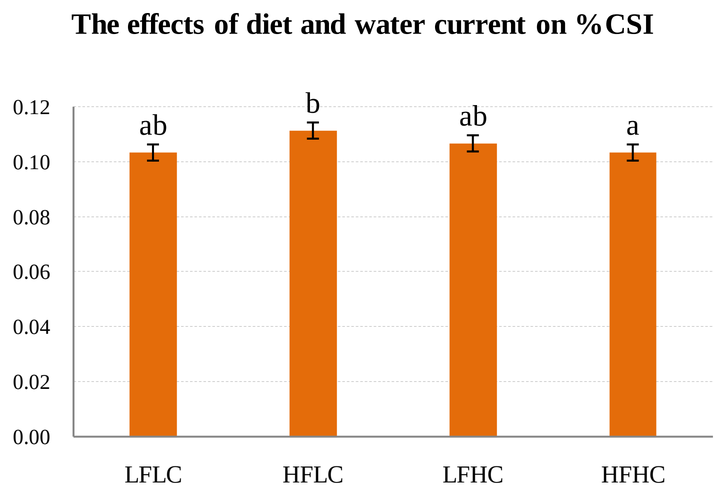

Fig. 8. The effects of diet and water current interaction on cardio-somatic index (CSI), mean ± S.E.M (n=3)

HFHC − high fat, high current; HFLC − high fat, low current; LFHC − low fat, high current; LFLC − low fat, low current

[a,b] Different superscripts indicate significant differences (P < 0.05) among treatment means

3.3 Whole body proximate composition

The initial proximate composition of the fish in the start population is shown in Fig. 9. The chemical composition of the fish at the start of the experiment was 31.9% DM, 13.5% crude fat, 17.2% crude protein, 1.9% ash, and 9.1% gross energy. At termination of the experiment, there was significant effect of diet on DM ($P = 0.005$), body fat level ($P = 0.009$) and gross energy ($P = 0.003$) between the start and end population (Fig. 9 _ A, B, E), whereas no significant differences were noted on protein and ash content (Fig. 9 _ C, D).

No significant differences were observed in proximate composition between fish from the start and from the termination of experiment that could be explained by water current. Neither was any differences observed between groups fed different diets or kept at different water currents. Raw data is presented in annex 3.

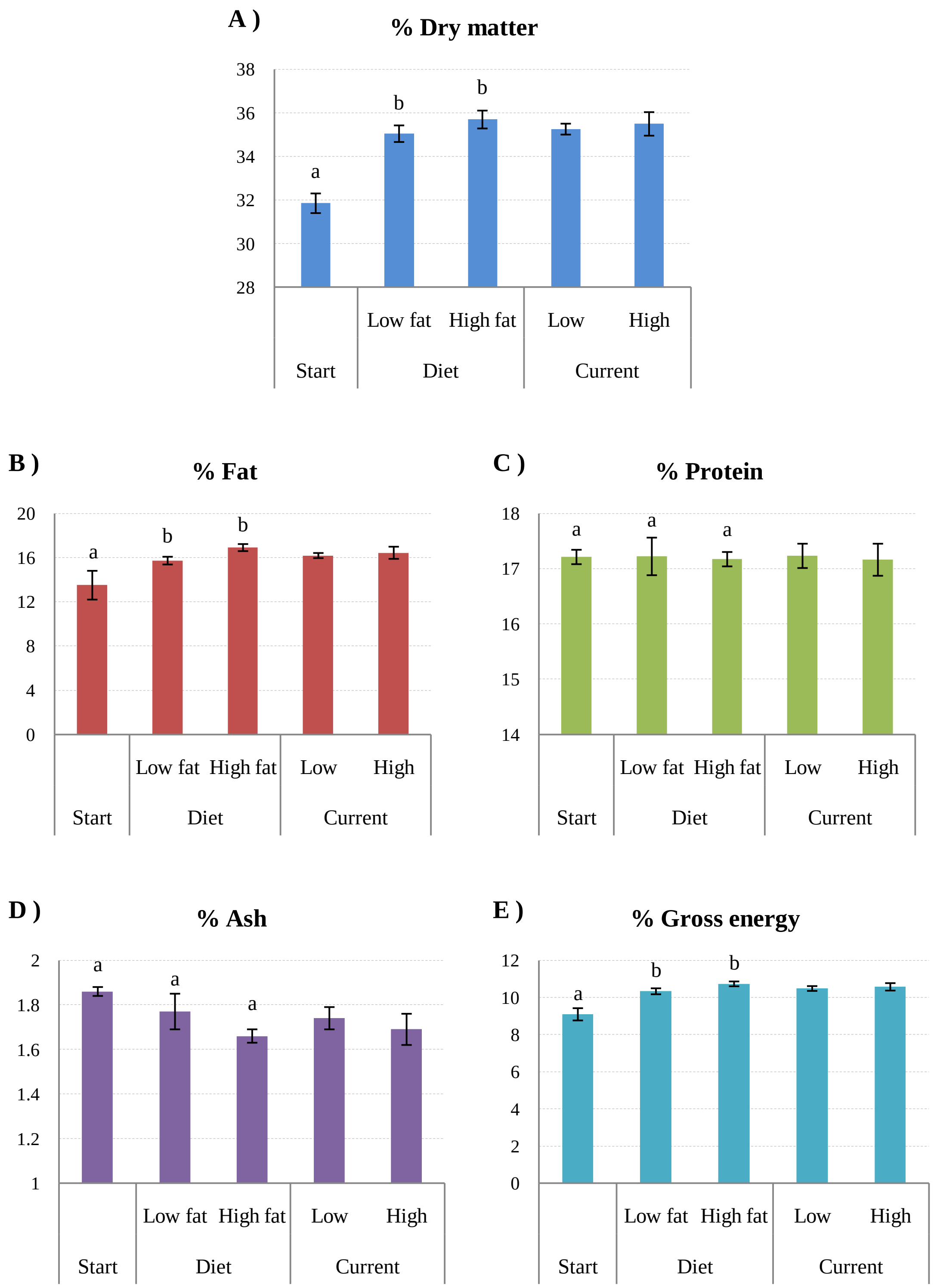

Fig. 9. The main effects of diet and water current on whole body compositions (% of wet weight) at the beginning and the end of the experiment, mean ± S.E.M (n=3)

[a, b] Different superscripts indicate significant differences between start and among dietary treatment ($P < 0.05$)

3.4 Fat content in organs and white muscle

No significant effects were observed for either diet or current treatment on the fat content of organs and white muscle (Figure 10_ A, B, C, D). Neither was any differences observed between groups fed different diets or kept at different water currents. Raw data is presented in annex 4.

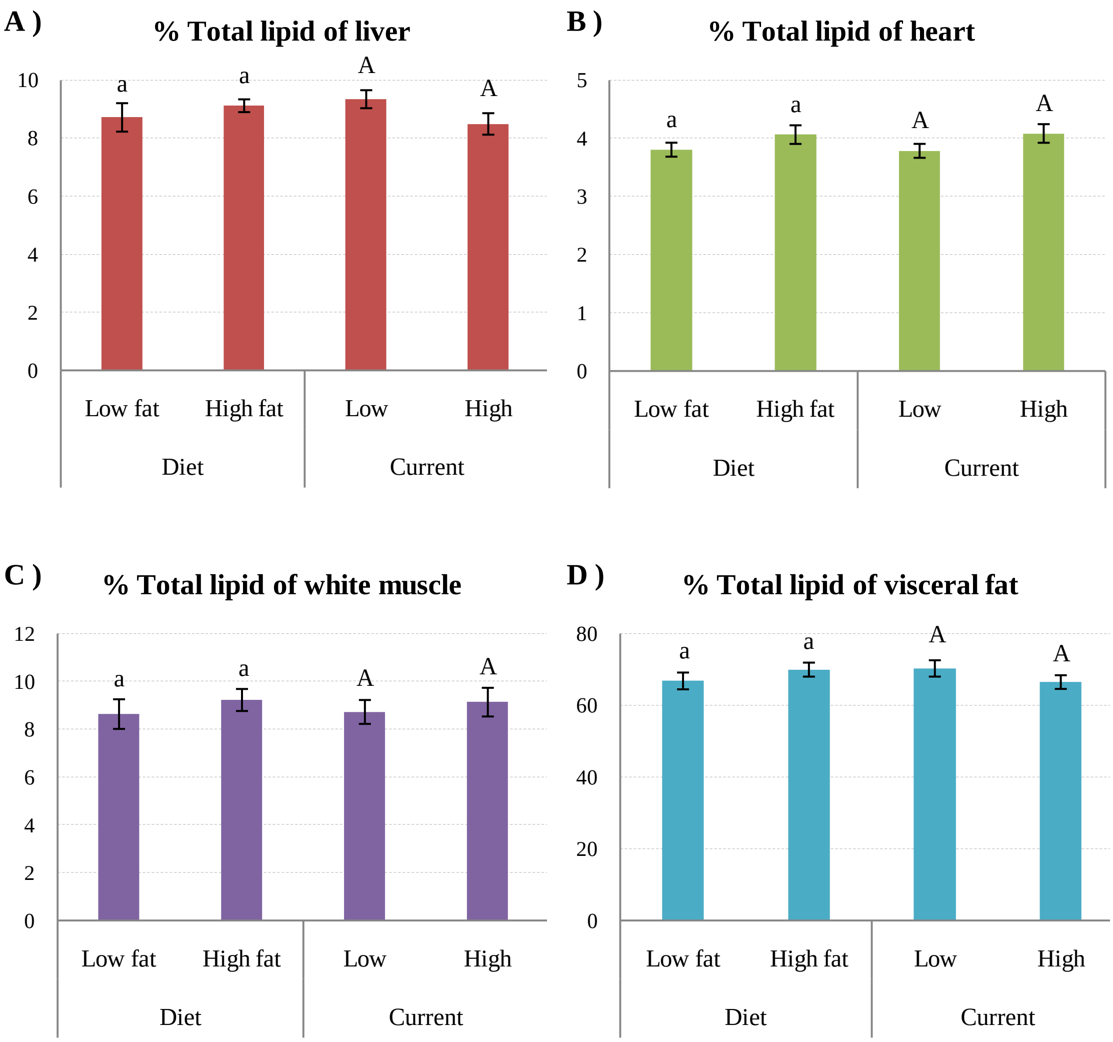

Fig. 10. The main effects as diet and water current on fat contents (%) of liver, heart, white muscle and visceral fat at the end of the experiment, mean ± S.E.M (n=3)

[a,b] Different superscripts indicate significant differences ($P < 0.05$) among dietary treatment means

[A,B] Different superscripts indicate significant differences ($P < 0.05$) among water current treatment means

3.5 Apparent nutrient digestibility

The apparent nutrient digestibility (ADC) is shown in Table 7. The ADC of fat tended to be affected by diet (P = 0.051) and water current (P = 0.057). Highest fat ADC was observed for fish fed the high fat diet and for fish kept at low water current. The ADC of ash was lowest for fish fed the high fat diet (P = 0.06) Nutrient digestibility was not affected by the interaction diet and water current. Raw data is presented in annex 5.

Table 7. The effects of diet and water current on apparent digestibility (%) of main nutrients at the end of the experiment, mean ± S.E.M (n=3)

	Diet			**Current**		
	Low fat	High fat	P-value	Low	High	P-value
Dry matter	70.42±0.51	69.72±1.69	0.73	70.02±1.00	70.12±1.47	0.96
Starch	63.10±2.18	59.77±3.72	0.56	62.25±2.90	60.12±3.72	0.69
Protein	87.92±0.41	86.50±1.19	0.35	87.13±0.75	87.28±1.11	0.92
Gross energy	83.16±0.43	84.03±0.92	0.45	83.88±0.59	83.34±1.00	0.66
Fat	95.97±0.48	97.05±0.29	0.051	97.03±0.19	95.98±0.53	0.057
Ash	0.57±4.95	-21.30±7.64	0.06	-12.20±7.38	-8.53±8.66	0.72

4. Discussion

4.1 Physical quality of the experimental diets

Pellets with the highest fat content showed the greatest expansion ratio (diameter) and lowest hardness under the compression test (Fig. 6). A lower expansion of the low fat diet was planned in order to ensure a sinking pellet. If both diets were produced with the same expansion and bulk density prior to coating of fat, the low fat diet would have a lower bulk density and most likely floating pellets. A more expanded pellet has more air pockets in the pellet structure, a necessity for absorption of oil post extrusion. The air-pockets are formed when steam and gas flash of the pellet due to the pressure reduction when the extrudate is conveyed from high pressure to ambient pressure as it leaves the die. The porous structure allows addition of fat post extrusion in vacuum coating systems. In line with several other previous investigations, the present result showed that the more expanded extrudate had a weaker structure. The present result is therefore in line with several investigations reporting an inverse relationship between expansion rate and hardness of pellet (Aarseth, *et al.*, 2006; Sørensen, *et al.*, 2009; Øverland, *et al.*, 2009). Aarseth and Prestløkken (2003) suggested that a greater proportion of large pores in the structure of brittle materials, such as pellets, may cause reduced strength because stress will be concentrated around larger pores when applying stress. Besides, physical characteristics of pellets such as durability, hardness and water stability should be taken into consideration in feeding experiments with fish. Recent research have suggested that physical quality of feed may interfere with feed intake and nutrient digestibility (Watanabe, *et al.*, 2001; Baeverfjord, *et al.*, 2006; Aas, *et al.*, 2009). Consequently, poor quality of pellets may lower nutrient utilization and feed intake, resulting in increased production cost.

4.2 Effects of dietary fat level

As reviewed in the introduction, dietary fats are a major provider of energy and essential fatty acids, especially for carnivorous species including salmonids. In addition, increasing fat content is reported to have a protein-sparing effect in salmon (Sargent, *et al.*, 2002). Previous studies have demonstrated that high fat diets can improve growth performance and feed utilization in Atlantic salmon (Hillestad,Johnsen, 1994; Hemre,Sandnes, 1999; Refstie, *et al.*, 2001; Solberg, 2004). In the present study, no differences were observed in growth performance or feed utilization between the two diets (Table 5).

Although the lack of dietary effects on growth performance was unexpected, these results are in line with a few other investigations reporting no effect of dietary fat content on growth performance (Hillestad, *et al.*, 1998; Karalazos, *et al.*, 2007). Hillestad, *et al.*(1998) reported no effect on growth when salmon (from 0.2–0.3 kg to 3–4 kg) was fed with low (22 %) or high (30%) fat content at similar feeding rates. Also Karalazos, *et al.*(2007) showed that salmon (~ 1168g) fed diet with low (32%) or high (36%) fat content at low temperature (4.2°C) had the same final weight.

The lack of dietary effects may be explained by a low feed intake and the general poor condition of the fish when the experiment started. It was decided to run the experiment although it was reported a few days before the start up of the experiment that the population of Atlantic salmon was diagnosed with CMS. Stress associated with injection of PIT tags, weighing and distribution of fish to the pens resulted in weaken fish and infection with ectoparasites. Consequently, mortality increased due to outbreak of CMS. Taken together, the health condition resulted in a lower appetite and feed intake than expected that easily could mask differences in dietary treatment.

High FCR in the experiment (Table 5) also indicate that control with feeding and feed intake could have been better. The plan was to feed the fish approximately 10% in excess with use of an automatic feeding system, and to collect uneaten feed with Lift Up. The feeding strategy was changed after the experiment started because the biomass in the pens was too low for the feeding system. The feeding system was not able to deliver accurate amount of small quantities. Instead of automatic feeding we had to use hand feeding. The salmon was therefore fed in two meals instead of a continuous feeding. Although was fed to apparent satiation, this strategy may have resulted in underfeeding because the fish was not offered feed when it was hungry.

The physical quality of the feed may also have been suboptimal explaining the high FCR of the diets. The physical quality of the diets, measured as DORIS durability and hardness, was lowest for the high fat diets (Fig. 6). The lower quality may have resulted in more small particles that was not eaten by the fish or recorded as uneaten feed.

Positive correlations between dietary fat content and fat deposition in fish have been reported in numerous studies with Atlantic salmon (Hillestad, *et al.*, 1998; Hemre,Sandnes, 1999; Refstie, *et al.*, 2001; Bendiksen, *et al.*, 2003a; Bendiksen, *et al.*, 2003b; Solberg, 2004; Hemre,Sandnes, 2008). Because fat is replacing water in a growing fish, body dry matter increases with increasing body fat (Bureau, *et al.*, 2002). In line with this, Rasmussen, *et al.* (2000), reported that Rainbow trout growing from 44 to 326 g fed high-fat feeds (31%) increased body fat content, concurrent with a reduction in whole body moisture and ash. A linear relationship between dietary fat level and fat deposition in fillet and visceral fat has been reported in previous studies. Solberg (2004) found that Atlantic salmon growing from 600 g to 4 kg, fed 36% oil in the diet had 10% higher carcass weight and 2% higher fat-content in the fillets compared to fish fed 26% oil in the diet. Increased deposition of visceral fat was also reported in salmon fed 30% fat in diet compared to those fed 22% fat in the diet (Hillestad, *et al.*, 1998). In contrary to the results reported from these former authors, no differences in fat content was observed in the present study that could be associated with the different fat levels in the diets (Fig. 9_A, B, C, D, E). The changes in chemical composition were mainly explained by the age of the fish, which is in line with earlier findings reported by Shearer, *et al.* (1994). No dietary effects on OSI and fat content in organs may be explained by the overall lack of dietary effects in this experiment (Table 6 ; Fig. 10_A, B, C, D).

The improved fat digestibility observed in the salmon fed the high fat diet (Table 7) is in line with other study (Bendiksen, *et al.*, 2003b). The greater fat digestibility was however, not reflected in improved energy digestibility, or improved weight gain.

4.3 Effects of high water current

A major difference between the previously reported studies and the one reported herein is that the present experiment was carried out under natural conditions in sea water. It was therefore not possible to standardize water speed to a fixed BL s^{-1}. The effect of tidal water current may be considered as interval training. As shown in Fig. 2, the water current changed over the day and varied slightly with depth in the pen. The low current pen had more even water current compared to the high current treatment. Under natural conditions the tidal current will also be influenced by wind and atmospheric pressure. Nevertheless, these results indicate that water current may have positive effects on growth performance.

The improved weight gain and growth performance of salmon kept at high tidal water current (Table 5) is in line with other investigations (Totland, *et al.*, 1987; Jørgensen,Jobling, 1993; Bugeon, *et al.*, 2003). Jørgensen and Jobling (1993) reported a significantly higher SGR in Atlantic salmon exercised at 1.0, 1.5 and 2.0 BL s^{-1} compared to fish kept in standing water (control). The highest SGR in the latter experiment was observed for the group swimming at 1.5 BL s^{-1}. Moreover, Bugeon, *et al.* (2003) observed that body weight of Brown trout held at 1 and 2 BL s^{-1} were significantly higher than those of controls < 0.1 BL s^{-1}. It is hypothesed that increased swimming activity, by increasing the water current velocity, may be associated with increased appetite resulting in increased feed intake.

Although FCR for the experiment was higher than expected for this size of fish fed extruded diets, the lowest FCR was observed for the fish kept at high water current, and was associated to the highest weight gain (Table 5). In contradiction to these findings, Kiessling, *et al.* (1994) reported poorer feed utilization for all-female Chinook salmon at different speeds of 0.5, 1.0, or 1.5 BL s^{-1}. Other studies have reported a negative effect on feed utilization in Rainbow trout as a response to intense training (Hernández, *et al.*, 2002).

No differences in CF, CSI, HIS or VFI were observed in the present experiment (Table 6). In contradiction, Bugeon, *et al.* (2003) observed that CF in brown trout increased in exercised fish, while Anttila, *et al.* (2010) reported a reduction in CF in trout with higher water speed. Kiessling, *et al.* (1994) found an increased HSI in all-female Chinook salmon with higher water current, whereas Bugeon, *et al.* (2003) found no effect of exercise on HIS in Brown trout. In contrast to the present study Gallaugher, *et al.* (2001) observed that heart weight of exercised fish was larger than non-exercised fish, and was also associated with higher oxygen uptake and transport, benefitting the cardiovascular system. To our knowledge limited knowledge exist on the effect of exercise on VFI. No studies were found that reported VFI in exercised fish.

No differences were observed in salmon body proximate composition between the two water currents (Fig. 9_A, B, C, D, E). The effects of training on body composition of salmonids have been reported in numerous studies (Totland, *et al.*, 1987; Jørgensen,Jobling, 1993; Hernández, *et al.*, 2002; Bugeon, *et al.*, 2003; Kiessling, *et al.*, 2005). Fish have to use more energy with increased swimming activity compared to slow movements (Brett, 1964). Body fat stores will be mobilized for energy production if energy content in the diet is too low to meet the additional requirement for swimming (Forster,Ogata, 1996). Consequently, proximate composition of body will change. Studies have reported a reduction in total body and muscle fat content in exercised salmonids (Patterson, *et al.*, 2004; Kiessling, *et al.*, 2005). These results are also in line with Christiansen, *et al.* (1989). The latter authors reported that Arctic charr kept at water velocities around 1.7 BL s^{-1} had lower body fat content and higher body protein content. In contrast, to these results, fat content in organs and white muscle did not vary significantly between low and high current treatments in the present experiment (Fig. 10_A, B, C, D). The present results are in line with Rasmussen, *et al.* (2011), who observed that fillet composition of Rainbow trout did not differ between exercised fish (0.9 BL s^{-1}) compared to fish kept in standing water (< 0.1 BL s^{-1}).

Except for fat digestibility, no effects of water current were observed on nutrient digestibility (Table 7). Fat, however, tended to be highest in the low current groups (Table 7). Overall fat digestibility was high in this experiment suggesting that highly digestible oil sources were used in this experiment. The lower fat digestibility observed in the high current group may be associated with a higher feed intake. Increased feed intake cause faster passage rate through the gastrointestinal tract, and may cause reduced digestibility when salmon have a high feed intake (Vens-Cappell, 1978).

4.4 Interactions between dietary fat level and exercise

The improved weight gain and growth performance of fish fed high fat and kept at high current suggest that there is a positive effect of high dietary fat in combination with training. This finding is supported by previous research with Yellowtail. Yellowtail fed high dietary fat content (20.3 %) and subjected to swim at 1.0 BL s^{-1}, had significantly higher weight gain and feed efficiency ratio compared to those fed lower dietary fats (13.2 %) and subjected to swim at < 0.3 BL s^{-1} (Yogata,Oku, 2000). On the contrary, Forster and Ogata (1996) showed that dietary fat levels (10.3% and 16.3%) in diets fed to Red sea bream had no effect on SGR, FCR, or nitrogen retention in combination with swimming speed (at 0.0, 1.5, and 3 BL s^{-1}). The interaction between swimming activity and fat content in diet fed to large Atlantic salmon is, however, not much investigated.

High mortality is one of the main challenges in Norwegian salmon industry during grow out phase in sea water, and this may be associated with heart inflammation (epicarditis) in Atlantic salmon. It is questioned whether CMS is associated with use of plant oils in high-fat diets (Hjeltnes, *et al.*, 2009). Seierstad, *et al.*, (2005) reported that salmon fed a diet containing a 50:50 mixture of fish oil and rapeseed oil had higher body weight, fish length and heart weight compared to the groups fed fish oil. Furthermore, histopathology associated with arteriosclerotic changes was observed in fish fed fish oil: rapeseed oil. The authors therefore suggested a possible interaction between fatty acid composition in the diet, heart weight and arteriosclerotic development in Atlantic salmon. The present results showed increased heart weight in salmon fed high fat kept at low water current (Fig. 8). Assuming that heart weight in fish fed diets with a mixture of fish oil:rapeseed oil as the fat source, is correlated to histopathology, the present results suggest that this oil blend should be avoided in high fat feed fed to salmon kept in low water current. Although no significant differences were observed in mortality among groups, the numerical values showed the greatest mortality in high fat and low current groups (Table 5).

5. Conclusion

In conclusion, the most important findings from the present study were that fish kept at high current had improved weight gain and feed utilization, while fat level in the diet had no effect on these parameters. The dietary effects in the present experiment were low mainly because of a low feed intake explained by CMS and high mortality. High fat diet in combination with training tended to improve growth rate. These results suggest that training in combination with high fat diet improve productivity at the fish farm.

Annex

Annex 1. Transcript of raw data from growth performance and feed utilization

Net pen	Feed*	Current*	Number of fish start	Average start weight	Number of fish end	Average final weight	Weight gain	Number dead fish	Percent dead fish	FCR	SGR	TGC
M14	1	1	109	1411.3	59.0	2535.8	1124.5	50.0	45.9	2.111	0.617	3.469
M15	2	1	110	1415.5	47.0	1894.3	478.8	63.0	57.3	3.593	0.307	1.642
M19	2	1	108	1414.7	33.0	2410.6	995.9	75.0	69.4	2.857	0.561	3.129
M20	2	2	107	1413.7	57.0	2657.0	1243.2	50.0	46.7	1.889	0.664	3.766
M21	1	2	108	1417.4	57.0	2459.1	1041.7	51.0	47.2	2.305	0.580	3.247
M22	1	1	113	1411.3	43.0	2467.9	1056.6	70.0	61.9	3.246	0.588	3.293
M25	2	2	107	1411.7	53.0	2674.2	1262.5	54.0	50.5	2.093	0.672	3.817
M26	2	1	110	1418.7	48.0	2511.9	1093.2	62.0	56.4	2.483	0.601	3.379
M27	1	1	110	1412.6	61.0	2443.4	1030.8	49.0	44.5	2.127	0.577	3.224
M28	1	2	109	1415.8	51.0	2622.3	1206.4	58.0	53.2	1.907	0.649	3.672
M29	1	2	107	1412.9	56.0	2388.1	975.3	51.0	47.7	2.416	0.553	3.076
M32	2	2	107	1411.5	46.0	3431.3	2019.8	61.0	57.0	1.597	0.935	5.542

* 1 means low energy diet or low current; 2 means high energy diet or high current

Annex 2. Transcript of raw data from condition factor (CF) and organo-somatic index (OSI)

Net pen	Feed*	Current*	Started length (cm)	Started weight (g)	Finished length (cm)	Finish weight (g)	Gain weight (%)	Heart index	Liver index	Visceral fat index	CF factor
M14	1	1	50.20	1434.80	60.10	2977.00	107.15	0.10	1.42	2.37	1.37
M15	2	1	50.60	1432.60	60.80	2927.00	104.95	0.12	1.29	2.43	1.30
M19	2	1	50.30	1381.60	61.30	3259.00	136.44	0.11	1.39	2.73	1.42
M20	2	2	48.60	1367.20	60.00	3002.00	119.42	0.10	1.33	2.39	1.39
M21	1	2	51.20	1521.00	61.80	3100.00	104.06	0.11	1.31	1.81	1.32
M22	1	1	51.00	1456.40	61.60	3032.00	108.64	0.10	1.35	2.51	1.30
M25	2	2	52.20	1531.20	63.00	3263.00	113.63	0.11	1.35	2.56	1.31
M26	2	1	49.60	1396.80	61.10	3143.00	126.62	0.11	1.32	2.22	1.37
M27	1	1	51.60	1454.40	61.80	3197.00	120.14	0.10	1.40	2.17	1.35
M28	1	2	52.00	1543.40	64.20	3842.00	148.68	0.10	1.52	2.57	1.45
M29	1	2	50.60	1462.40	62.30	3293.00	126.48	0.11	1.38	1.85	1.36
M32	2	2	50.60	1466.40	62.10	3254.00	122.09	0.10	1.35	2.26	1.36

* 1 means low energy diet or low current; 2 means high energy diet or high current

Annex 3. Transcript of raw data from chemical analysis of whole body composition

Net pen	Feed*	Current*	Fat %	Nitrogen %	Protein %	Ash %	Dry matter %	Energy
M14	1	1	15.8	2.59	16.21	1.86	34.52	10.04
M15	2	1	16.9	2.77	17.34	1.70	35.43	10.55
M19	2	1	16.3	2.78	17.40	1.64	34.95	10.61
M20	2	2	18.1	2.75	17.17	1.73	37.30	11.17
M21	1	2	16.7	2.89	18.06	1.86	36.48	10.72
M22	1	1	15.7	2.75	17.20	1.94	35.19	10.38
M25	2	2	17.4	2.72	17.02	1.58	35.65	10.74
M26	2	1	16.8	2.80	17.51	1.59	36.35	11.01
M27	1	1	15.6	2.84	17.74	1.71	35.09	10.30
M28	1	2	16.3	2.60	16.23	1.40	35.38	10.79
M29	1	2	14.2	2.86	17.89	1.82	33.65	9.75
M32	2	2	15.9	2.66	16.60	1.73	34.51	10.27
Start 1	0	0	14.8	2.73	17.08	1.84	32.31	9.42
Start 2	0	0	12.2	2.77	17.33	1.88	31.41	8.76

* 1 means low energy diet or low current; 2 means high energy diet or high current

Annex 4. Transcript of raw data from fat content of organs and white muscle

Net pen	Feed*	Current*	Liver %	Heart %	White muscle %	Visceral fat%
M14	1	1	10.37	3.80	7.24	70.52
M15	2	1	8.66	3.60	8.22	72.44
M19	2	1	8.38	3.90	9.11	62.06
M20	2	2	9.13	3.38	7.83	78.84
M21	1	2	9.83	4.23	9.23	70.62
M22	1	1	9.67	3.79	10.68	66.99
M25	2	2	8.73	4.24	8.17	66.38
M26	2	1	8.54	4.46	9.08	66.34
M27	1	1	8.79	4.27	10.32	70.36
M28	1	2	7.84	3.59	7.20	58.47
M29	1	2	9.86	3.59	11.27	71.81
M32	2	2	7.17	4.34	8.73	65.31

* 1 means low energy diet or low current; 2 means high energy diet or high current

Annex 5. Transcript of raw data from apparent nutrient digestibility

Net pen	Current*	Feed*	Replicate	Fat	Nitrogen	Protein	Ash	Dry matter	Starch	Organic matter	Energy
15	1	2	1	97,6	83,7	83,7	-36,0	65,6	56,1	73,2	81,5
19	1	2	2	97,1	86,7	86,7	-31,1	69,5	54,0	77,1	84,6
26	1	2	3	97,3	88,8	88,8	-7,0	72,5	69,5	78,5	85,9
20	2	2	1	97,6	83,5	83,5	-32,3	66,0	47,7	73,4	81,7
25	2	2	2	97,0	85,4	85,4	-31,8	68,3	60,2	75,9	83,4
32	2	2	3	95,7	90,9	90,9	10,4	76,4	71,1	81,4	87,1
14	1	1	1	96,2	88,3	88,3	8,7	71,7	70,2	77,3	83,7
22	1	1	2	96,9	86,8	88,1	3,4	71,0	58,0	77,0	83,7
27	1	1	3	97,1	85,8	87,2	-11,2	69,8	65,7	77,0	83,9
21	2	1	1	96,4	85,2	86,7	13,3	69,0	59,9	73,9	81,6
28	2	1	2	95,2	86,3	87,6	-17,4	69,2	61,7	76,9	82,9
29	2	1	3	94,0	88,5	89,6	6,6	71,8	-	77,6	-

* 1 means low energy diet or low current; 2 means high energy diet or high current

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