Norwegian University of Life Sciences
Faculty of Veterinary Medicine and Biosciences
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Master Thesis 2015
30 credits

Variation in Digestibility of Protein and Lipid among Individual Atlantic Salmon

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15/08/2015

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Acknowledges

I would like to express my deep gratitude to my supervisor and co-supervisors, Dres. Bjarne Gjerde, Nils Kristian Afseth and Bjarne Hatlen for their guidance and valuable advices. Especially thanks to my main supervisor (Bjarne G) for his patience to answer all my questions and discussion throughout this thesis work.

I would like to acknowledge all the people at Nofima Marine Research Center (Ås and Sunndalsøra) for their help and the great time I enjoyed there. I also have to thanks to Karl Osterhus, Ann Gunn Bradli and Per Marton Holland for their help and friendly welcome at Ewos-Dirdal.

Thanks to my family for the unconditional love, support and encourage me to achieve this goal today. Finally, thanks to all my friends for make me always smile.

Mariela.

Ås, August 15th, 2015.

Abstract

A group of 60 pit tagged post smolt Atlantic salmon chosen randomly from a pool of 50 families (34 represented), with an initial body weight of 440 g, were reared in a single tank for 56 days. Fish weight gain was 414 g and specific growth rate 1.2% d^{-1} during the experimental period. Individual apparent digestibility coefficient (ADC) was determined from three faecal samples of each fish (stripping) obtained during the experiment. ADCs mean and standard deviation (SD) for the first stripping (n=57) was 90.8% (SD=1.4%) for protein and 95.0% (SD=1.1%) for lipid; for the second stripping (n=56), 90.0% (SD=1.5%) for protein and 94.8% (SD=1.1%) for lipid, and for the third stripping (n=54) 88.5% (SD=2.5%) for protein and 93.9% (SD=2.0%) for lipid. Intraclass correlations (repeatability) for ADC of lipid varied from 0.24 to 0.5 and of protein from 0.00 to 0.02. These results indicate significant genetic variation in digestibility of lipid in Atlantic salmon, but not for protein. Therefore it should be possible to obtain a favorable genetic gain for ADC of lipid, but not for ADC of protein.

Key words: Individual Atlantic salmon (*Salmo salar*), proteins, lipids, apparent digestibility, intraclass correlation, genetic variation.

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List of Abbreviations

ADC	Apparent Digestibility Coefficient
ATP	Adenosine Triphosphate
CL	Crude Lipid
CP	Crude Protein
DE	Digestible Energy
DM	Dry Matter
DP	Digestible Protein
EFA	Essential Fatty Acids
ENL	Endogenous Nitrogen Gut Losses
FAO	Food and Agriculture Organization of the United Nations
FCR	Feed Conversion Ratio
FER	Feed Efficiency Ratio
GE	Gross Energy
GH	Growth Hormone
HUFA	High Unsaturated Fatty Acids
IGF	Insulin Like Growth Factor
IGFBP	Insulin Growth Factor Binding Proteins
ME	Metabolizable Energy
NE	Net Energy
NIRS	Near Infrared Spectroscopy
NMBU	Norwegian University of Life Sciences

PUFA	Polyunsaturated Fatty Acids
RMSECV	Root Mean Square Error of Cross Validation
SGR	Specific Growth Rate
TDC	True Digestibility Coefficient
XRFS	X-ray Fluorescence Spectroscopy

1 Introduction

In Atlantic salmon farming, feed expenses account for about half of the total production cost in the grow-out phase (fiskeridir, online), and therefore feed efficiency (g weight gain/g feed intake) become the most important economic trait as the improvement of it lead to diminish production cost and, in parallel, to reduce waste production which is associated to environmental impact. Feed efficiency has been enhanced through feed manufacturing technologies, controlling and or monitoring the physical factors in the rearing system (temperature and oxygen concentration, for example) on which feed efficiency depends and indirectly through selection for increased growth rate (Thodesen et al., 2001). However studies on selection not always match in results.

A basic consideration to augment feed efficiency is provide the right nutrients in the right amount and proportion, thus it will have a properly flow through the consecutives physiological processes of digestibility, metabolizability and net deposition. Particularly in the last decade, studies have put focus to test novel feed ingredients and different proportions of the ingredients for formulated diets. For any of this cases the digestibility must be measured, because digestible nutrients will enhance feed efficiency values, as more nutrients are available for productive functions.

Digestibility trials with fish require faeces collection and chemical analysis of both the feed and the faeces samples for the nutrients of interest, as well as for an inert indicator (e.g. yttrium oxide) added to the diet, since the total amount of excretions (faeces and ammonia) can not be measured. These kinds of tests are viable since the number of faeces samples required is not so big (usually around 20, as each sample is a pool of the collected faeces from individual fish reared in a replicated tank or cage). Consequently, the number of chemical analyses necessaries to

determine the Apparent Digestibility Coefficient (ADC) of nutrients in feed trials becomes economically bearable.

Estimation of ADC for individual fish is a different case, it requires the measurement of individual feed intake and faeces, which is possible only through rearing the fish individually (Nikki et al., 2004) with the disadvantage that social interactions among the fish are lost resulting in biased ADC estimates (Martins et al., 2008). For a group of fish reared in a tank or cage it is possible to record the amount of wasted and thus the feed intake in separate tanks as in a feed trial or in selective breeding study with fullsib families, and from which parameters as feed efficiency for each group for a given period of time can be determined. However, the amount of faeces over the same period of time cannot be quantified which means that an inert indicator is always required to determine ADC in fish.

The determination of ADC for individual fish may be also restricted by the small quantity of faeces for the chemical analyses, in particular if ADC for several nutrients (e.g. both protein, fat, energy and feed additives like astaxanthin) is required. This may be compensated for by obtaining the faces samples from larger number of fish or from repeated stripping of the same fish, which brings some disadvantages (Stone et al., 2008).

Nevertheless, in research related to selective breeding programs, the number of sample must be large (typically pooled samples from > 200 families or individual samples from > 2000 fish). Consequently, the use of the traditional chemical analysis for the determination of ADC in such studies implies an extremely high cost and, obviously, a significant limitation in the sample sizes.

The above facts make it impossible to start a selective breeding program to directly improve feed efficiency traits in fish (Gjedrem, 1983). However, if ADC could be obtained from faeces samples from individuals or families at a low cost, this could

be a first step to select directly for improved feed efficiency in fish, providing that ADC show genetic variation and not unfavourably correlated to other important traits (e.g. feed intake and growth).

On another hand, Near Infrared Spectrometry (NIRS) can be run with samples less than 1 g, beside, it has been successfully proved as a reliable method to predict digestibility in cows (Decruyenaere et al., 2012), small ruminants (Decruyenaere et al., 2009) and rabbits (Nuñez-Sanchez et al., 2012). Considering the obvious differences that a trial on aquatic media has (issues in total faecal collection, for instance), by the appropriate control of the feed regime and faeces samples collection perhaps it could be feasible (and very valuable) to develop an accurate prediction model by this simple and inexpensive method to determine macro nutrients digestibility from individual salmon, since by our own knowledge not publications related to the topic exists until now.

The main objective of the present study is to assess the feasibility to predict macronutrients digestibility (protein and lipid) from individual Atlantic salmon utilizing NIRS system and to obtain a first estimate of the magnitude of the genetic variation in ADC for the mentioned nutrients. This requires the development of a reliable prediction equation for protein and lipid in faeces samples as well as for an inert indicator in the samples. The development of the necessary prediction equations is the objective of a parallel master thesis at NMBU (Kwarteng, 2015), while the quantification of the variation in ADC of protein and lipid among individuals and among repeated stripping of the same individuals is the main topic of this study.

2 Literature Review

2.1 Digestibility

Digestibility, by definition, is the amount of eaten food that does not appear in the faeces and, therefore, is absorbed in the gastrointestinal tract (Stein et al., 2007) and thus the nutrients availability for maintenance (basal metabolism), growth, movement and reproduction.

In fish, as well as in most animals, the digestion depends mainly of the hydrolytic enzymes activity that catabolizes the molecules degradation through hydrolytic reactions. As many other enzymes, the digestives enzymes also have a degree of specialization related to the kind of chemical bond it has to hydrolyzate. Therefore, it is important to remark that the nutritive value of certain ingredient not only depends of it chemical composition but also of the digestive enzymes the animal has. The hydrolyzates compounds give the essential nutrients to the individual as amino acids, fatty acid and glucose, which will be absorbed and integrated to the blood stream.

The procedure to measure digestibility include the chemical composition of the given feed and the faeces. When total feed intake and faeces from an individual are exactly recorded in a certain time is called direct methods, whereas the partial feed and faeces samples collection with the feed containing a digestion inert indicator is named indirect methods. Digestion inert indicator is a non-digestible substance which is added to the diet, allowing determine the digestibility by calculations depending on the ratio of the indicator in the faeces and feed samples. Unlike the terrestrial animals, the total faeces collection in fish trials is a very demanding task, by this reason it must resort to an indirect method.

Faeces collection by stripping the last part of the intestine is commonly used in carnivorous fish. Another techniques such as faeces suction and intestinal dissection

are also possible; all methods have the disadvantage that the samples contamination with endogenous material may occur, which bring an underestimation of the nutrients digestibility, specially proteins (Bureau and Cho, 1999). Other techniques that include the faeces collection naturally released by the fish in the water media have the disadvantage of overestimation of the nutrients digestibility as consequence of nutrients leakage in the water (Kitagima et al., 2010).

Faeces contain undigested food and endogenous unabsorbed residues (secretions from body origin, discharged into the digestive tract as mucoproteins, digestive enzymes, etc. together with the residues from microflora that inhabit the digestive tract [Nyachoti et al., 1997; Sanz et al., 1994]). The faecal nitrogen, excluding that from ingested nutrients, is named endogenous nitrogen gut losses (ENL) (Bureau and Cho, 1999). Having this acquaintance related to the faeces contains, a difference between apparent digestibility and true digestibility emerges. Digestibility measured for that part of faeces that not include ENL is referred as true digestibility; apparent digestibility does not eliminate ENL, being the difference between intake and output. Nevertheless, the apparent digestibility is taken as reliable and representative value and thus what is used in digestibility trials, since the difference between apparent digestibility coefficient (ADC) and true digestibility coefficient (TDC) is as small as 5% (approx.), furthermore, the difference become minimal when the fish ingest a diet with high quality proteins (Hardy, 1997; Gatlin, 2010).

The difference in nutrients digestibility usually is the factor that mostly affect the nutrients utilization as energy source and therefore for growth. It confirm that the individual digestibility of the main nutrients contained in the diet, as well as digestible energy values, must be used in order to calculate the nutrients availability, because the main goal in diet formulation is to reach the highest

proportion of energy retained for growth in comparison with the gross energy intake.

2.2 Fish bioenergetics

The basal energetic requirements for fish is much lower than for terrestrial animals because fish are poikilotherms, which mean to expend energy in body temperature maintenance is not necessary. Beside, to live in aquatic environment implies that the gravity force will not act as strongly as on shore and consequently aquatic animals do not require strong body structures, which derive in energy saving for body build. In the same context, the motion (swim) and to keep the body position in the water requires less energy than on the ground. Finally, nitrates wastes excretion demand less energy utilization for fish than terrestrial animals because terrestrials need to transform the ammonia (result from protein catabolism) into less toxic substances before being excreted. As this process is not necessary for fish, it allow them to obtain 10% to 20% more energy from protein catabolism (Brett and Groves, 1979).

Through the catabolism and oxidation of nutrients contained in diet, the fish get net chemical energy, which will be released and used to keep vital processes and growth (anabolism). From the total chemical energy released from the nutrients contained in the diet (gross energy) a big fraction is lost and eliminated by the faeces; the energy remained in the body (digestible energy) is not ready yet to be used for the fish, some process (deamination, for example) must occur and will cost some energy, the remained energy is named metabolizable energy, but digestion and absorption will also take some energy reflected in heat increment (low value for fish). After all this process, the portion of energy remained (net energy) is that available and used for the fish in maintenance, gluconeogenesis, activity (including reproduction) and growth (Klekowsky and Duncan, 1975). For salmonid species, the

sum of the ingested energy lost as no digestible feed (faeces), metabolic excretion and heat is around 45% (Figure 1).

Intraspecific variation in the energetic loss depends of several factors as composition and digestibility of the ingredients, feed regime, water temperature, size and physiological stage of the fish and other factors that together will influence the nutrients requirement of the fish (Table 1). Further, the variation in basal metabolism is correlated to the metabolic cost faced during digestion (Millidine et al., 2009).

Fish does not utilize directly this free released energy, because it is attached to phosphoric bonds of adenosine triphosphate (ATP) that are highly energetic and the main driver force of the biochemical life processes.

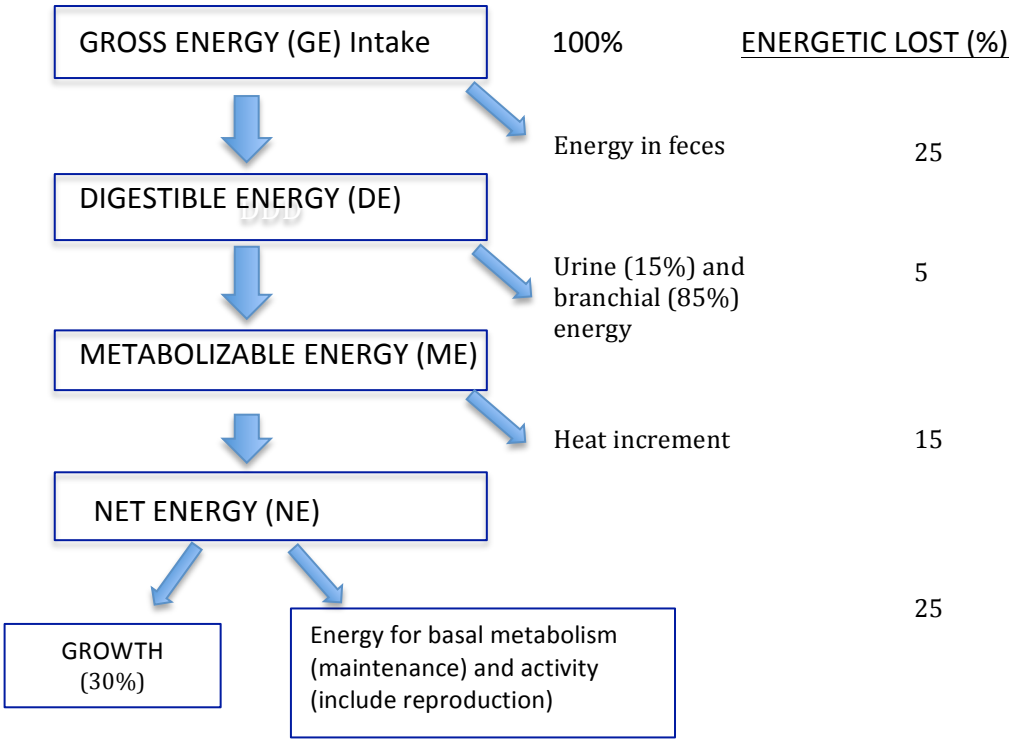

Figure 1. Evaluation of Feed Energy Value.
Source: own elaboration based on Tacon, 1987

Table 1. Factors that influence the nutrients (protein – energetic) fish requirements

FACTOR	REASON	SOURCE
Water Temperature	Increasing water temperature will increase fish feed consumption and, therefore, metabolic rate, consequently the requirement of energy for maintenance will increase.	Brett and Groves, 1979; Lowell 1998
Fish Size	Metabolic rates, and consequently the requirement of energy for maintenance, decrease as the fish size increase.	Brett and Groves, 1979; Lowell 1998
Physiological Stage	Energetic requirement increase during the reproduction activity periods. Nutrients requirement differs in fresh and salt water stage.	Wooton, 1985; Lowell 1998
Water Flow	Increasing the water flow will increase the energetic requirement to keep the fish position in the water column.	Brett and Groves, 1979; Knights, 1985
Water Quality and Stress	Contaminants, increased salinity, low concentration of dissolved oxygen and high density (confinement) increase the energy requirement for maintenance.	Talbot, 1993; Knights, 1985; Lowell 1998
Diet formulation and ingredients quality	The individual quality of each ingredient affect the diet formulation and feed nutritional and physical quality	Lowell, 1998
Environmental factors	Example: photoperiod. In dark environment the nutrients requirement is lower.	Lowell, 1998

2.3 Fish growth

Growth is a factor that has primary importance for economic success, since it is related to weight gain as consequence of proper nutrients absorption, the way to promote it is having a diet formulation that contains proteins and lipids of high digestibility in the proper amount (Caballero et al., 1999) and rearing the fish in as best as possible environmental conditions to avoid any disruption that can exacerbate an appropriate metabolism, thus maximize the protein rate deposition.

As general (biological) concept, growth is a multifactorial and complex regulated process that involves the flesh hypertrophy (size increases) and hyperplasia (amount increases) (Pecl and Moltschaniwskyj, 1997). Growth can be divided in two concepts:

- Somatic growth, which includes the organism improvement in longitudinal dimensions as result of cells reproduction and cells substances apposition.
- Mass growth, which is related to volume increases due to the energies reserves accumulation.

Both depends on many different physiological factors, linked to the genetic charge inherited from parents, which give to every individual the specific capacity to assimilate and utilize the ingested nutrients, and behavioural factors related to the opportunity the fish have to acquire the required nutrients for optimal development. By this way, the nutrients consumed are used to build new cell structures (anabolism) and energy obtainment (catabolism), with both as complex coupled processes that depend on each other and make metabolism together.

An unique characteristic in fish as compared to other vertebrates, is that both hyperplasia and hypertrophy contribute to muscle growth beyond post-larval stage and, even under optimal conditions, growth will be not linear. Growth is affected by extrinsic factors mainly related to rearing parameters (temperature, pressure, osmotic conditions and contaminants) as well as intrinsic patterns like tension, innervation or activity (Mommsen, 2001). Abundant literature is available about the factors and patterns that can affect salmon growth, as photoperiod (Boeuf and Le Bail, 1999); digestible protein (DP) digestible energy (DE) ratio and feeding level (Azevedo et al., 2002); temperature, feed fat content and oil source (Bendiksen et al., 2003; Karalazos et al., 2011) for instance.

Another peculiar characteristic of fish is their capacity to accumulate functional protein for storage that at the end make the fish more efficient when additional muscle is present. Not aquatic vertebrates have muscle fibre arranged to run or fly,

that make the muscle mass concentrate in some areas (legs or chest), but fish, oppositely, have a good muscle mass distribution and the special disposal of the muscles fibres around the body allow them to keep the tissue functionality and use the accumulation of functional protein as a way to reserve energy (Mommsen, 2001).

Several hormones through complex processes and interactions regulate the growth. Somatic growth (including energy metabolism) is mainly controlled by the GH/insulin like growth factor (IGF). The system is constituted by the growth hormone (GH) that promotes protein accretion increasing it rate synthesis in organs (like liver, stomach, gills and heart) (Björnsson, 1997) and tissues; GH receptors; Insulin like growth factor 1 (IGF1) and Insulin like growth factor 2 (IGF2), that are similar acting in the metabolic process of muscle growth mainly by the uptake of amino acids into the muscle, inducing mitogenesis that improve it, together with muscle protein synthesis at the same level that GH do; IGF receptors and IGF binding proteins (IGFBP) (Mommsen, 2001).

Arginine is a basic but versatile amino acid that act as building block for proteins and is involved in several metabolism routes; it is essential for the synthesis of polyamides (that are extremely related to increase muscle mass) and creatine (which is fundamental for muscle growth since it is the molecule where this tissue storage the energy) (Mommsen, 2001).

2.4 Macronutrients in salmon diet

The feeding habits of any species reflect it digestive tract anatomy, adapted to intestinal function, developing specialized anatomical and physiological features. Salmon, as carnivorous fish, have a J-shaped stomach and short intestine ($1_{body\ length}$: $0.8_{intestine}$) with the capacity to intestinal amino acid transport and absorption, but not at all glucose (Buddington et al., 1987). The specie also counts a blind ending sacs (pyloric caeca) that allow them optimize digestion and high lipids absorption, that make it able to effectively utilize wax esters.

Protein and lipids are the main macro-ingredients that a salmon diet must content (Figure 2). The amount and quality of nutrients ingested have direct impact on fish growth. The amount of proteins and lipids in the diet must be in the proper ratio to avoid any disequilibrium that would leads in the incapacity to lean tissue accretion and proper body structures, or use amino acids as energy source for basic functions, which is not profitable in concept of cost-benefit because greater amount of ATP is required to obtain energy from these components. Besides, since the nutritional value for any compound diet is measured by the digestibility of it individual ingredients (Luptasch et al., 1997; Allan et al., 2000) nutrients quality must not be underestimated, as it must be good enough to supply the fish needs. If the lipid quality is not optimal, again the consequence will be the use of amino acids as source of energy instead intended for growth. Besides, it is of major importance to consider the possible interactions between different nutrients, it could lead in a serious health diminished or benefits. Increasing the levels of dietary lipids (up to 24%) the efficiency of protein utilization will be higher (FAO, online), for example. In salmon, due the lack of fish oil, different vegetables oil has been tested and in different proportions, at the moment the conclusion is that is not possible to replace more than 50% fish oil without fish health diminished (inflammatory responses). Furthermore, since the intestine has fundamental importance acting as barrier to pathogenic microorganisms and as selective permeable barrier for

absorption and osmoregulation of nutrients (Buddington et al., 1997) proper feed is required to maintain the fish in the optimal desirable conditions. Reveco et al. (2014) proved that the diet has direct effect in the population of the intestinal bacteria, it could be linked to the inflammatory effect that soybean meal has in the distal intestine.

2.4.1 Proteins

Around 21% of the salmon flesh is protein (Ytrestøyl, Aas and Åsgard, 2015). As a carnivorous species it requires large amount of protein in its diet. However, as this amount depends on the amino acid composition of the diet, the fish do not require a specific amount of proteins, but an equilibrate mix of amino acids. The protein in the diet must provide the 10 essential amino acids the fish requires and nitrogen for the non-essential amino acids synthesis (Halver, 2002a). Salmon is adapted to utilize the protein excess, which compensates the incapacity to digest and metabolize carbohydrates due to the deficiency of specialized enzymes digestion it has (Navas, 1997). Starch is the only polysaccharide able to be digested by salmons, through endogens enzymes, but it must be previously gelatinized. It is added to the diet due to the bond capacity it has and should not be included in a proportion bigger than 10% of the salmons compound diet (Storebakken, direct talk), even that, commercial salmon feed include more than it is recommended to improve playability and stability of the feed.

Digestible protein to energy ratio requirement for salmon depends of it stage and size, but in general is ranging around 18 g/MJ (Einen and Roem, 1997), and a protein content in diet about 40% (FAO, online), constituting the main cost among feed ingredients (Figure 3).

The proteins metabolism follows some extremely complex paths. In very shorts words, once the protein is consumed, it is digested and hydrolysed to release the amino acids, which are absorbed mainly at the level of the anterior portion of the

digestive tract and distributed trough the blood to all the organs and flesh, where they are used for new tissue proteins synthesis, transaminated into other amino acids, catabolized to provide energy used in gluconeogenesis or lipogenesis, or used in the synthesis of other non-protein nitrogenous molecules (Halver, 2002b).

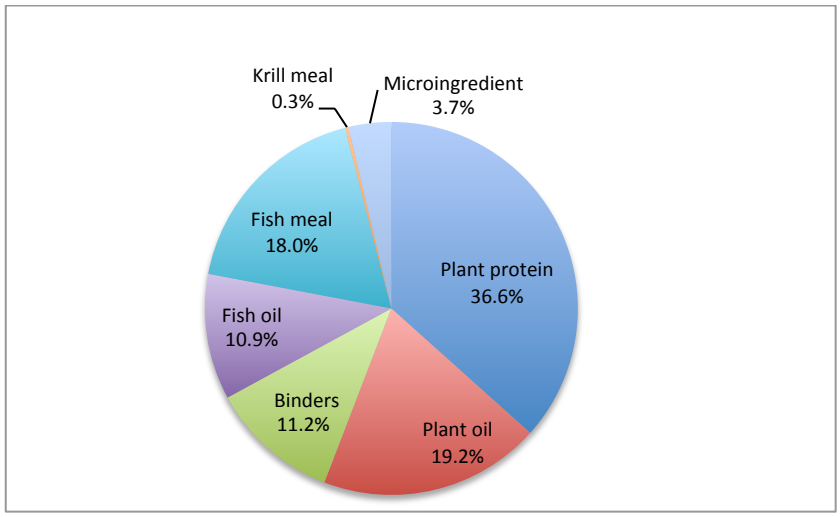

Figure 2. Commercial salmon feed composition. (Own elaboration based on Ytrestøyl et al., 2015)

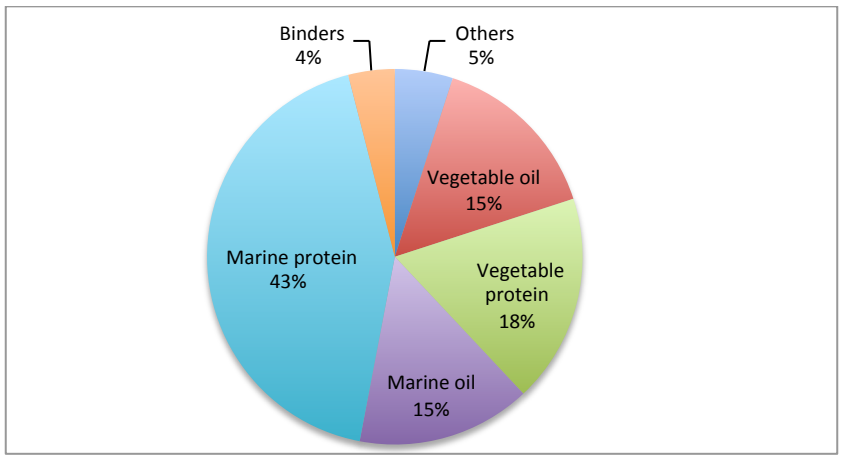

Figure 3. Feed component cost. (Own elaboration based on Ewos data)

2.4.2 Lipids

Lipids have great importance in salmon diet, supplying energy and essential fatty acids (EFA), but also involved as bioactive components (Schiller, 2012). It inclusion has increased from 10% in 1970's to ~ 30-40% at current (Tacon et al., 2008). The

major lipids available to salmon are triglycerides and wax esters. The specie has the characteristic that once the fatty acid in the diet are assimilated it can be modified by the fish through a metabolic process of elongation (C addition to extend the fatty acid chain) and desaturation (increase the number of double bonds in the fatty acid chain). It need fatty acid n-3 series (20:5n-3 EPA and/or 22:6n-3 DHA) to maintain the long chain high-unsaturated fatty acid (HUFA) required level deposited in the muscle, but flesh fatty acid composition highly depend of fatty acid composition in the diet (Torstensen et al., 2005). As most marine species, salmon is not able to synthesize *de novo* polyunsaturated fatty acid (PUFA) because they have limited activity of Δ^5 and Δ^6 desaturases (Monroig, Tocher and Navarro, 2013). Since the lipids in the diet differs in chain length and unsaturation, consequently they have different melting point and polarity, thus, one of the reason because temperature is a key factor in it utilization. A not proper fatty acid level in the diet, or an alteration in adequate rearing conditions can affect the survival, growth and pigmentation of salmons (Olsen et al., 2005).

In his review Tocher (2003) takes a general assumption that lipid digestion, absorption and lipoprotein formation seems similar in fish than in mammals, besides, summarize that the pathways of lipid synthesis in fish intestine is still uncertain. The lipid homeostasis (balance between intake, transport, storage, biosynthesis, metabolism and catabolism) acts under a very complex regulation, since each one of the processes must work and be controlled independently in a cell specific manner and at the same time in co-junction with each one of the others processes at the whole body level as well as in a specific tissue, keeping it extremely sophisticate balance.

Atlantic salmon use the liver as the organ where the main fraction of lipids metabolism and transport occur and, differing that other species, it has not the capacity to store it. Lipids absorption occurs mainly in the anterior intestine (duodenum) and pyloric caeca, where is the highest lipolytic activity, however it can

be absorbed along the entire portion of the intestine in lessening quantity. Pancreatic lipase and bile salt are released to the intestine being the main responsible of the lipids digestion, where free fatty acid and glycerol are the result from the luminal hydrolysis of the triglycerides. The lipids, stored in the enterocytes, are transported as lipoproteins to the circulatory system to be delivered to the liver or directly to the liver through the portal system (Schlenk and Benson, 2001).

2.5 Apparent digestibility coefficient (ADC)

Since in salmon diet the large amount, most costly and that one with greater impact in growth are proteins, abundant literature is related to it digestibility (Sugiura et al., 1998; Hillestad et al., 1999; Yamamoto et al., 2007; Sajadi and Carter, 2008), to get a list of studies related to protein digestibility see Sales (2008). Researchers have good knowledge about different protein source and it ADC, results may differ mainly depending on protein source, faecal collection method utilized and inert indicator added to the diet. Standard values of ADC from different sources of proteins are summarized in table 2. (*)

Lipids ADC are in a different stage. In comparison among all nutrients contained in a diet, lipids digestibility differ depending to the composition itself (degree of saturation, chain length, melting point of fatty acid and the source) (Hua and Bureau, 2009), which make impossible establish a standard ADC for lipids. It is based that reports in the literature shows different and, sometimes, contradictories results. Recently, Krontveit et al. (2014) reported a modification of lipids digestibility over the time, whereas Huguet et al. (2015) reported an insignificant difference on the same criteria, but methodologies used in these trials were different. Cho and Slinger (1979) reported an ADC of fish meal lipids as 97%, same than reported by Bureau and Cho (1999) but poultry by-product lipid source of 83% (in rainbow trout). In general terms is only possible make mention that digestibility

of lipids in fish is reduced when saturation and chain length increase (Torstensen et al., 2000; Caballero et al., 2002) and, from different research conclusions, can be assumed that the lipids ADC for fish is high, ranging over 80% (Huan and Bureau, 2009).

Table 2. Apparent digestibility coefficients (ADC) of different protein sources for Atlantic salmon

Ingredient	Crude protein (%)	ADC (%)
Fishmeal LT94, Norway	77.5	95.8
Fishmeal Atlantic Herring, Canada	74.5	94.2
Fishmeal Anchovy, Peru	66.5	94.4
Fish Soluble Protein Concentrate (CPSPG)	71.7	95.5
Poultry by-product meal	59.7	81.5
Poultry feather meal, hydrolysed	82.5	71.6
Meat meal, defatted, steam cooked	55.8	85.0
Blood meal, spray dried	89.8	70.6
Corn gluten meal	59.9	88.9
Soybean meal, dehulled	49.8	83.4
Soy protein concentrate	68.7	93.8
Canola meal	38.9	76.8
Brewer yeast	41.8	87.4
Wheat gluten	79.5	98.0
Pea protein concentrate	49.1	90.4
Lupin meal, white	38.6	88.9

Source: FAO, online.
(*) Faecal sampling method as well as the inert indicator was not specified.

2.6 Genetic programs and feed efficiency

In the early 1970s, Akvaforsk, Norway started selective breeding programs for Atlantic salmon and rainbow trout (Gjedrem, 2010). In those first family based programs, selection was practised for increased growth and lower proportion of precocious males and grilse. Gradually more traits have been included in the breeding objective (table 3).

Atlantic salmon is the specie that shows the highest response to selection for growth rate, with 17.8% per generation (5 estimates) (Gjedrem and Morten, 2015). From this trait, another correlated responses are expected to bring some improvement because genetically are highly associated (Kolstad et al., 2004), feed efficiency for instance.

Feed efficiency ratio (Kg gain/Kg feed)(FER), the unit of biomass generate from unit of feed consumed, is a trait difficult and expensive to record because it require to register the feed intake on a large number of families over a long period which is extremely expensive and impossible for individuals because with an isolated fish part of the variability in feed efficiency derived from the group interaction will be missed (Martins et al., 2008), although is highly promising that growth rate and FER have a positive genetic correlation (Gjedrem and Morten, 2015). During 5 generations of growth rate selection in Atlantic salmon, Thodesen et al., (1999) found that feed efficiency has been improved by 20% and 40% in feed intake, protein retention increased with 9% and energy retention with 14%. Thodesen et al. (2001) report a correlation between FER and growth of 0.79 that is a bit higher than the 0.6 reported by Kolstad et al. (2004).

It is important to remark the big impact that even a little improvement in feed utilization has on production costs and in many other subjects related to sustainability. Quantitatively, considering that already in the 5^{th} generation the improvement in feed conversion ratio (Kg feed/kg gain) (FCR) was around 23%, now (11^{th} generation) it should be 30% at least, meaning to save 5 to 6 billions NOK/year or 0.12 millions tons of feed (Gjedrem by direct talk).

Table 3 Develop of breeding traits

Trait	Phenotype		Year - class
	Own	Sibs	
Growth	x	x	1972
Sexual maturity	x	x	1980
Survival	x	x	
Disease resistance			
Furunculosis		x	1989
ISA		x	1992
IPN		x	1997
PD		x	
Carcass quality			
Body fat	(x)	x	1993
Filet color		x	1990
Carcass yield	x	x	2001
Animal welfare			
Cardiac abnormality		x	
Vertebrae deformities	x	x	1992
Abdominal adhesions		x	
Melanin deposit		x	

2.7 Near Infrared (NIR) and X-ray fluorescence (XRF) spectroscopy, general characteristics and works principle

2.7.1 Near infrared (NIR) spectroscopy

It is a non-destructive analytical technic, which has gained ample acceptance among others similar methods mainly because of the numerous advantages it poses as low cost, fast, accurate and reliable, samples are easy to handle, multiple attributes can be analysed simultaneously and the use of any chemical agent is not necessary (Klaypradit et al., 2011). The general works principle relates the light absorbed by a sample to its chemical and physical composition, providing spectral data which contain integrated information of the samples as the absorption responses from all it components, as well as some measuring noises (Ishikawa et al., 2012).

2.7.2 X-ray fluorescence (XRF) spectroscopy

Is a non-destructive analytical technic that allow determine the elemental composition of different elements (until 40 at the same time). Easy to handle, portable, reliable and fast are some of the features found in the system. The spectrum from the characteristic fluorescence x-ray (energy) emitted by each specific element in the sample is measured that allow determining the chemistry of those elements and their relative concentration (in a range of 1.25 KeV up to 85 KeV) when it is illuminated by x-ray. The device also measure the elastic (Raleigh) and inelastic (Compton) scatter x-ray emitted by the sample to define the estimated density and percentage of the light elements in the sample (See figure 4).

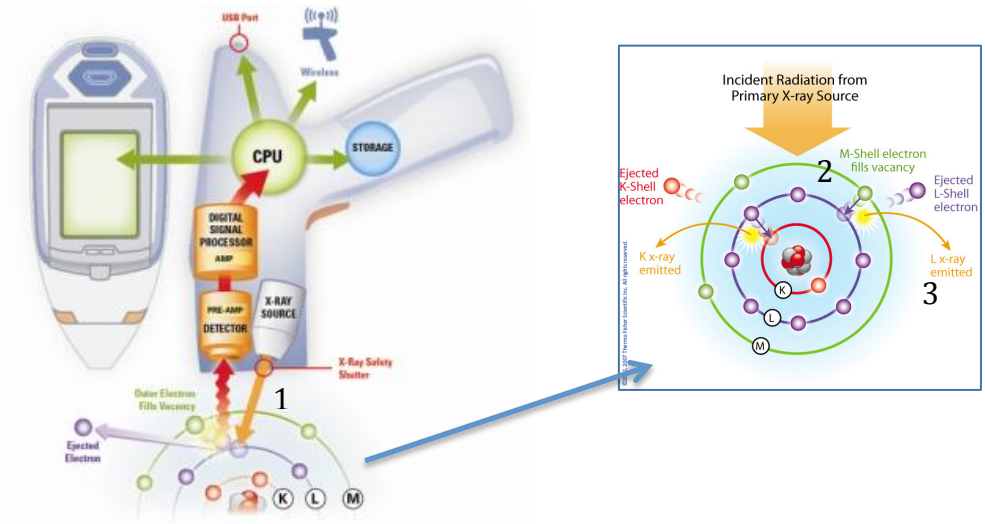

Figure 4 X-ray fluorescence spectroscopy works principle.
Source: Niton, online.

1 A fluorescent x-ray is created when an x-ray of sufficient energy strikes an atom in the sample, dislodging an electron from one of the atom's inner orbital shells.

2 The atom regains stability, filling the vacancy left in the inner orbital shell with an electron from one of the atom's higher energy orbital shells.

3 The electron drops to the lower energy state by releasing a fluorescent x-ray, and the energy of this x-ray is equal to the specific difference in energy between two quantum states of the electron.

3 Material and Methods

3.1 Fish and rearing conditions

The first part of the present study was carried out at Nofima, research station Sunndalsøra (62º 40'N, 08º 33'E), Norway, over a period of 56 days, from 26 of August to 21 of October 2014. The fish specie used was Atlantic salmon (*Salmo salar*), from the breeding company Salmobreed that had been started in February 2013.

During the third week of July of 2014, a random sample of 60 PIT-tagged fish was netted from a tank with a total of 1390 fish of 50 fullsib families. The sampling resulted that the 60 fish were from 34 of the 50 families. The fish, with a mean body weight of 440.2 g (SD 38.7 g), were placed into an indoor octagonal tank 3.3 m^3 (2m diameter), supplied with salt water previously filtered through 10 µm sieve and UV treated. Water mean temperature was 11.9 ºC (min 8.0 ºC, max 14.8 ºC) and O_2 concentration, regulated by magnetic valves, kept in the range of 87% - 90% during the 56 days experimental period. Before start of the experiment the fish were accustomed to the rearing system for two weeks. From 26th August the fish were fed 6 times per hour, 24h $days^{-1}$, until satiation by 20% overfeeding, with a 4.5 mm extruded diet (see Table 1) produced at Nofima, Aquafeed Technology Centre, Bergen). The feed was provided by an automatic-mechanical feeder device.

Yttrium lll oxide (Y_2O_3; Alfa Aesar Karlsruhe, Germany, with a purity of 99.9%) was mixed with the dry feed ingredients prior to extrusion as the inert non-absorbed reference substance (indicator).

Fish were treated in accordance with the Norwegian Animal Welfare Act.

Table 4. Formulation and proximate composition (%) of salmon feed.

Fish meal [a]	38.53
Soy Protein Concentrate 16/13	12.00
Fish oil (herring) O1/13	10.00
Rapseed oil O1/11	12.00
Horse beans 53/13	5.45
Wheat 3/14	8.00
Sunseed meal 88/12	3.33
Wheat gluten 36/13	5.00
Betafin T 4/13	1.00
Soy lecithine T21/13	1.00
Vitamin mix T3/13[b]	2.00
Mineral mix T1/14[c]	0.52
Monosodiumphosphate T49/10 (24% P)	1.00
Carop. Pink (10%) T 35/10	0.01
Yttrium oxide T20/13	0.15
Composition (%)	
Moisture	4.8
Dry Matter	95.20
Ash	7.49
Nitrogen (7.05 * 6.25)	44.06
Energy	23.38
Crude Fat	28.10
Yttrium Oxide (Y_2O_3)	0.10

[a] NorseNat LT. [b] Vitamin Mix: D3, C, B12, E, thiamin, riboflavin, pyridoxine-HCl, calcium pantothenate, biotin, folic acid, niacin, menadione bisulfite. [c] Mineral mix: Magnesium, potassium, zinc, iron, manganese, copper.

3.2 Sampling

The first collection of faeces samples (stripping) took place on 2th October, 37 days after the fish got the experimental feed. The second collection took place on 15th October (13 days after the 1st stripping) while the third collection took place on 21th October (6 days after the 2nd stripping). The collection of faeces was performed by, first, sampling randomly a few fish at a time from the tank and placed them in a small container with FINQUEL vet. 1000 mg/g (Trikainmesilat) to be anaesthetized. Then, the belly was wiped off cautiously, using towel paper, to avoid cross contamination with water and/or mucus during the fish handling, after which the fish were stripped for faeces carefully following the procedure reported by

Austreng (1978). Individual body weight was recorded on August 26 and at 2nd and 3rd stripping. All raw faecal samples (n= 173) were weighed by an analytical balance (Metter Toledo AB204-S, deviation of ± 0.0001 g), labelled and liofilizated at -40 ºC (freeze dried) after which the weight of the dry matter (DM) was recorded. Thereafter, the samples were stored at -20 ºC until the prediction of crude protein (CP), crude lipids (CL) and yttrium (Y_2O_3) could be obtained.

Originally, only two faeces samplings 14 days apart were planned. However, an additional third sampling was done as the two Master students was not possible to participate previously in the experiment.

3.3 Chemical analyses of nutrients in feed

The second part of the trial was conducted at Nofima, Ås (59º 39'N, 10º 45'E), Norway.

DM of the experimental feed was determined after drying loss to constant weight in an oven at 103 °C for 24h (ISO 6496: 1999). Ash content were determined after combusted until constant weight at 550 °C for 16-18h (ISO 5984: 2002). C P content (N x 6.25) was determined using automated Kjeldahl method (Kjeltech Auto System, Tecator, Höganäs, Sweden) (ISO 5983). Crude fat content was determined using the Soxhlet method (Soxtec HT6, Tecator, Höganäs, Sweden; after HCl hydrolysis) as described by Folch et al., 1957. Yttrium were analysed at Eurofins, Moss, Norway by inductively coupled plasma optical emission spectrometry (ICP-OES) on a VARIAN vista Pro instrument (Varian Inc., Palo Alto, California).

3.4 Prediction of macronutrients and Yttrium Oxide (Y_2O_3) in faeces

In this study, the proteins and fat values of the faeces samples were obtained from a prediction equation developed in a parallel thesis (Kwarteng, 2015) with Near Infrared Spectroscopy (NIRS) spectra as the explanatory variables. Details about technical procedures, calibration and the corresponding prediction equations can be found in the mentioned thesis, but with a short summary in the following.

3.4.1 Prediction of proteins and lipids in faeces

The dried faeces samples were finely ground using a manual mortar.

Calibration of proteins was estimated using 180 freeze-dried fish faeces samples from 10 different feeding trials. Predicted proteins were validating using 23 new fish faeces samples from previous experiments performed at Nofima (ÅS).

Lipids calibration was done with a total of 115 fish faeces samples coming from 9 different feeding experiments.

NIRS spectra acquisition for each sample was done by placing each sample in a small crystal ring cup in the NIRS system (XDS spectrometer monochromator, XM-1000, FOSS Electric, XDS Rapid Content Analyser, Höganäs, Sweden), and the absorption data recorded. Each sample was randomly divided in three parts, the NIR spectra of each part ran twice and the corresponding spectra averaged.

Analytical software of spectra was done using Vision spectral analysis for windows (Copyright 2006 FOSS NIR system, INC, Denmark) and imported to Unscramble X (version 10.3) statistical analysis software (CAMO Process AS, Oslo, Norway) for data processing and calibration elaboration.

3.4.2 Prediction of Yttrium Oxide (Y_2O_3) in faeces

Inert indicator (Y_2O_3) was determined at Ewos Innovation facilities, Dirdal (58º 49'N, 6º 11'E), Norway, using a Thermo Scientific XL3 NITON X-ray fluorescence (XRF) spectroscopy analyser. The sample name, spectrum and elemental composition were recorded automatically. Each faeces sample was ran twice, using all amount (mean 0.3 g) of faeces available. Yttrium content was obtained using an existing faeces/feed model prediction developed by EWOS. However, as this prediction equation was developed for a lower Y_2O_3 value (up to 550 ppm) than the samples in this study (mean 3500 ppm), the predicted values were obtained by extrapolation of the data, therefore were compared with values from chemical analysis of some samples. The result allowed declare it as acceptable (See appendix 4).

3.5 Calculation of growth performance

Fish growth performance was calculated for each growth period (start to 2nd stripping, 2nd stripping to 3rd stripping and start to 3rd stripping as:

Weight gain (g/fish) = final weight – initial weight.

Specific growth rate (SGR, % day) = 100 x [ln (final mean weight) – ln (initial mean weight)] x days^{-1}.

3.6 Calculation of apparent digestibility coefficient (ADC)

ADC was calculated as:

ADC = [(*a-b)/a*] * 100 ; where *a=%* nutrient (protein or fat) in feed divided by % Y_2O_3 in feed.

and *b=%* nutrient in faeces divided by %Y_2O_3 in faeces.

3.7 Statistical analysis

The recorded and calculated data were first subjected to simple statistic analyses (mean, standard deviation, maximum and minimum values, and Pearson correlation coefficients between some selected variables.

For the predicted protein and fat ADC, the repeatability (the measurement of consistent individual differences) for each of them was calculated as $\sigma^2_x / \sigma^2_x + \sigma^2_e$, where σ^2_x is the variance component between fish, and σ^2_e is the variance component between the three stripping within fish. These variance components were obtained from one-way analyses of variance with digestibility of each nutrient as the dependent variable and fish ID as a random effect in addition to the residual error, and which can be written as:

$$Y_{ij} = \mu + F_i + E_{ij}$$

where Y_{ij} is the *j*th ADC of the *i*th individual, μ is the overall mean (constant), F_i is the effect of the individual fish (random) and E_{ij} is the error (random effect of the three stripping within fish).

The statistical analyses were performed using SAS/STAT software, version 9.4 for Windows. Copyright © SAS Institute. Inc.

4. Results

For this trial feed conversion is assumed acceptable regarding that the amount of uneaten feed was in the expected range, based that the fish were fed *ad libitum*.

During the experiment the mortality was low (5%), three fish died, one before the second stripping and two before the third stripping. The stripping procedure was done without issues, except that few fish with no faecal material at the second and third stripping. Faeces samples were collected for all individuals at the first stripping, while during the second and third stripping faeces could not be obtained from four and five of the fish, respectively.

The literature agreed that there is not an optimal procedure for fish faeces sampling. In the stripping method we used there is a risk to get an underestimation of ADC, as was mentioned in the literature review. The amount of faeces obtained in the third, and last, stripping was clearly lower (40%) than from the two previous strippings. This could be due to a human factor (it was done by a student, but supervised by the research technician that did the two first strippings) or by the fact that the third stripping was performed only six days after the second stripping, while the second was performed 14 days after the first. However, the differences affect only the size of the sample but not the possibility to produce a bias on it estimation since the results for protein content in faeces was not correlated to the amount of faeces ($r= 0.04$ for protein and $r= 0.4$ for lipids, data not shown).

4.1 Descriptive statistics for traits recorded at each stripping

Descriptive statistics for the traits recorded at each of the three faecal strippings are given in Table 5.

The mean values of the weight faeces samples (for both wet and dry) were quite similar in the first and second stripping but the standard deviation bigger at the second stripping. The mean weight of faeces from the third stripping is clearly lower than the previous two stripping but the standard deviation similar to the second stripping.

From the data we can say that the mean dry matter of the three faeces samples were similar, with a little bit lower value at 2nd stripping. Details about it calculations are not shown but the means for dry matter were 14.6%, 13.3% and 15.2% for the first, second and third stripping respectively. Details from individual performance can be find in appendix 1.

Fish growth was irregular, some individuals showed great performance while others not so prosperous reflected by the relatively low correlation coefficient between fish weight at start and at second stripping (0.4) and at start and third stripping (0.4). A high correlation is observed at second and third stripping (0.9) due to the short gap between sampling (6 days). The total weight gain from individuals was concentrate in the range between 400 g to 500 g (n=23) (figure 5). As was expected, a high correlation between total weight gain and SGR was obtained (r = 0.94).

The means values for SGR are similar in the three lapses it was calculated, again with a value slightly low at the time between second and third stripping, even that, the means indicate an acceptable growth during the experimental period.

Table 5. Descriptive statistics for the traits recorded at each of the three strippings

	N	Mean	SD	Min	Max	Time (days)
Fish body weight (g)						
Tagging	60	440.2	38.7	383	547	0
Stripping 1	nd(*)	nd	nd	nd	nd	37
Stripping 2	59	806.6	93.0	502	1020	50
Stripping 3	57	852.8	105.2	547	1092	56
Fish weight gain (g)						
Tagging to 2º stripping	59	367.1	84.5	53	592	50
From 2º to 3º stripping	57	43.7	20.9	-7	102	6
Tagging to 3º stripping	57	414.3	96.9	98	664	56
Specific Growth Rate (SGR) (% d^{-1})						
Tagging to 2º stripping	59	1.2	0.2	0.2	1.8	50
From 2º to 3º stripping	57	0.9	0.4	0.2	1.8	6
Tagging to 3º stripping	57	1.2	0.2	0.4	1.7	56
Weight faeces samples raw (g)						
Stripping 1	60	2.39	0.69	0.76	3.92	37
Stripping 2	56	2.40	0.98	0.79	4.76	50
Stripping 3	55	1.45	0.88	0.09	4.47	56
Weight faeces samples dry (g)						
Stripping 1	60	0.35	0.11	0.08	0.57	37
Stripping 2	56	0.32	0.15	0.10	0.78	50
Stripping 3	55	0.22	0.14	0.008	0.68	56
Protein Content in faeces (%)						
Stripping 1	60	15.8	1.1	10.7	17.6	37
Stripping 2	56	15.2	1.0	13.0	17.9	50
Stripping 3	55	16.0	1.3	11.1	18.8	56
Lipids Content in faeces (%)						
Stripping 1	60	5.5	0.9	3.7	9.1	37
Stripping 2	56	5.0	0.9	3.2	7.8	50
Stripping 3	55	5.4	1.5	1.4	12.4	56

(*) nd: no data.

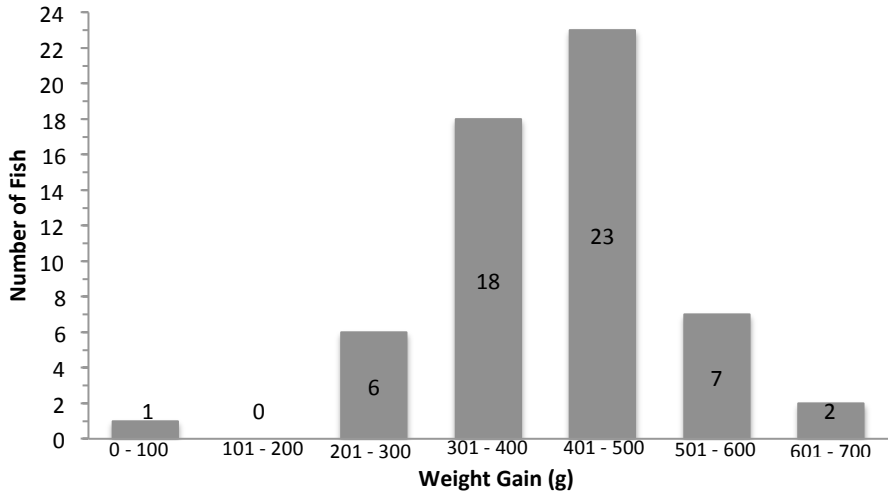

Figure 5. Distribution of total weight gain (from start to third stripping)

Protein and fat content in faeces (% DM) showed similar mean values and standard deviations at the three strippings. However, the standard deviation for lipids content at the third stripping was 0.6 % higher than at the previous two strippings, even that the weight of dry faeces and proteins content is less correlated to the weight of the faeces and the lipids content.

4.2 ADC

Means values of ADC for proteins and lipids were in an acceptable level for all the three measures and very similar each other. The ADC values for protein are concentrate around the 90% and the ADC values for lipid much more concentrated around the 95% (figure 6 and 7 respectively). However, for both protein and fat the standard deviation at the third stripping was around one percent unit higher than at the two previous strippings and ADC decrease after each stripping (see Table 6 and Figure 8) following the same tendency than the indicator (appendix 3) and different tendency than the amount of nutrient in faeces (table 2).

Table 6. Resume of fish macronutrients ADC (%).

	Stripping	Nº Fish	Mean	SD	Min	Max
Protein	1	57	90.82	1.37	85.70	92.96
	2	56	89.96	1.49	85.78	91.62
	3	54	88.49	2.50	79.46	91.79
Lipids	1	57	95.01	1.12	90.41	97.10
	2	56	94.82	1.09	91.49	96.30
	3	54	93.94	1.99	86.83	97.00

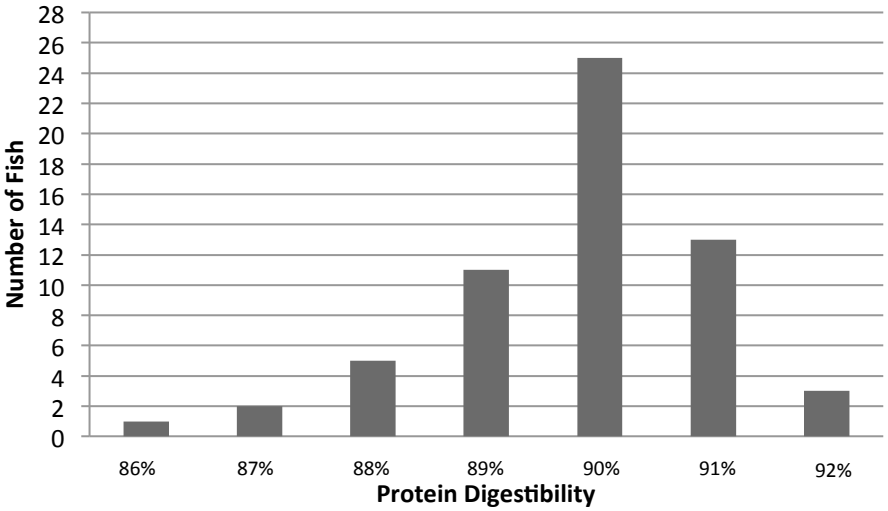

Figure 6. Distribution of individual ADC of protein (from mean values)

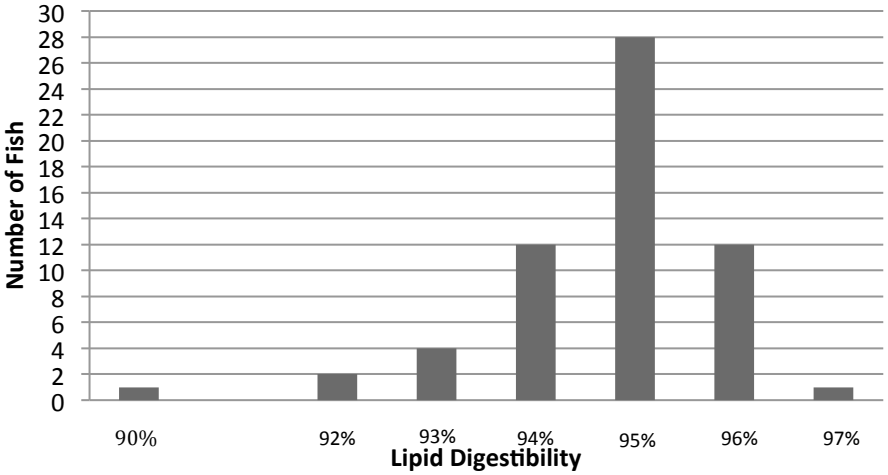

Figure 7.Distribution of individual ADC of lipid (from mean values)

Individual proteins and lipids ADC from each measurement is showed in figure 8

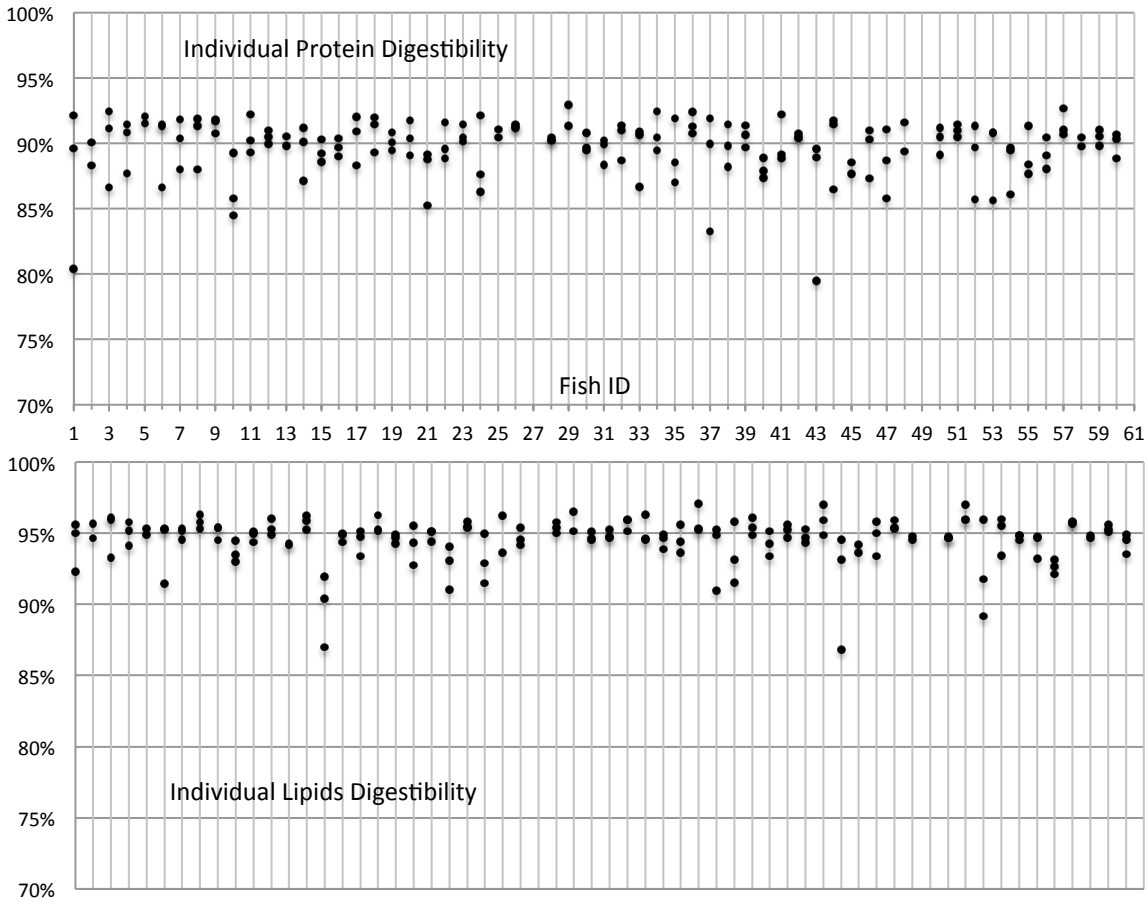

Figure 8.ADC of each fish at three strippings.

4.3 Correlations between growth and ADC

The regression coefficient (b) of the total weight gain (from start to third stripping) on their average protein ADC of the three strippings was close to zero and not significantly different from zero (Figure 9). The correlation coefficient (r) between the average of protein ADC of each fish and total weight gain was low (0.04) and not significantly different from zero (Figure 9).

Similarly, the regression coefficient of the total weight gain on their average lipid ADC of the three strippings was close to zero and not significantly different from zero (Figure 10). The correlation coefficient between average of lipid ADC of each

fish and total weight gain was low (-0.13) and not significantly different from zero (Figure 10).

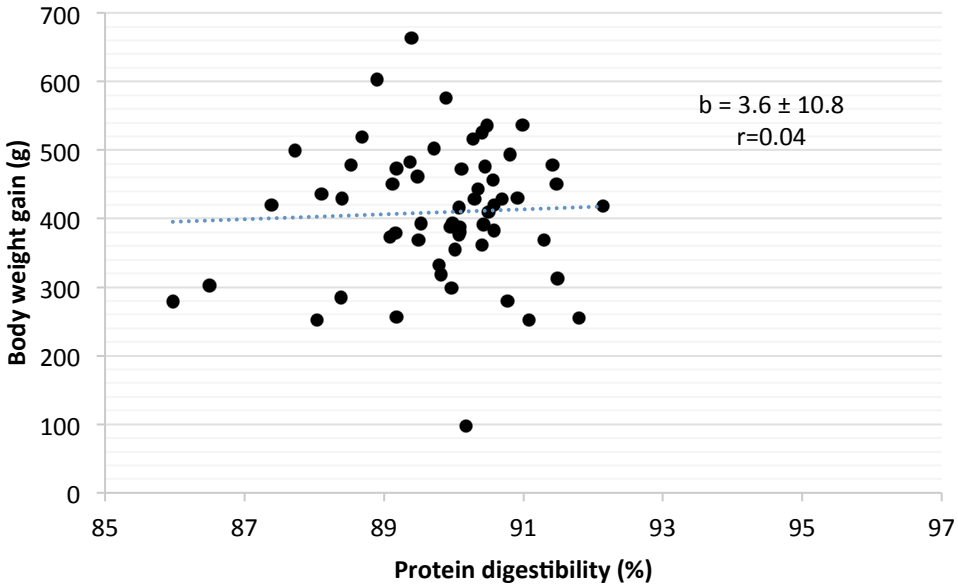

Figure 9. Regression of weight gain on protein ADC (from mean values of individuals)

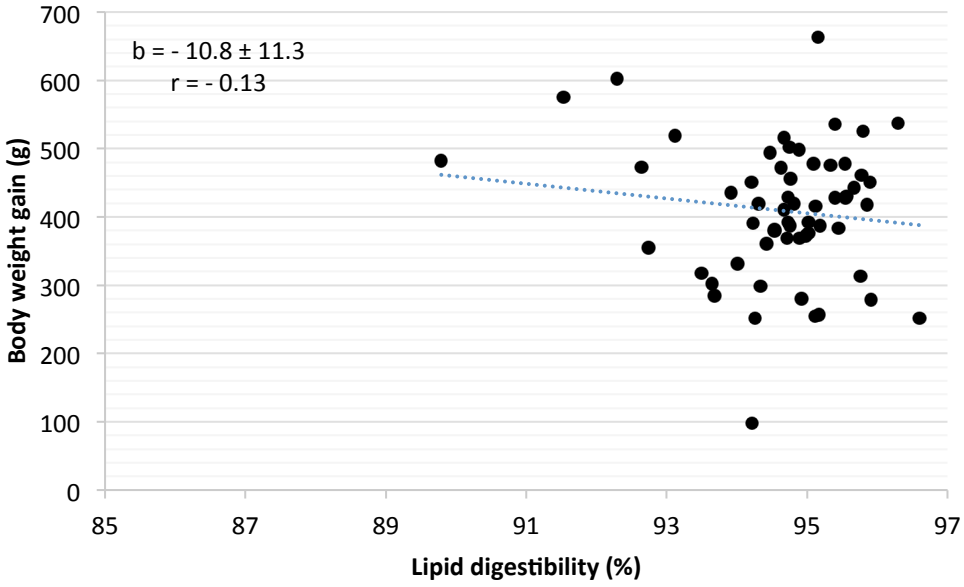

Figure 10. Regression of weight gain on lipid ADC (from mean values of individuals)

4.4 Repeatability (Intraclass correlation (ICC))

The calculated intraclass correlation for ADC for protein and lipid are given in Table 7. The ADC for proteins was close to zero, while those for lipid ranged from 0.24 (second and third stripping) to 0.50 (first and second stripping). These results strongly indicate a significant genetic variation of medium magnitude for ADC of lipid in Atlantic salmon, while the genetic variation in ADC for protein is not present, indicating, perhaps, a strong environmental effect.

Table 7. Intraclass correlation for ADC of protein and lipid obtained from the three strippings.

PROTEIN	Stripping			
	1, 2 and 3	1 and 2	1 and 3	2 and 3
Fish ID	0.00	0.05	0.00	0.17
Residual	4.29	2.15	5.35	4.54
Intraclass correlation	**0.00**	**0.02**	**0.00**	**0.04**
LIPIDS	1, 2 and 3	1 and 2	1 and 3	2 and 3
Fish ID	0.79	0.61	0.98	0.65
Residual	1.51	0.61	1.85	2.08
Intraclass correlation	**0.34**	**0.50**	**0.34**	**0.24**

5 Discussion

All fish were reared in the same tank, the diet formulation was qualitative and quantitatively covering the nutritional requirements for salmon in post-smolt stage and the feed strategy appropriate for the trial (*add libitum,* 24h d^{-1}). This is supported by the mean values obtained in fish weight gain during the 56 days (414.3 g) and the SGR that, in the present study, were higher than those reported by Bjerkeng et al. (2007) and similar to the values obtained by Aas et al. (2006) for the control diet. Therefore, the rearing conditions seems to be adequate for the normal develop of the fish and not considered as a reason that itself affects the individual fish performance. It is also supported with the fact that the fish had acceptable feed intake, thus the nutrients contained in faeces samples should allow to get reliable and repeatable ADCs estimation (Bureau and Cho, 1999).

The amount of dry faeces samples obtained per fish at each stripping (see appendix 1) were sufficient large for obtaining prediction of protein and lipid based on NIRS spectra. The detection of protein and lipid in the faeces samples was accurate with a root mean square error of cross validation (RMSECV) of 1.6 (R^2=0.97) for protein and RMSECV of 0.7 (R^2=0.92) for lipid as compared to the observed standard deviation of 8.4 (protein) and 2.4 (lipid) for the two calibration data sets (Kwarteng, 2015), which is more than enough to accept it as reliable prediction equations for protein and lipid in fish faecal samples. This shows that even for those samples with extremely low amount of dry faeces (0.04 g) the method is feasible to obtain digestibility coefficients of nutrients for individual fish. Use of chemical analyses requires much large amount of faeces and may include the collection of faeces over several days depending on the size of the fish (Glencross et al., 2007). Beside, NIR method allows to obtain ADC for several different nutrients from the same sample and in a non destructive way, while chemical analyses will require a separate sample for each nutrient without the possibility to reuse the sample.

The ADC means values of protein (table 6) are in the line with those reported for Aas et al. (2006) rearing Atlantic salmon of ≈170 g (initial weight) at water temperature similar than this study. In comparison with the values reported by Karalazos et al. (2011) our results are a bit higher, but the salmons used in the study were bigger and the trial was testing different diets. It lead us to assume our ADC values for protein as acceptable. Beside, the faecal collection by stripping is the more suitable and reliable method for determination of ADCs of protein, as Stone et al. (2008), who studied the effect of repeated stripping on rainbow trout, concluded.

As was mentioned in the literature review, lipids digestibility rang over 80%. The means values for ADC of lipid obtained in this study (table 6) are over these values and in the line with results obtained for Huguet et al. (2015) rearing Atlantic salmon of ≈55 g in Australia. It is our knowledge that lipids are easily metabolized than proteins, but is important to keep in mind what researches confirm, that lipid digestibility is highly dependent of the source and by it chemical and physical properties (Menoyo et al., 2005; Huan and Bureau, 2009; Huguet et al., 2015), despite that considerations our values for ADC of lipid are acceptable.

After all, our results indicate that the method utilised to collect the faeces samples as well to predict the amount of nutrients in faeces are appropriate to obtain reliable ADC values of protein and lipid. Beside, the three faeces collections were started approximately at the same time of the day (about 10 am) to standardize the procedure as much as possible. Further, Carter (2003) compared two inert indicators (being Yttrium oxide one of them) and proved that Y_2O_3 is perfectly suitable as inert digestibility indicator in Atlantic salmon for lipids components and in general, Y_2O_3 is accepted widely as an appropriate indicator in the measurement of digestibility for fish trials (Austreng et al., 2000; Hatlen et al., 2015).

Despite the small difference in mean values obtained in ADC for protein and lipid for the three stripping, a tendency to a decrease in the values over time was observed. The decrease in the number of fish from the first to the third stripping is only three, therefore this should not bring an important difference. The reason of the tendency to decrease ADC is unclear, there are many factors that could affect the digestibility in fish. A possible reason could be the fact that at the third stripping (only six days after the second) the fish were not sufficiently recovered that is reflected in a higher SD of ACD at the third stripping and a less amount of faeces, bigger SD, therefore bigger variation. In addition, the SGR is lower between the second and third stripping (see table 5) with 3 fish that even decreasing it weight during the same period (data not shown) which suggest that the feed intake was reduced and perhaps some fish did not eat at all, therefore they did not growth. The results suggest that digestibility may vary over the time within an individual and between the individuals at a given time.

To our best knowledge, this is the first study on individual digestibility for fish. Our results show that the repeatability for lipids are moderate (0.24 – 0.50) which indicate a substantial genetic variation in ADC of lipid in Atlantic salmon, meaning that ADC of lipid can be improved through selective breeding. Thodesen et al. (2001), conclude that through selection for increased growth the feed utilization can be improved. Then, the question that emerge is about the genetic correlation between digestibility and feed efficiency. To answer this question requires an additional study where both digestibility and feed efficiency can be recorded on sufficient large number of families.

The repeatability for ADC of protein is very low or zero, meaning that the genetic variation is very low and thus no scope for genetic improvement of ADC of protein in Atlantic salmon. This is in the line with Thodesen et al. (2001) who found no significant effect of Atlantic salmon family on the ADC of protein. However, in rainbow trout Austreng and Refstie (1979) reported a significant difference in

protein digestibility between different families of rainbow trout. Rasmussen and Jokumsen (2009) agreed, concluding that there exist significant variation among rainbow trout families in growth and digestibility.

To deduce the difference in the results of repeatability for ADC of lipid and protein is difficult to assess since (as is mentioned in the introduction) digestibility can be affected by a number of factors and the ingredients are processed by different metabolism. The sampling error of the estimates is low since the studied fish population was relatively large (60 fish), all fish were reared in the same tank and short period of time (56 days) to reduce the temporary environmental effect and assume the trait constant, so it should not lead us to impair the results.

The close to zero regression coefficient of total weight gain on ADC of both lipid and protein and the close to zero correlation between these variables suggest that ADC have no effect on growth. Assuming that feed consumption is positively correlated to growth, the result suggest the possibility that fish with low ADC may have a higher feed intake and thus better growth than a fish with high ADC but lower feed intake.

In conclusion, the results in this study indicate significant genetic variation in digestibility of lipid in Atlantic salmon, but not for protein. Therefore it should be possible to obtain a favorable genetic gain for ADC of lipid, but not for ADC of protein.

As digestibility coefficient can be obtained on live individual fish, the selection for increased lipid digestibility can be performed using a combined selection procedure; i.e. selection both between and within families thus obtaining higher selection intensity and thus higher genetic gain than using family selection only. This is also a feasible procedure as digestibility coefficients can be obtained on a large number of individuals using NIR technology and the prediction equation developed by Kwarteng (2015), and thus at a much lower cost than using chemical analyses as was the alternative prior to this study.

Appendices

Appendix 1: Raw values from the 60 individuals at the 3 times sampling.

Fish ID	Fish weight start	Fish weight 2º stripping	Fish weight 3º stripping	Weight raw faeces			Weight dry faeces		
				1º Stripping	2º Stripping	3º Stripping	1º Stripping	2º Stripping	3º Stripping
1	443	840	863	3.41	2.39	0.94	0.47	0.29	0.13
2	411	668	667.5	2.81	1.55	0.32	0.35	0.17	0.02
3	499	869	915	3.02	2.99	0.79	0.44	0.39	0.12
4	463	812	856	1.66	1.63	1.15	0.25	0.22	0.17
5	547	802	Die	2.60	1.20	Die	0.44	0.21	Die
6	452	790	784	2.46	0.79	0.29	0.40	0.11	0.04
7	422	742	799	2.98	2.09	1.10	0.48	0.32	0.13
8	505	960	1030.5	3.25	1.55	1.38	0.42	0.21	0.18
9	493	908	971	2.18	2.92	1.52	0.30	0.40	0.23
10	398	670	700.5	1.48	1.94	0.94	0.21	0.20	0.11
11	395	759	815	2.82	3.52	1.87	0.48	0.49	0.35
12	462	929	998	2.80	4.33	0.61	0.40	0.61	0.10
13	449	502	547	0.76	3.48	2.77	0.08	0.46	0.48
14	498	908	959	1.73	2.19	0.83	0.23	0.30	0.09
15	513	910	996	3.34	4.76	4.47	0.57	0.78	0.68
16	435	880	937	2.39	3.41	2.59	0.35	0.47	0.39
17	396	721	757	2.50	1.95	0.82	0.44	0.27	0.14
18	471	875	901	1.91	3.51	1.96	0.33	0.44	0.33
19	400	847	872	3.58	4.40	3.62	0.49	0.52	0.48
20	455	784	846	1.67	1.24	0.83	0.25	0.15	0.10
21	456	892	955	3.04	3.45	0.68	0.36	0.38	0.07
22	464	777	819	2.30	2.11	1.10	0.33	0.29	0.15
23	431	828	859	3.22	1.96	2.92	0.46	0.26	0.44
24	436	907	955	1.63	0.89	1.08	0.29	0.11	0.17
25	389	669	Die	1.66	1.19	Die	0.19	0.16	Die
26	477	820	846	2.16	2.94	1.02	0.35	0.46	0.17
27	478	Die	Die	2.15	Die	Die	0.35	Die	Die
28	452	842	880	2.85	2.91	1.22	0.39	0.43	0.21
29	437	814	855	0.94	Empty	1.70	0.15	Empty	0.33
30	422	765	809	2.68	2.51	2.54	0.40	0.30	0.37
31	484	832	853	1.20	2.21	1.35	0.16	0.27	0.18
32	413	793	856	3.92	3.49	2.42	0.47	0.56	0.33
33	428	1020	1092	1.33	3.12	1.00	0.13	0.42	0.10
34	424	863	918	2.51	3.63	1.37	0.42	0.56	0.21
35	402	752	781	2.94	0.99	1.04	0.45	0.12	0.16
36	448	877	898.5	3.19	2.84	2.87	0.36	0.40	0.42
37	421	685	706	1.94	1.98	0.53	0.33	0.27	0.08
38	401	687	719	3.66	3.74	1.36	0.53	0.43	0.16
39	420	754	803	2.33	2.14	0.90	0.37	0.33	0.12
40	474	712	726	1.58	1.68	1.22	0.23	0.19	0.18
41	498	840	885	2.91	1.79	2.69	0.43	0.23	0.42
42	386	785	842	2.19	3.33	1.30	0.32	0.44	0.21
43	393	640	672	1.97	1.42	0.44	0.33	0.11	0.04
44	388	881	963.5	2.68	2.29	1.02	0.49	0.40	0.17
45	503	884	939	2.06	Empty	2.07	0.32	Empty	0.27
46	417	762	810	1.99	2.42	0.92	0.32	0.34	0.13

47	383	808	861	2.67	2.75	0.86	0.39	0.31	0.11
48	426	804	836	2.02	1.85	0.48	0.31	0.29	0.03
49	438	697	690	1.65	Empty	0.09	0.17	Empty	0.01
50	404	861	920	2.09	2.68	2.62	0.32	0.38	0.48
51	503	977	1040	2.27	1.48	2.04	0.32	0.20	0.30
52	405	906	1008	3.70	1.38	1.03	0.53	0.16	0.16
53	433	770	806	1.68	0.82	0.75	0.22	0.10	0.09
54	383	765	812	2.43	3.10	1.32	0.38	0.34	0.21
55	413	817	864	2.07	3.51	1.20	0.32	0.51	0.17
56	426	852	899	2.51	2.14	1.64	0.44	0.26	0.27
57	420	709	733	2.69	3.42	2.65	0.42	0.48	0.38
58	475	822	856	3.03	1.39	1.75	0.51	0.22	0.31
59	431	862	907	2.24	2.09	1.81	0.34	0.29	0.30
60	422	682	720.5	2.14	1.09	1.02	0.31	0.14	0.16

Appendix 2 Means values of Indicator (Y_2O_3) content (%) in faeces from individuals

Fish ID	Mean	SD
1	0.34	0.13
2	0.34	0.06
3	0.38	0.09
4	0.35	0.03
5	0.37	0.02
6	0.33	0.13
7	0.37	0.06
8	0.38	0.05
9	0.38	0.03
10	0.28	0.05
11	0.39	0.04
12	0.37	0.01
13	0.35	0.04
14	0.34	0.05
15	0.33	0.00
16	0.37	0.01
17	0.36	0.06
18	0.39	0.04
19	0.34	0.02
20	0.35	0.07
21	0.30	0.02
22	0.34	0.05
23	0.37	0.03
24	0.32	0.09
25	0.36	0.04
26	0.37	0.03
27	0.40	nd
28	0.37	0.02
29	0.45	0.04
30	0.36	0.03
31	0.33	0.02
32	0.38	0.05
33	0.33	0.08
34	0.38	0.05
35	0.32	0.10
36	0.40	0.05
37	0.32	0.11
38	0.36	0.04
39	0.38	0.01
40	0.29	0.01
41	0.37	0.06

42	0.36	0.02
43	0.27	0.09
44	0.33	0.09
45	0.32	0.02
46	0.35	0.06
47	0.34	0.06
48	0.37	0.04
49	0.38	nd
50	0.35	0.02
51	0.41	0.05
52	0.30	0.07
53	0.34	0.08
54	0.33	0.05
55	0.33	0.05
56	0.30	0.03
57	0.40	0.03
58	0.35	0.02
59	0.36	0.02
60	0.36	0.05

Appendix 3. Resume of indicator (Y_2O_3) content in faeces from the three strippings

Yttrium Oxide (%)	N	Mean	SD	Min	Max
Stripping 1	57	0.39	0.04	0.24	0.48
Stripping 2	56	0.34	0.04	0.25	0.41
Stripping 3	54	0.32	0.06	0.16	0.42

Appendix 4. Validation of the values from X-ray spectrometry of the indicator (Y_2O_3) content in faeces

Fish ID	X-ray spectrometry	Chemical analysis	Difference
3	0.461	0.410	0.051
12	0.379	0.341	0.038
32	0.408	0.375	0.033
36	0.447	0.361	0.086
38	0.392	0.351	0.041

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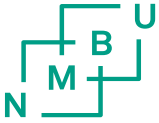

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