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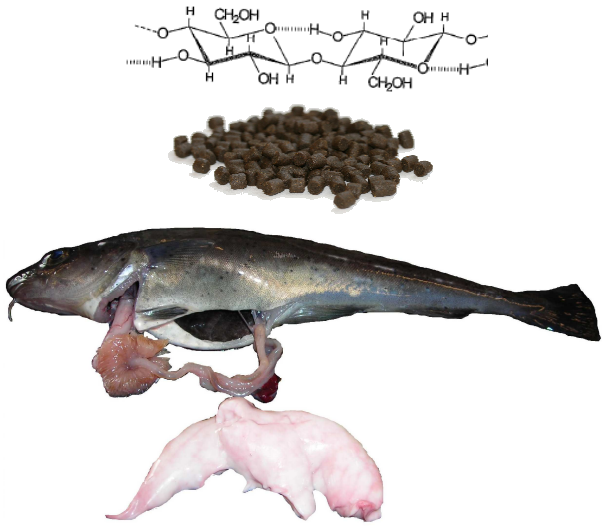
- effects on growth, feed intake, liver size and digestibility of nutrients



Anette Lekva

Master Thesis in Nutrition of Aquatic Organisms in Aquaculture

Institute of Biology
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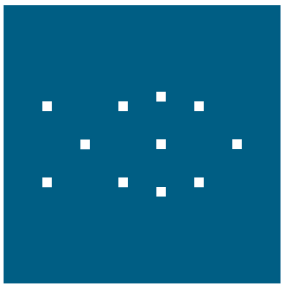

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Anette Lekva

Abbreviations

ADC	Apparent digestibility coefficient
ADP	Adenosine diphosphate
AER	Ash efficiency ratio
Ag	Silver
ALAT	Alanine aminotransferase (GOT)
APV	Ash productive value
As	Arsenic
ASAT	Aspartate aminotransferase (GPT)
ATP	Adenosine triphosphate
Ba	Barium
Cd	Cadmium
CF	Condition factor
Co	Cobalt
Cu	Copper
FCR	Feed conversion ratio
Fe	Iron
FM0	100% fish meal as protein source, plus 0% α-cellulose
FM6	100% fish meal as protein source, plus 6% α-cellulose
FM12	100% fish meal as protein source, plus 12% α-cellulose
FM18	100% fish meal as protein source, plus 18% α-cellulose
GIT	Gastro intestinal tract
GOT	Glutamate Oxaloacetate Transaminase (ALAT)
GPT	Glutamate-Pyruvate Transaminase (ASAT)
Hb	Haemoglobin
Hct	Haematocrit
Hg	Mercury
HSI	Hepatosomatic index / liver index
ICP-MS	Inductive coupled plasma mass spectrophotometer
LER	Lipid efficiency ratio
LOQ	Limit of quantification
LPV	Lipid productive value
MCH	Mean cell haematocrit
MCHC	Mean cell haemoglobin concentration
MCV	Mean cell volume
Mn	Manganese
Mo	Molybdenum
NAD	Nicotinamin adenine dinucleotide
NSP	Non starch polysaccharides

Pb	Lead
PE	Protein to energy ratio
PER	Protein efficiency ratio
PP0	50% plant protein and 50% fish meal as protein source, plus 0% α-cellulose
PP6	50% plant protein and 50% fish meal as protein source, plus 6% α-cellulose
PP12	50% plant protein and 50% fish meal as protein source, plus 12% α-cellulose
PP18	50% plant protein and 50% fish meal as protein source, plus 18% α-cellulose
PPV	Protein productive value
SD	Standard deviation
Se	Selenium
SER	Starch efficiency ratio
SGR	Specific growth rate
Sn	Tin
SPC	Soy protein concentrate
SPV	Starch productive value
Sr	Strontium
V	Vanadium
Zn	Zink

1 Abstract

Atlantic cod (*Gadus morhua*) is known to grow large livers, especially farmed cod, but commercially, muscle growth is preferred, due to profits. Therefore, main focus in this feeding trial was to achieve lowered liver size, without compromising growth, feed utilization, digestibility of nutrients and elements, and maintain health. To accomplish this, indigestible fibre was used to dilute energy in feed. Atlantic cod were fed increasing α-cellulose inclusions (0, 6, 12 and 18%), in sustainable diets based on 50% plant ingredients (soy protein concentrate and wheat gluten) plus 50% fish meal as protein source (PP), or diets based on 100% fish meal as protein source (FM). The initial average cod live weight was 138 g, and the feeding trial lasted for 14 weeks.

Good growth and feed utilization were obtained in all diet groups. At the end of the feeding trial growth was equal in all diet groups, but feed intake was higher for cod fed α-cellulose. The similar energy intake, even though dietary energy concentrations differed, and the dietary digestible macro nutrients (protein/fat/carbohydrates) and protein to energy (PE) showed similar ratios, indicate that the cod adjusted its feed intake in accordance to both energy and protein amount. The liver index (HSI) was not affected by increased α-cellulose. Digestibility of fat decreased with increased α-cellulose, in disagreement with increased lipid efficiency ratio (LER). Digestibility of protein was not affected by α-cellulose. Dry matter digestibility decreased with increased α-cellulose, in accordance with increased dry matter in faeces. There were variation in element concentrations between plant based and fish meal based diets. Some element digestibility results were negative, which might be due to presence of elements in water, although the diet is the main source of elements for fish. Most element digestibility results were not affected by increased α-cellulose. Though, Mn and Ba digestibility increased with increased α-cellulose, for cod fed PP diets, but not FM diets. Cod health was good, and macro composition of whole body and liver was not affected by α-cellulose.

Our study reports that it seems hard to manipulate energy deposition in cod, even when using α-cellulose as energy dilution in diets. Our results also show that cod tolerated up to 18% α-cellulose inclusions, both in combination with FM and PP, and that the cod compensated by higher feed intake to satisfy its need for energy and protein. Although, cod fed 18% α-cellulose had more faecal waste, which may be a local environmental challenge.

2 Introduction

2.1 General introduction

2.1.1 Cod farming and feed resources

Atlantic cod have historically been important for fisheries. Interest for cod farming rose in the late 90-ties when fisheries quotas dramatically decreased. However, today the north east Atlantic cod stocks have increased (Langaard *et al.*, 2008), and the quotas increased 8% from 2008 to 2009. Volumes of farmed cod are still low, nevertheless, cod farming competes with the global aquaculture industry for feed ingredients (Torrissen, 2008). Feed constitute approximately 60-80% of the operational costs in global intensive aquaculture (FAO, 2006), partly due to expensive marine feed ingredients (fish meal and fish oil). In addition, wild fish is exploited to the maximum for most species, and thereby marine ingredients are resources that will not increase. Cod need a diet high in protein, and low in fat and carbohydrates. According to Rosenlund *et al*. (2004) cod feed should contain 50-60% protein, 13-20% fat and less than 15% digestible carbohydrates (based on dry weight), to maintain good growth, and relative low liver size (<12%). Though, the nutrient requirement differs some with the cod's life stage. Protein in cod diets has until recently been based on expensive high quality fish meal, which has not been a sustainable feed. Cod farming is expected to increase about 17 % annually, which predicts a production of 50 000 cod in 2017 (Torrissen, 2008). World aquaculture in 2004 produced 46 million tonnes in total for all species. To keep up current level of seafood consumption per capita it is estimated that aquaculture needs to reach 80 million tonnes world wide by 2050 (FAO, 2006). Therefore, even more sustainable feed ingredients should be used in fish farming. Plant feed ingredients are available at low prices in large quantities, but do not meet the nutrient requirement in the same manner as marine ingredients. Plant ingredients e.g. contain anti nutrients, and have more carbohydrates and indigestible components than fish meal (Francis *et al.*, 2001). Increases of these components may have negative effects on feed utilization. If more alternative ingredients shall be used, it has to be without considerable adverse effects on fish performance and health.

2.1.2 Cod gut waste and liver size

Remnants (gut waste, head, skin and bones) in cod production today constitute up to 60% of produced biomass (Hansen and Kjerstad, 2008), and the liver is a considerable amount of the gut waste. Farmed cod livers are mostly found to be larger than wild cod livers (Jobling, 1988; Grant *et al.*, 1998; Gildberg, 2004; Mørkøre, 2005). The market value for cod liver is not as high as the market value for cod muscle, therefore it is desirable that cod use protein for muscle growth and reduce liver growth. Liver size can be reduced by starvation (Hemre *et al.*, 1992; Jobling *et al.*, 1994; Karlsen *et al.*, 1994) and lower feeding frequencies (Dos Santos *et al.*, 1993). To reduce feeding to near starvation will result in reduced muscle growth, which will not be economically for the farmer. For Atlantic salmon (*Salmo salar*) energy dense diets, up to a certain level, have resulted in good growth and feed utilization (Hemre and Sandnes, 1999). The same strategy will however not function for cod, as this species stores all surplus energy in the liver. Researchers have shown that a high fat content in cod feed gives enlarged liver (1986; Lie *et al.*, 1988; Jobling *et al.*, 1991; Hemre *et al.*, 1992; Jobling *et al.*, 1994; Morais *et al.*, 2001; Rosenlund *et al.*, 2004; Karlsen *et al.*, 2006). Also increased dietary starch seems to increase fat and glycogen deposition in liver (Hemre *et al.*, 1989). The fact that high energy diets increase liver size is in agreement with liver size being reduced by using diets high in protein and low in energy (Rosenlund et al. 2004).

Muscle growth is probably driven by protein amount and composition (Hemre *et al.*, 1989; Karlsen *et al.*, 2006). The small amount of fat that is found in cod muscle is mostly membrane lipids (Lie, 1991). However, all metabolic mechanisms and growth demands energy, meaning that a proper protein to energy (PE) balance in the feed is needed, to avoid loss of body mass. Experiments with feed based on fish meal and fish oil in different amounts gave varying liver growth in cod, and it was indicated that to hold liver growth around 10-12% the relationship between protein to energy should be around 3 to 1 (Rosenlund *et al.*, 2004). This diet only functioned partly in big scale. Therefore, a new strategy was needed to better predict liver growth compared to muscle growth, especially since high quality ingredients were used in that experiment.

2.1.3 Cod feed utilization

Atlantic cod is an epibenthic-pelagic (Agbayani, 2001) and opportunistic species. Hence, in nature cod feed on a variety of fish and invertebrates from pelagic and benthic depending on which feed is accessible, the cod's life stage, location, spawning success of the prey and seasonal variations (Link and Almeida, 2000; Orlova *et al.*, 2005). In pelagic zones, cod feed on animals like capelin, herring, polar cod and crustaceans. While in benthic zones, the diet can consist of worms, molluscs, echinoderms and cructaceans. Thus the Atlantic cod is used to a variable diet in nature, and in addition does e.g. crustaceans and molluscs contain large amount of carbohydrates (chitin and glycogen). This could be why farmed cod show a high tolerance to plant ingredients (Albrektsen *et al.*, 2006; Hansen *et al.*, 2006; Refstie *et al.*, 2006a). The cod's natural prey does of course not contain same types of carbohydrates as plants. Anyway, experiments show that it is possible to include up to 50% plant protein in cod feed (Hansen *et al.*, 2007b) without any major adverse effects on growth and feed utilisation. But not without limitations, because there was a diarrhoea like condition in cod fed 100 % plant protein (Olsen *et al.*, 2007). However, digestion of starch in cod depends on amount consumed, source and physical state of the starch (Hemre *et al.*, 1990). Further, plant ingredients can create problems related to other components, especially the anti nutrients and fibres might inhibit digestion of nutrients.

Cod probably tolerate plant material in the diet better than salmonid species. In experiments with salmon elucidating the effect of soybean meal inclusions, there were found severe changes in the gastrointestinal tract (GIT) (Van Den Ingh *et al.*, 1991; Francis *et al.*, 2001; Krogdahl *et al.*, 2003). Plant protein diets gave no detectable enteritis changes in cod intestine (Hansen *et al.*, 2006; Refstie *et al.*, 2006b; Olsen *et al.*, 2007), plus there were found a significant number of bacteria in the lower intestinal tract. It was speculated if bacteria may have a digestive function and maybe explain why no enteritis changes were observed. Herbivore fish have caecal pouches for microbial digestion, and Seppola *et al*. (2006) found similar distal fermentation chambers in cod. Atlantic cod were found to compensate the high anti nutrient and fibre contents in diets by increasing feed intake and growth of the intestines to keep good somatic growth (Refstie *et al.*, 2006a; Hansen *et al.*, 2007a). Ergo, growth was not reduced even though it resulted in lower feed utilization and protein retention. In accordance with the idea that protein growth in animals most likely is regulated through control of food intake (Webster, 1993; Jobling *et al.*, 1994), but energy may be consumed in

excess, that means that fat deposition is much less strictly regulated. Based on the appearance and function of the cod GIT, it is classified among the omnivorous species. Intestine bacteria, larger intestines and more carbohydrate digesting enzymes are probably major reasons why herbivorous and omnivorous tolerates plant ingredients better than carnivorous. Experiments with cod have shown that there is no gain in exceeding one large meal every 24 hour (Hansen *et al.*, 2006). Atlantic cod have a flexible gastro intestinal tract (GIT), which can be filled to huge volumes when feeding frequencies are low, hence cod will maintain good somatic growth and liver growth (Jobling *et al.*, 1994). That could be why a specific strategy to manipulate the energy deposit in cod, with non-digestible fibre in feed, might lead to success.

2.1.4 Energy dilution of feed

Low energy fish diets are observed to result in quicker return of appetite than a energy dense diets (Jobling *et al.*, 1991; Jobling and Hjelmeland, 1992). This means that fish eat more if the feed have less energy. Fish regulates feed intake closely connected to the PE ratio in the diet, pointing to the importance of balancing both protein and energy. With a too low protein compared to energy content, muscle growth will be reduced and fat deposits can increase (Einen, 2001). Though, with a too high PE, some protein will be directed towards energy in metabolic processes, instead of being used for muscle growth.

Energy diluting with bone meal and crab by-products in feed is previously investigated with cod, giving increased feed intake and growth (Toppe *et al.*, 2006), although there was no effect on the liver index (HSI). However, studies with cellulose as indigestible bulk fillers are performed on other fish species. Cellulose inclusions up to 20% in feeds for European seabass (*Dicentrarchus labrax*) did not affect growth performance, but feed intake increased and HSI tended to decrease (Dias *et al.*, 1998). Rainbow trout (*Salmo gairdnerii*) fed up to 30% cellulose also increased feed intake to maintain growth (Bromley and Adkins, 1984). In another trial with rainbow trout (*Oncorhynchus mykiss*), up to 15% dietary cellulose did not affect growth, feed intake, apparent digestibility of crude protein, crude fat, total starch or ash (Hansen and Storebakken, 2007). Red drum (*Sciaenops ocellatus*) with 20% cellulose inclusions showed low fat deposition compared to red drum fed less cellulose, which may indicate that excess energy resulted in fat deposition (Turano *et al.*, 2002). However, HSI, dry matter or protein were not significantly affected. Experiments with tilapia (*Oreochromis*

niloticus) have shown that cellulose inclusions up to 9% may improve growth performance, FCR and PER, and produce leaner fish compared to fish without cellulose inclusions (Al-Ogaily, 1996). Although other studies with 10% and 20% cellulose for rainbow trout (*Salmo gairdnerii*) and tilapia (*Oreochromis mossambicus*), reported growth depression and reduction of feed intake (Hilton *et al.,* 1983; Dioundick and Stom, 1990).

The idea behind energy dilution has however its history from studies with fast growing broiler chicken. Therefore some background about fibre in broiler chicken diets is included. Soluble fibre in mammals and poultry tend to increase digesta viscosity and retard absorption of nutrients (Krogdahl *et al.*, 2005), while insoluble fibre tend to increase digesta transit, resulting in reduced absorption time. In experiments with broiler chickens some dietary NSP (non starch polysaccharides) increased digestion viscosity and reduced performance (Razdan *et al.*, 1997; Jozefiak *et al.*, 2004). Digestibility of protein and fat has also shown to be reduced with increased NSP content (Smits *et al.*, 2000; Saki and Alipana, 2005). Availability of some minerals were also affected by high dietary viscosity (Mohanna *et al.*, 1999). Smits *et al*. (2000) implied that if the viscous properties of NSP were eliminated in broiler chicken feed then the nutrient value of some fats would be improved. Jozefiak *et al*. (2004) found that it was likely not total NSP that influenced intestinal viscosity in broiler chickens, but the NSP type. In addition maybe enzyme supplementation, since diets with viscous components and microbial enzymes probably prevented formation of viscous chyme. Chickens fed more fibre increased length and weight of GIT (Jorgensen *et al.*, 1996; Saki and Alipana, 2005), and feed intake increased. NSP explained 86-96% of the metabolic energy variation in feed (Jorgensen *et al.*, 1996), which indicate that NSP is a good predictor of dietary energy content. Increasing raw fibre in feed reduced metabolic energy per metabolic weight unit, and chickens retained more energy as body protein and consequently less as body fat.

NSP in diets for broilers and some fish species shows that there are both positive and negative effects, depending on type and amount of NSP. With correct NSP we could perhaps reduce energy deposition in cod too. Adding inert fillers in feed will keep PE ratio constant, but by dilution, the proportion of individual nutrients and energy will be lowered. The indigestible cellulose we added as an energy dilution agent in the feed in this master study is claimed to be inert, and to result in no gut damage, at least in broilers. Therefore, the need to elucidate how it affected nutrient availability for cod, or resulted in gut damage or not, and how it affected liver growth, were urgent.

2.2 Feed components

2.2.1 Plant ingredients

Plant ingredients often have high carbohydrate levels, are imbalanced in essential amino acid compositions, lack several of the essential long chain fatty acids and other important nutrients such as phosphorus, contribute with a high content of non digestible components and anti nutrients (Francis *et al.*, 2001). This increase of possible negative components and lack of essential components may have negative effects on energy utilization, digestion viscosity, nutrient digestion, and secondary metabolic responses. Anti nutrients present in soybean meal are; protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, anti vitamins, allergens and fibres. Protease inhibitors and lectins affect protein digestion and utilization. There are processing techniques that can reduce damaging effects from anti nutrients in feed, such as dry and wet heating, solvent extraction and enzyme treatment. This has to be done with caution, since the treatment can alter important feed nutrients too.

Soy protein concentrate (SPC) was used in the present master thesis and this SPC is specially designed for the animal feed industry (Appendix 1, Figure 9.4 and 9.5), and contain 65% crude protein. In other trials with dietary SPC, it is found to have no negative effects on feed utilization and digestion, in certain concentrations, for salmon (Refstie *et al.*, 1998) and cod (Hansen *et al.*, 2006). This is due to the production process for SPC, which inactivates anti nutritional factors and removes soluble carbohydrates; hence the soy protein becomes highly digestible. Anyway, there is some trypsin inhibitor activity and antigen activity in this SPC, and for example, phytic acid can inhibit trypsin activity in salmon (Denstadli *et al.*, 2006). However, Francis *et al*. (2001) reported that protease inhibitors, phytates and antigens at normal levels in fish diets were unlikely to affect growth performance, but soluble NSP and saponins seem to be more important to be aware of in practical aquaculture nutrition. Compared to fish meal the amino acid profile of soy protein is quite well balanced (Dersjant-Li, 2002), but soy protein concentrate contain less methionine and lysine compared to fish meal, so crystalline amino acids were added to meet requirements by NRC (1993). Wheat gluten was also added as a protein source in the feed in the present trial (Appendix 1, Figure

9.6 and 9.7.), and wheat gluten has high protein content (80%). Hansen *et al*. (2006; 2007a) found that SPC combined with wheat gluten has better feed utilization and digestibility than other plant sources used in their cod diets, that is why these ingredients are used in the present trial. Wheat was added in all diets, as a binding agent in pellets. The indigestible fibre Vitacel® R 200 was added as the energy diluter of the feed (Appendix 1, Figure 9.1., 9.2. and 9.3.). Vitalcel is a highly purified powdered α-cellulose (99.5% cellulose), and claims to give optimal effects on intestinal flora, better feed conversion ratios, increased protein digestibility, lower mortalities, less diarrhoea diseases, better vitality and healthiness of the animal populations. However, this is at low inclusion levels (0.35-2.0%), and Vitacel has not been tried in cod diets before.

2.2.2 Fibre

Fibres, sugars, starch and glycogen are carbohydrates (Coultate, 2002). Carbohydrates are normally added in small amounts in fish feed, for energy and as binding agents (in pellets), and are reported to have protein sparing effect (Hemre *et al.*, 1995). Carbohydrates in fish feed mostly come from plants, since fish is low in glycogen. Cellulose, hemi-cellulose, lignin, gums, seaweed polysaccharides, pectin, resistant starch and inulin are different fibre types (Coultate, 2002). Bindings between fibre units are strong, often linked as β-linkages, and therefore not available for intestinal enzymes (Hemre, 2001). Fibres can be characterised by not being broken down by digestive enzymes in the small intestine of mammals (Burkitt, 1979). Although, most fibres are partly broken down by bacteria in the large intestine of omnivores, such as humans, and maybe cod (Hansen *et al.*, 2006). Colon bacteria ferment sugar to get energy for growth giving many types of end products, which can be absorbed by the host. Different plant sources can have different types and amounts of fibre, therefore it is important to know which carbohydrate types that are present in the plant feed ingredients and how these interact, before adding them to fish feed.

All fibres (except lignin) are polysaccharides, which can be classified into soluble and insoluble fibres. Pectin and gum are soluble, hemi-cellulose is partly soluble and cellulose is insoluble. Cellulose is an important component of all plant cell walls, and is built up by at least 3000 β-D-glucopyranose units (a sugar), which are linked together by β-1,4-glycosidic

linkages, and strong hydrogen bindings (Coultate, 2002), illustrated in Figure 2.2.1. Chitin, (a polysaccharide, found in e.g. crustaceans) is similar to cellulose (Campbell and Farrel, 2006). Chitin has the same bindings (β-1,4-glycosidic) as cellulose, and chitin may be described as cellulose with one hydroxyl group on each monomer (N-acetyl-β-D-glucosamine).

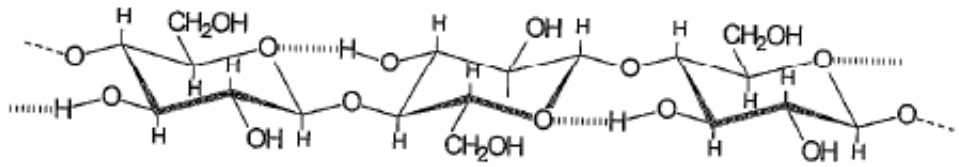


Figure 2.2.1. Cellulose chair structure, β-D-glucopyranoses with 1-4 glycosidic bonds and hydrogen bonds (Coultate, 2002).

Glucopyranose units are arranged in linear molecules and cellulose molecules form microfibrils in plant tissues. This is a stable and ordered structure which gives cellulose strength to be insoluble. Herbivore animals can to some extent utilize cellulose, because they have specialized microorganisms in parts of their digestion system (Burkitt, 1979; Coultate, 2002). These microorganisms can secrete enzymes which can hydrolyse cellulose and release free glucose. Further, the microorganisms get energy from fermenting glucose to short chain fatty acids, for example butyric acid, which can be absorbed and utilised by animals. Anyway, cellulose digestion is a slow process, and this is probably why herbivores have such a large GIT, compared to carnivores.

Diets high in fibre give health benefits for humans, for example lower number of bowel disorders (Burkitt, 1979; Coultate, 2002). Fibre improves the musculature of the gut wall, reduces the time potentially carcinogens spend in the bowel, and it is indicated by research that dietary fibre is probably good for more. Still, one should be careful to regard high fibre intake as only beneficial, because, for example phytic acids can complex divalent cations and cause calcium deficiencies. Furthermore, NSP might act as an anti nutrient, by reducing utilization of other nutrients rather than supplying nutrients (Krogdahl *et al.*, 2005). However, there is little information about anti nutritional factors of NSP in fish.

2.2.3 Elements

When replacing fish meal with plant ingredients it is important to also consider utilization of essential elements/minerals (Storebakken *et al.*, 2000b). There are essential and non essential minerals (Lorentzen *et al.*, 2001). Essential electrolytes are important for the fluid balance in fish (sodium (Na), potassium (K) and chlorine (Cl)). Essential bone minerals are calcium (Ca), phosphorus (P), magnesium (Mg) and sulphur (S). Essential trace elements are arsenic (As), zink (Zn), iron (Fe), manganese (Mn), copper (Cu), iodine (I), selenium (Se), fluorine (F), chromium (Cr), cobalt (Co), molybdenum (Mo), vanadium (V), silicon (Si), nickel (Ni) and tin (Sn). Some non essential elements, as the heavy metals arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg), can be very toxic. However, almost all minerals can be toxic with a too high intake. Therefore, the requirement and upper limit of minerals need to be determined. Trace elements are needed in smaller amounts than electrolytes and bone minerals. There is not total agreement about which minerals that should be considered essential, but it is mostly agreed that if a deficiency can be proven when the mineral is left out of the diet and that the symptoms disappears when the mineral is supplied again, then the mineral is essential. Deficiencies in fish can be reduced growth, poor feed utilization, reduced appetite, cataract, decreased mineralization of bones, etc. Knowledge still lacks about essential minerals, especially in fish. Though, essential minerals described for other animals may also be important for fish, 7 macro minerals and 16 trace minerals have been demonstrated as essential in 1 or more animal species (Davis and Gatlin, 1996). Knowledge about trace elements in fish are mainly limited to Fe, Cu, Mn, Zn and Se (Davis and Gatlin, 1996; Watanbe *et al.*, 1997), the fish requirement for these, Co and I is listed in Table 2.2.1.

Table 2.2.1. Fish requirement ranges for the trace elements; iron, copper, manganese, zinc, cobalt, selenium and iodine. The table is copied from Watanbe *et al*. (1997).

Mineral	Requirement [a]
Iron	30–170
Copper	1–5
Manganese	2–20
Zinc	15–40
Cobalt	0.05–1.0
Selenium	0.15–0.5
Iodine	1–4

[a] Expressed as mg mineral kg^{-1} dry diet.

Water is an important source for many minerals, e.g. Ca, Mg, Na, Fe, Zn, Cu, I, Se (Lorentzen *et al.*, 2001), which is also observed in experiments with both cod and salmon (Lied *et al.*,

1982; Storebakken *et al.*, 1998b; Ward *et al.*, 2005). Fish may absorb minerals with gills and intestine, however, the diet is the main source (Lall and Bishop, 1977). The mineral content in sea water is quite constant, and fish drink water as a part of their osmoregulation. To keep homeostasis of minerals, the fish have regulating mechanisms, either absorption gradients dependent on need (e.g. Cu, Zn, Fe) or a constantly high absorption with secretion of excess minerals (e.g. Se, I). Absorption regulation of Fe, Zn and Cu, mainly take place in the intestine. Ions from these metals are first transported through microvillis into the intestinal cell, where they are bound to specific intracellular carrier proteins, and transferred over the basal membrane into the blood.

Bioavailability of nutrients in feeds is defined as the nutrient part that is digested, absorbed and enters the biologic system or nutrient storage in fish. Absorption of minerals can have huge variations, depending on which chemical state the minerals are in, inside the intestine. Interactions with other feed ingredients also affect element availability (Hilton, 1989). Therefore, feed ingredient types used will influence the need for adding extra minerals to the diet. Element absorption also differs among species, e.g. coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*Oncorhynchus mykiss*) examined by Sugiura *et al*. (1998). A high bioavailability is reckoned as good for essential minerals, because less is needed to cover the requirement. Also, a low content and high utilization will reduce the mineral stream through fish, which is environmentally friendly. For toxic minerals, low bioavailability is an advantage, and of course a low content in diets. To determine digestibility of various trace elements (Al, As, Co, Mo, Se, Sn) have been found difficult, in salmon (Ward *et al.*, 2005), due to too low concentrations to provide accurate information. In addition some digestibility results often provided negative, probably due to presence of these elements in the water. Digestibility of Mn and Zn in cod is found to have no significant difference between diets with soy bean meal and fish meal (Førde-Skjærvik *et al.*, 2006).

Essential trace elements analysed in this master thesis are Co, Cu, Fe, Mn, Mo, Se, Sn, V and Zn. Other elements analysed are strontium (Sr), silver (Ag) and barium (Ba). Heavy metals analysed are As, Cd, Pb and Hg. Therefore some background information about these elements is included in the two following headlines; essential elements and undesirable elements. As is listed as both a heavy metal and an essential element; here it is listed as an undesirable, as only total As is determined, while the possible essential As form is arsenobetaine (Amlund *et al.*, 2006).

2.2.4 Essential elements

Zn is the trace element that is most abundant in fish; it exists in all organs and tissues (Watanbe *et al.*, 1997; Lorentzen *et al.*, 2001). In biologic systems Zn appears as a divalent ion, which easy forms complexes with amino acids, peptides and proteins. Zn is a part of the metabolism of proteins, carbohydrates and nucleic acids, regulates synthesis of protein and is a specific cofactor of several enzymes. Fish utilize Zn from both feed and water, but from feed seems to be better utilized. Deficiencies of Zn can give low digestibility of protein and carbohydrates, fin damage and cataract. In addition, Zn separates from the other elements with reduced growth as an early sign of deficiency. This is probably due to Zn not having easy turnover storages in the body and that Zn is a part of functions that directly control the protein synthesis, ergo the growth. Adding Zn above the minimum limit seems to be necessary in fish meal based diets, since a high content of bone minerals (Ca and P) inhibit Zn absorption (Watanbe *et al.*, 1997; Lorentzen and Maage, 1999). Plant ingredients like soybean meal can also reduce Zn absorption (and other divalent minerals). Studies with salmon show that increased amounts of phytic acid inhibits Zn absorption (Storebakken *et al.*, 2000b; Denstadli *et al.*, 2006).

Mn is important in functions of many enzymes (Lorentzen *et al.*, 2001), e.g. enzymes in the mitochondria, which take part in the metabolism of fat, carbohydrates and protein. Absorption from water is not likely, since the Mn content in seawater is very low (Davis and Gatlin, 1996). In addition, bioavailability of Mn from fish meal is uncertain (Lorentzen *et al.*, 1996), hence, it should be added in feed. Mn has a huge safety range in salmon, meaning that it can be added in excess, without severe effects. Absorption of Mn is hard to determine, considering that a large part of Mn in the faeces is likely to be endogenic Mn from bile. Deficiency of Mn leads to weight reduction, deformities, etc. High dietary Ca an P can reduce absorption of Mn (Watanbe *et al.*, 1997).

Fe exist in all cells, and is normally bound to proteins (Davis and Gatlin, 1996; Watanbe *et al.*, 1997; Lorentzen *et al.*, 2001), and homeostasis of Fe is controlled by intestinal absorption. Most Fe in fish and animals exist as haem-Fe, and the bioavailability of haem-Fe is higher than non-haem-Fe, in salmon (Andersen *et al.*, 1997). Fe is abundant in many foodstuffs, in animals as well as plants, however, haem-Fe in e.g. myoglobin from meat or fish is better absorbed than Fe from plant feeds (Coultate, 2002). Bone minerals in fish meal inhibit

absorption of both haem-Fe and non-haem-Fe, in salmon (Lorentzen and Maage, 1999). Reduced haemoglobin concentration is the most known sign of Fe deficiency.

Cu is essential for many functions studied in mammals, it is a part of several proteins and it is necessary for optimal function of the immune system (Watanbe *et al.*, 1997; Lorentzen *et al.*, 2001), and deficiency of Cu has led to reduced growth. Absorption of Cu is thought to be well regulated in fish (Berntssen *et al.*, 2000), because absorption of Cu decreases with increasing Cu in feed for salmon. The intestine is important in regulating Cu homeostasis in fish, and high retention of Cu, from feed in to the intestinal tissue, leads to increased apoptosis in intestinal cells. Availability of Cu depends on the physiological state of the animal and amount of metabolic antagonists of Cu (e.g. Zn, Fe, Cd, Mo), competing for binding sites on proteins responsible for mineral absorption and/or synthesis of enzymes, however details in fish are little known.

Se is an important component in enzymes, anti-oxidant functions and is a part of the metabolism of I (Watanbe *et al.*, 1997; Lorentzen *et al.*, 2001). Fish absorb Se from both water and diet, and high levels can be toxic and the safety range is narrow. Deficiencies of Se can give reduced growth, increased mortality, etc. A feed with more than 30% fish meal will in theory cover the Se requirement for rainbow trout (*Oncorhynchus mykiss*) (NRC, 1993). However, the feed might contain components which may reduce the Se bioavailability.

Co is a component of cyanocobalamin (vitamin $_{B12}$) which is a conenzyme for enzymes associated with synthesis of haemoglobin and muscle protein. Therefore, dietary Co is essential for growth and haematology in fish (Watanbe *et al.*, 1997). Co can be absorbed from the diet and from surrounding water.

Knowledge about other essential minerals as Mo, V and Sn is limited (Watanbe *et al.*, 1997; Das, 2000). Deficiencies are demonstrated in mammals, although, studies in fish are not performed (Lorentzen *et al.*, 2001).

2.2.5 Undesirable elements

As is found in most marine organisms (Lorentzen *et al.*, 2001; Coultate, 2002). Accumulation of As increases with increasing salinity, and As can damage fat and carbohydrate metabolism. Toxicity of As depends on chemical state and valence. Marine organisms mostly contain organic pentavalent As (arsenobetaine), which is stable and less toxic than trivalent As. Arsenobetaine is possible essential for fish, according to studies with salmon and cod (Amlund *et al.*, 2006). Cd contamination mostly comes from industrial pollution (Baldisseretto *et al.*, 2005), and contaminated food in water is a more important source than water itself. Absorption of Cd increases when the dietary concentration is low (Harrison and Jefferson, 1992), and Cd is accumulated in the kidney, liver and intestines (Berntssen *et al.*, 2000). Damage from Cd can be increased stress hormones and reduced carbohydrate metabolism. Elevated dietary Ca can protect against dietary and waterborne Cd uptake in rainbow trout (Baldisseretto *et al.*, 2005).

Hg occurs in different forms; free metal (Hg^0), inorganic Hg (Hg^{2+}), salts, and alkyl-Hg compounds (Coultate, 2002), these forms have very different toxicity and metabolism. Organic compounds of Hg and Hg salts are most hazardous. Alkyl-Hg compounds mostly come from industrial pollution. Methyl-Hg will accumulate to dangerous levels, even though if the pollutant is inorganic Hg salt or the free metal (by anaerobic methane producing bacteria in sediments). Hg biomagnifies up marine food chains, since predatory fish can accumulate Hg (Morel *et al.*, 1998). Cod is found to readily absorb dietary methyl-Hg (Amlund *et al.*, 2007). Pb is less toxic to marine organisms compared to other metals, however, fish contain low amounts of Pb (NIFES, 2009). Organic compounds of Pb are most toxic, but most Pb compounds have very low water solubility, which is the major factor for the poor absorption of Pb from the gastrointestinal tract in humans (Coultate, 2002). Though, Pb can inhibit the formation of haemoglobin. Absorption of Pb increases when the Ca concentration in feed is low and Pb is mainly accumulated in bone tissue.

Ba, Sr and Ag are not found to be vital for fish or other animals, therefore are they assumed to be toxic or at least non essential for fish, although reports of this are not found, so they could be essential in small amounts. Concentrations of Ba in otoliths, are one of more element concentrations used as identification to locate cod nursery areas (Gibb *et al.*, 2007). Toxicity of Ag occurs mainly in aqueous phase, depending on concentration and form (Ratte, 1999). Sr and Ag are little studied elements in marine biological samples (Ratte, 1999; Das, 2000).

2.3 The digestive system and health

2.3.1 The digestive system

Nutrients are digested and absorbed in the gastrointestinal tract (GIT) of fish (Buddington *et al.*, 1997). The GIT also have many other functions; important for the water and electrolyte balance, a source of hormones which regulate digestion and metabolic processes, and a part of the immune defence. Figure 2.3.1. show some parts of the GIT for a juvenile cod.

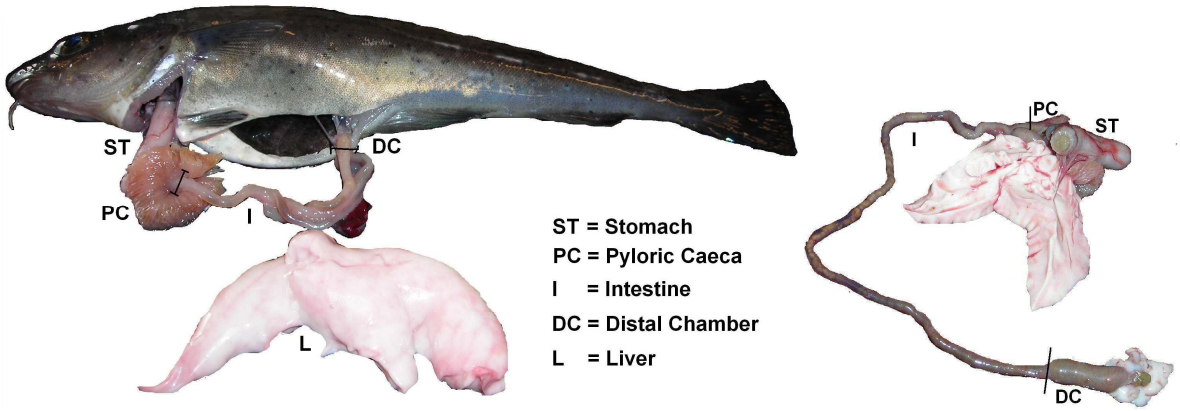


Figure 2.3.1. Stomach (ST), pyloric caeca (PC), intestine (I), distal chamber (DC) and liver (L) to a juvenile cod about 100 g and 20 cm long (Austevoll 10.10.07).

The GIT starts with throat (pharynx), gill opening and gullet (oesophagus) which lead food to the stomach (Bishop and Odense, 1966; Jobling and Hjelmeland, 1992; Kryvi and Totland, 1997). Below oesophagus lies the stomach and intestinal system fastened with a thin transparent membrane. Cod have a curved stomach, and the stomach "stores" feed and starts the digestive processes. The stomach surface area consists of mucosa cells and one cell type which secretes pro-forms of pepsin and hydrochloric acid (HCl). Pepsinogen is activated into pepsin when the pH is below 6. Hydrogen ions (H^+) and chloride ions (Cl^-) are formed to HCl in the stomach. Pylorus is in the end of the stomach, and the pyloric sphincter empties stomach contents in portions into the intestine. Cod have a high number (~700) of narrow pyloric caeca situated in a short section in the intestine right after the pyloric sphincter (Refstie *et al.*, 2006b). Pyloric caecas increase absorptive surface area in the intestine (Buddington *et al.*, 1997). In addition, folds and villis in the intestine mucosa also increase the intestine surface. The intestines main function is enzymatic hydrolysis and food absorption

(Jobling and Hjelmeland, 1992). At the end of the intestine, the distal chamber has a clear separation from the intestine, with a clear thickening of the mucosal wall, and the distal chamber ends in anus. The liver lies in front of the lumen and produces bile and stores fat and glycogen as energy reserves. Bile is stored in the gall bladder, close to pylorus and the intestine, and enters in the intestine close to the pyloric caecas. Pancreas lies between the pyloric caecas, and secretes digestive enzymes in to the intestine.

2.3.2 Digestion of nutrients

Protein and carbohydrates are hydrolysed by enzymes secreted from pancreas. Protein digestion starts with denaturation and pepsin action in the stomach, continuing in the intestine by the action of trypsin and various peptidases. There are specific enzymes for different carbohydrates and proteins. This hydrolysing process gives peptides and oligosaccharides, which are further hydrolysed to amino acids and sugars, which are transported into enterocytes. In carnivorous fish, protease activity is likely to be connected to diet composition (Buddington *et al.*, 1997). However, carbohydrase appears to be genetically low. That is why a too high amount of starch will affect digestion negatively (Hemre, 2001). Digestion of carbohydrates varies between types of meal used (different meals contain different sugars and bindings of these), molecule size, amount of carbohydrates, carbohydrate types, fish species, life stadium, temperature and feed intake. Most fish have the enzyme amylase, a starch digesting enzyme (Jobling and Hjelmeland, 1992). Fish secrete chitinase, but it seems like most chitin digesting enzyme activity comes from intestinal bacteria. Chitinase activity in Atlantic cod reaches a peak when it feeds on crustaceans, a big part of this is probably because of high chitinase and chitin concentrations in the prey (Krogdahl *et al.*, 2005). Digestion of carbohydrates in fish have been extensively researched, but the information about processes in carbohydrate digestion and absorption is still not fully understood for any species in aquaculture production (Krogdahl *et al.*, 2005). Amino acids in cod are mainly absorbed in the pyloric caeca and intestine, but absorption continues along the entire intestinal tract (Lied *et al.*, 1982; Lied and Solbakken, 1984). Digestion of lipids starts in the pyloric caeca and intestine. Bile emulsifies lipid to micelles, so the lipids get more available for enzymatic cleavage. Then fatty acids can be absorbed into enterocytes.

2.3.3 Fibre; a digesting inhibitor?

Fibres are not broken down by digestion enzymes in the first part of the intestine, but are partly hydrolysed by bacteria in the last part of the intestine of omnivores. E.g. cellulose is practically indigestible for most fish, and generally it is believed that most cellulose digesting activity (cellulase) in fish intestine come from bacteria. Though, whether cellulase have endogenous or exogenous origin is still discussed (Krogdahl *et al.*, 2005). Soluble fibre thickens water layers in the intestine, which reduce absorption of water soluble nutrients (Figure 2.3.2.) (Hemre, 2001). Further, soluble fibre can disturb digestive enzymes, which results in reduced digestion of carbohydrates and protein. In addition, soluble fibre can break micelles which are necessary for good fat absorption (Krogdahl *et al.*, 2005). In tilapia (*Oreochromis niloticus*), soluble fibre has caused diarrhoea like faeces, in contrast to the insoluble fibre which enhanced faeces stability (Amirkolaie *et al.*, 2005). Due to these negative effects from soluble fibre, the amount used in feed binders is kept to a minimum. Insoluble fibres increase feed flow rate through the intestine (Hemre, 2001), and fish get less time to digest nutrients. This further confirms that too much fibre is preferred to be avoided.

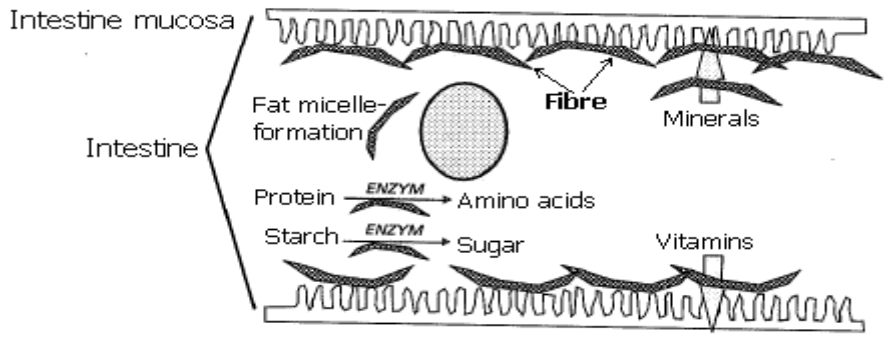


Figure 2.3.2. Fibre interacting with nutrients in fish intestine, copied from Hemre (2001).

Feed utilization in salmon was reduced with high dietary carbohydrate inclusion (Hemre *et al.*, 1989; Hemre *et al.*, 1995), indicating negative influence from undigested starch, since excess starch probably behaved like indigestible fibre in the intestine. Soluble fibre in diets for tilapia increased digestion viscosity, reduced growth and digestibility of protein, fat and starch (Amirkolaie *et al.*, 2005). In contrast, moderate dietary cellulose did not affect digesta viscosity, growth and digestibility. In addition, when cellulose plus the soluble fibre were added in feeds, it seemed like cellulose alleviated negative effects from the soluble fibre. Experiments with both salmon and chicken found that some soybean products can negatively affect digestion (Refstie *et al.*, 1999). This could be due to anti nutritional effects from NSP, which cause high viscosity in chicken guts, and increased water content in salmon guts.

2.3.5 Health parameters

Haematocrit (Hct), red blood cell count (RBC) and haemoglobin (Hb) are haematological parameters that are important when evaluating fish health (Sandnes *et al.*, 1986; 1988; Waagbø, 2001). Low values of haematological parameters probably indicate anaemia. Anaemia is a symptom that occurs in many diseases; from nutritional, parasite, environmental stress, environmental pollution, viral or bacterial origin. This means that irregularities in fish health are likely to be detected with haematological analyses, but not the cause. Although, mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) in combination with Hb, RBC and Hct give valuable information on diagnostics of the origin of the anaemia. Haematological parameters in fish vary depending on seasonal variations, water temperature, age, nutrition (Sandnes *et al.*, 1988; Waagbø, 1999) and maturation. Therefore it may be hard to control if haematological parameters are normal or not. Anyway, there are haematological values found in cod experiments (Lie *et al.*, 1990; Hemre *et al.*, 2002; Rosenlund *et al.*, 2004; Olsen *et al.*, 2007), to compare our results with.

Aspartate aminotransferase (ASAT) and alanine amintransferase (ALAT) normally occur in low levels in blood plasma (Racicot *et al.*, 1975). ASAT and ALAT are non plasma organ specific enzymes, which are used as indicators for organ damage. When cell membranes are intact, these enzymes are found in low concentrations in plasma. When there is cell damage, enzyme activity increases in the blood plasma. Determination of these enzymes is efficient in diagnosis of liver and kidney diseases in fish. Total protein in blood plasma increases when the fish is dehydrated (Sandnes *et al.*, 1986), but when total protein is low, it is a less specific diagnosis of a disease. Blood glucose in cod has been reported to increase with increased dietary carbohydrate (Hemre *et al.*, 1989; Rosenlund *et al.*, 2004), this means that higher carbohydrate inclusions have to be done with caution.

Sick or stressed fish eat and grow less, and have poor feed utilization (Einen, 2001). Therefore, elucidation of fish health during feeding the trial is needed, to know that it is experimental feed affecting fish performance, and not the fish being sick or stressed. Elucidation of fish health is of course also performed to find potential adverse effects from experimental feed.

3 Aim of the study

The aim of the study was to find possible effects from including increasing amounts (0, 6, 12 and 18%) of non digestible fibre (α-cellulose), as an energy diluter, in Atlantic cod diets; sustainable diets holding 50% plant meal (PP) plus 50% fish meal (FM) as protein source, or diets holding 100% fish meal as protein source.

Main focus was to control liver sizes, without compromising total growth, utilization of nutrients or fish health.

Further questions were; are there differences between cod fed a plant based diet and a fish based diet and are there effects from the increasing α-cellulose, on any of the following:

- Growth (SGR and weight gain) and condition factor (CF)
- Feed utilization (feed intake, energy intake, protein intake and FCR)
- Size of liver, muscle and gutted fish
- Protein and lipid utilization
- Digestibility of protein, lipid and dry matter
- Digestibility of elements (As, Co, Cu, Fe, Mn, Mo, Se, Sn, V, Zn, Sr, Ag, Ba, Cd, Pb and Hg)
- Concentration of elements in diets
- Fish health
- Composition of whole body and liver

4 Materials and methods

4.1 Feeding experiment

4.1.1 Progress

The feeding trial was runned at Austevoll Aquaculture Research Station (Institute of Marine Research, Norway) and the feed were produced by Skretting ARC (Stavanger, Norway). The feeding trial started 1.Nov.2007, when the cod had an average weight of 138g (±4g) and lasted until 5.Feb.2008 (totally 97 days), with average final weight of 312g (±11g). Totally 1444 cod were randomly distributed into 16 different tanks (from 89–93 cod per tank) three days before the feeding trial started, since this is a stressing procedure. Few cod died during the trial (0–5 cod per tank). Progress overview of samplings and measurements from the feeding trial is given in Table 4.1.1.

Table 4.1.1. Progress overview of samplings and measurements during the feeding trial.

Procedure	Date:	Days	Weeks	Length	Weight	Organ samples
First sampling	10.Okt.2007 *	0	0	X	X	X
First weighing	29.Okt.2007 **	19	2.7	X	X	
Start feeding trial	01.Nov.2007 ***	3	0.4			
Mid weighing	18.Des.2007	47	6.7	X	X	
Final sampling & weighing	05.Feb.2008	49	7.0	X	X	X
Total	From first sampling	118	16.9			
Total	From feeding trial starts	96	13.7			

* 10.Okt. - 1.Nov.2007: Adjustment to rearing conditions.
** 29.Okt.2007: Measurement and experimental set up.
*** 1.Nov.2007: Feeding experiment starts.

4.1.2 Rearing conditions

Fish tanks were dark green, 1.5 m in diameter and 1 m deep. The trial was runned using light continuously. Oxygen concentration in water was measured in outlet water, and adjusted to always hold more than 89% saturation. Water was taken from 165m depth with a stable temperature around 8°C and salinity around 35‰, this were obtained throughout the trial. Atlantic cod juveniles used in this trial were produced in a closed inlet (Parisvatnet), from a local stock, and they were hatched in spring 2007. Until the trial started the cod were fed a commercial cod diet (Amber Neptun, Skretting AS, Stavanger, Norway), declared to contain 52% crude protein and 18% crude fat, mainly originating from high quality marine raw materials. Cod juveniles arrived at Austevoll three weeks before the feeding trial started and adjusted to rearing conditions. Before the feeding trial started, the cod was also randomly redistributed in all fish tanks. The fish were fed once a day by an automatic feeder (Storvik skiveautomat, Storvik Aqua AS, Sunndalsøra, Norway), and each feeding lasted about 1.5 hour from 0900 in the morning. Outlet water was split, most water went out as "waste", but water with waste feed was filtered, and waste feed was dried over night at 65°C and then weighed dry. Dead fish were registered, weighed and removed every day.

4.1.3 Experimental design

Vitacel R 200 Superfine (J. Rettenmaier & Söhne, Rosenberg, Germany, data sheet in Appendix 1; Figure 9.1., 9.2. and 9.3.), an α-cellulose, was added from 0 to 18 % in diets, resulting in a regression design (Figure 4.1.1.). Totally 8 different diets were fed in duplicate.

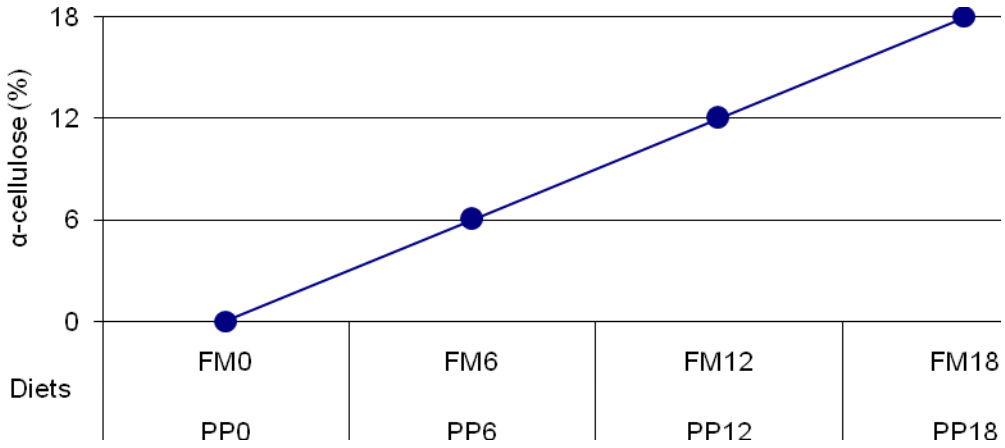


Figure 4.1.1. Inclusion levels of α-cellulose in the 8 different experiment diets and protein sources. Abbreviations: FM = fish meal, PP = 50 % plant protein & 50 % fish meal, 0, 6, 12 & 18 = inclusions of α-cellulose.

The feed receipt is shown in Table 4.1.2. Fish meal used was Norse LT (Vedde Herring Oil Factory, Egersund, Norway) and plant materials used in diets were Soycomil (ADM, Speciality Ingredients BV, Koog an de Zaan, Netherlands, data sheet in Appendix 1, Figure 9.4. and 9.5.) and wheat gluten (Gluvital 21040, Cerestar, Charlottenlund, Denmark, data sheet in Appendix 1, Figure 9.5. and 9.6.). Additional amino acids were added to all PP based diets; the amino acids added were DL-methionine (Degussa, Hanau, Germany) and L-lysine (Ajinomoto Eurolysine, Paris, France). Wheat was added in all diets. Fish oil was added in all diets, to balance total lipid and fatty acids to be equal. Nutrient requirement recommendations followed NRC (1993); vitamin and minerals (Premixes) were added (Proprietary composition, Skretting ARC, Stavanger, Norway) and mono-sodium phosphate (Trouw Nutrition, Boxmeer, Netherlands) was added in diets with 50 % plant protein. Yttrium was added in all diets as an inert indicator to calculate digestibility of nutrients.

Table 4.1.2. Feed receipt for the experimental diets.

	Diets*							
Ingredient (g/kg)	FM0	FM6	FM12	FM18	PP0	PP6	PP12	PP18
Fish meal	713	670	627	585	362	341	319	297
Soycomil	0	0	0	0	185	174	163	152
Wheat gluten	0	0	0	0	148	139	130	121
Wheat	181	170	159	148	130	122	114	106
Fish oil	102	96	90	83	131	123	115	107
Premixes	3.2	3.0	2.8	2.6	3.2	3.0	2.8	2.6
DL-Methionine	0	0	0	0	3.3	3.1	2.9	2.7
L-lysine	0	0	0	0	8.2	7.7	7.2	6.7
Mono-sodium-phosphate	0	0	0	0	29	27	26	23
Yttrium premix	1.10	1.03	0.97	0.90	1.10	1.03	0.97	0.90
Vitacel	0	60	120	180	0	60	120	180
Sum	1000	1000	1000	1000	1000	1000	1000	1000

*Abbreviations: FM = fish meal, PP = 50 % plant protein & 50 % fish meal, 0, 6, 12 & 18 = inclusions of α-cellulose.

All diets were analysed after the trial ended. Table 4.1.3. and Table 4.1.4. show analysed concentrations of nutrients and elements in diets.

Table 4.1.3. Analysed concentrations of dietary fat, protein, starch, ash and dry matter, and indigestible fibre, energy and PE (protein/energy) ratio calculated from the analysed nutrients.

Diets*	Indigestible fibre** %	Fat g/100g	Protein g/100g	Starch g/100g	Ash g/100g	Dry-matter g/100g	Energy*** $kJ \cdot g^{-1}$	PE-ratio* $mgP/ kJ \cdot g^{-1}$
FM 0	0.1	18.3	52.4	12.2	8.1	91.1	21.6	24.3
FM 6	5.0	16.9	50.2	11.6	7.8	91.5	20.5	24.6
FM 12	9.7	16.6	47.7	11.0	7.6	92.6	19.6	24.3
FM 18	15.1	15.1	44.8	9.7	7.1	91.8	18.1	24.7
PP 0	3.0	21.3	50.7	10.2	7.6	92.8	22.0	23.1
PP 6	9.3	18.7	48.2	9.9	7.2	93.2	20.3	23.7
PP 12	12.7	17.4	44.6	8.9	6.7	90.3	18.8	23.7
PP 18	17.7	15.5	43.1	8.2	****	****	17.6	24.5

*Abbreviations: PE = protein to energy ratio, FM = fish meal, PP = 50 % plant protein & 50 % Fish Meal, 0,6,12 &18 = % cellulose inclusions.
** Indigestible fibre = 100% - (%water + %fat + %protein + %starch + %ash).
*** Total energy ($kJ \cdot g^{-1}$) = protein (24 $kJ \cdot g^{-1}$) + fat (38 $kJ \cdot g^{-1}$) + starch (17 $kJ \cdot g^{-1}$), total energy values by Jobling and Hjelmeland (1992).
**** Analyse failed, a mean from the others are used in further calculations.

Table 4.1.4. Analysed concentrations of elements in diets.

	Elements (mg/kg) *																
Diets*	Y	Ag	As	Ba	Cd	Co	Cu	Fe	Hg	Mn	Mo	Pb	Se	Sn	Sr	V	Zn
FM 0	65	<0.01	3.7	0.88	0.19	0.08	7.3	200	0.09	25	0.24	<0.04	<0.2	<0.04	26	0.08	160
FM 6	68	<0.01	3.6	1.10	0.18	0.14	8.1	190	0.08	27	0.12	0.12	<0.2	<0.04	24	0.12	170
FM 12	65	<0.01	3.4	1.00	0.17	0.05	7.6	170	0.07	24	0.08	<0.04	<0.2	0.05	23	0.07	170
FM 18	64	<0.01	3.1	1.20	0.16	0.07	7.4	220	0.07	26	0.11	<0.04	<0.2	0.07	21	0.08	160
PP 0	80	<0.01	2.4	3.40	0.13	0.19	10.0	290	0.04	54	0.87	<0.04	<0.2	<0.04	17	0.34	180
PP 6	66	<0.01	2.6	3.20	0.12	0.17	8.8	230	0.04	47	0.78	<0.04	<0.2	0.04	16	0.31	160
PP 12	63	<0.01	2.4	3.10	0.11	0.16	7.7	240	0.04	46	0.70	<0.04	<0.2	0.05	15	0.28	140
PP 18	58	<0.01	2.1	3.30	0.10	0.16	7.4	230	0.03	45	0.72	<0.04	<0.2	0.07	14	0.25	130

*Abbreviations: FM = Fish Meal, PP = 50 % Plant Protein & 50 % Fish Meal, 0,6,12 &18 = % α-cellulose inclusions, Y=yttrium, Ag=silver, Ba=barium, Cd=cadmium, Co=cobalt, Cu=copper, Fe=iron, Hg=mercury, Mn=manganese, Mo=molybdenum, Pb=lead, Se=selenium, Sn=tin, Sr=strontium, V=vanadium and Zn=zinc.

4.2 Sampling procedures

There were two samplings during the trial. First sampling (10.Okt.2007) was before the feeding trial started and before the cod were distributed in different tanks. Final sampling was when the feeding trial ended (05.Feb.2008). At the final sampling all fish were weighed (grams) and length (cm) measured. There were also weighing and length measurements of all the cod three days before the feeding trial started and in middle of the trial (18.Des.2007), but at those days no fish were sampled for further investigations.

At first sampling, 20 randomly selected fish were killed with a blow to the head, and then weighed and length measured. Livers were dissected and weighed on all 20 fish, to calculate the liver index (HSI). Carcasses and livers for all 20 cod were pooled and homogenized at NIFES with a kitchen machine (Braun K 3000). These two samples were stored at –20 °C, until whole body and liver analyses were performed.

Final sampling was performed 12 hours after last feeding, for each tank. Same procedures as in initial sampling were carried through at final sampling. However, at final sampling, 16 cod from each of the 16 tanks were used. From each tank, 10 cod were used for whole body and faeces samples, and 6 cod for blood and liver samples. Fillet weights were also measured on the 6 cod and all 16 cod were used to measure HSI and gutted weight. The cod were also dissected for brain and intestine samples, but these results will not be a part of this master thesis. Blood samples were taken from the caudal blood vessels, just behind the ventral fin (Vena caudalis), with heparinized syringes. There was one blood sample from each cod and this was divided in three parts. First, two parts of the blood samples were centrifuged (Haemofuge, Heraeust Christ) at 3000 rpm, less than one hour after blood samples were taken. One part was used to measure Hct on individual samples, and from the second part the plasma was pooled to one sample per tank. The plasma samples were then frozen on liquid nitrogen and stored at -80 °C until analyses were completed 16 days after sampling. The third part was used to measure RBC and Hb at NIFES one day after sampling, keeping samples at 4 °C. The 10 faeces samples from the same tank were pooled to one sample per tank. Faeces

were sampled by stripping the last part of the intestine of the cod, as described by Hemre *et al*. (2003). Faeces were stored at -20 °C, until analyses were performed.

An overview of samples taken and what they were analysed for is listed in table 4.2.1. All analyses were carried through at NIFES during spring 2008, autumn 2008 and winter 2009.

Table 4.2.1. Samples taken and what they were analysed for.

Samples taken	Sampling date	What different samples were analysed for					
Whole body	10.Okt.07	Protein	Fat	Dry matter	Glycogen	Ash	
Whole body	05.Feb.08	Protein	Fat	Dry matter	Glycogen	Ash	
Feed	05.Feb.08	Protein	Fat	Dry matter	Starch	Ash	Elements***
Faeces	05.Feb.08	Protein	Fat	Dry matter	Starch		Elements***
Liver	05.Feb.08	Protein	Fat	Dry matter	Glycogen		
Blood	05.Feb.08	Haematocrit		Haemoglobin	Red blood cell count		
Plasma	05.Feb.08	ASAT*		ALAT**	Plasma glucose		

*Alanine aminotransferase.
**Aspartate aminotransferase.
***Yttrium, silver, barium, cadmium, cobalt, copper, iron, mercury, manganese, molybdenum, lead, selenium, tin, strontium, vanadium and zinc.

4.3 Analytical methods

To get reliable results the analytical methods have good quality insurance and some are accredited. There are quality controls and calibrations that must be performed together with the analyses of samples. How to get good quality assurance of analytical methods are described in Appendix 2.

4.3.1 Determination of total protein in liver, whole body, feed and faeces

Total protein was measured in homogenates of liver, whole body, feed and faeces. Most nitrogen in a fish comes from protein, therefore is total nitrogen analysed. This method is based on the principle that mean content of nitrogen in amino acids are 16 %, and when total nitrogen is determined it can be multiplied with 6.25 to find approximate protein concentration. Total nitrogen was measured with a nitrogen analysing instrument (LECO-FP528) according to Leco FP-528 manuals and AOAC official methods of analyses (1995), 16 th. Ed. Metode 992.15: "Crude protein in meat and meat products, combustion method" This method is accredited at NIFES.

In the nitrogen analyser, samples were burned with oxygen in a combustion tube at 950 °C. The carrier gas helium leads CO_2, water and nitrogen through a reduction tube where copper reduces oxygen from nitrogen. Water and CO_2 were also removed and only nitrogen gas and helium (carrier gas) were left. Nitrogen content was measured with a thermal conductivity detector which measures deviance from helium's normal heat conduction ability. Samples were quantified with a calibration curve. Reactions that took place in the nitrogen analyser are following:

Reactions in combustion tube:

$R\text{-}NH_2 + O_2 + \text{sample} \rightarrow N_2 + NO_x + H_2O + SO_x + CO_2$

Reaction in reduction tube:

$NO_x + H_2O + CO_2 + SO_x + \text{Cu (solid)} + \text{He (carrier gas)} \rightarrow N_2\text{ (gas)} + H_2O\text{ (gas)} + CO_2\text{ (gas)} + \text{S (solid)} + \text{CuO (solid)} + \text{He (carrier gas)}$

4.3.2 Determination of fat in liver and whole body

Total fat in liver and whole body was measured after etylacetate extraction according to Lie (1991), and this method is accredited at NIFES. This is a gravimetric method, which means that samples are weighed before and after extraction of fat with etylacetate. An aliquot from samples were taken out and were filtrated, so there was only fat and etylacetate left. Then etylacetate was steamed off, fat was weighed and calculations back to total fat concentration in samples was performed according to the following formulae:

$$\text{Total fat in sample} = \frac{Etylacetate\ (ml) * Fat\ (g) * 100}{Weighed\ sample\ (g) * (Aliqout\ (ml) - (1{,}1 * Fat\ (g))}$$

4.3.3 Determination of fat in feed and faeces

A more accurate method is needed to find total fat in feed and faeces, due to more complex matrix. Therefore, acid hydrolysis was used, as described by the EU commission directive 98/64/EC in the Official journal of the European community (nr L257/23, 19.Sept.98, part B); Community methods of analysis for the determination of amino acids, crude oils and fats, and olaquindox in feedingstuffs and amending Directive 71/393/EEC (http://eur-lex.europa.eu), Tecator application note AN 301, REV 3.0 “Solvent Extraction using the Soxtec System” and Tecator application note ASN 3427 “The extraction of total fat in feed”. This method is accredited at NIFES.

This is a gravimetric method. When analysed, free fat from samples were pre-extracted with n-heptan, and then centrifuged. The solid residue was taken away from the extract, and then n-heptan steamed off so that the extraction residue (free fat) could be measured. To remove possibly bound fat from the solid residue, this was hydrolysed with hot HCl (Hydrochloric acid) and n-heptan, in an incubator. Then the heptan phase was transferred to an extraction cup and the solid residue was transferred to a LLE-column (Varian CHEM ELUT CE1010 Column, capacity to 10ml, art.nr12198007, Holger Technolgy). In the LLE-column fat was extracted with petroleum and was then also transferred to the extraction cup, where the solvent steamed off and the extraction residue (fat) was weighed. The two extraction residues were added up and then calculated back to total fat.

4.3.4 Determination of glycogen in liver and whole body and starch in feed

Digestible carbohydrate in liver, whole body and feed was determined according to Hemre *et al*. (1989) using a modified method originally described by Murat & Serfaty (1974) and Holm *et al*. (1986). At the final stage glucose was measured in a MAXMAT^{TM} PL (Multi-purpose diagnostic analyser, version 3.0.0, revision 8. MAXMAT S.A, France). This method is not accredited at NIFES.

In principle samples were enzymatically hydrolysed with a heat stable α-amylase (thermamyl) and amyloglukosidase, to get free glucose. In MaxMat an automated reaction took place between glucose and a glucose reagent solution containg two enzymes; hexokinase and glucose-6-phosphate-deshydrogenase (G6PDH), the following reactions took place in MaxMat:

$$\text{D-glucose} + \text{ATP} \xrightarrow{Hexokinase} \text{Glucose-6-phosphate} + \text{ADP}$$

$$\text{Glucose-6-phosphate} + \text{NAD}^{+} \xrightarrow{\text{G6PDH}} \text{D-gluconate-6-phosphate} + \text{NADH} + \text{H}^{+}$$

Then the formed NADH was measured photometric at two wavelengths; main: 340 and associated: 450, as 1 molecule of glucose forms 1 molecule of NADH. Glucose was quantified using a calibration curve. Then glucose concentrations were used to calculate digestible carbohydrate concentrations in samples, with the following formulae:

$$\text{Starch/glycogen in sample (mg/g)} = \frac{\text{Result from MaxMat } (mmol/L) * 180.18 * 0.9}{\text{Sample weight } (g) * \text{Total volume } (ml)}$$

180.18 = calculation factor from mmol/L to mg/L

0.9 = correction factor from glucose to glycogen/starch/digestible carbohydrates

4.3.5 Determination of dry matter in feed, whole body, liver and faeces

Dry matter was analysed in feed, whole body and liver, according to NMKL (Nordic committee on food analysis) method 23, 3.edition, 1991, UDC 637.5. This method is accredited at NIFES. Samples were dried in 104 °C to find dry matter. This is a gravimetric method and dry matter concentration was calculated with following formulae:

$$\text{Dry matter (g/100g)} = \frac{Dryed\ sample\ (g) * 100}{Wet\ sample\ (g)}$$

Faeces were freeze dried to determine dry matter, since the same faeces samples were afterwards used for analysing elements. To freeze dry, samples were frozen at -20 °C and water was extracted from the frozen sample by vacuum in the freeze dryer (Christ Gamma 1-16 LSC), where the water goes from ice to vapour. This is also a gravimetric method and dry matter was calculated with following formulae:

$$\text{Dry matter (g/100g)} = \frac{Freeze\ dried\ sample\ (g) * 100}{Wet\ sample\ (g)}$$

4.3.6 Determination of ash in feed and whole body

Ash was analysed in feed and whole body, according to NMKL (Nordic committee on food analysis) method 23, 3.edition, 1991, UDC 637.5. This method is accredited at NIFES. Samples were burned at 550 °C to find ash content. This is a gravimetric method and ash content was calculated with the following formulae:

$$\text{Ash (g/100g)} = \frac{Burned\ sample\ (g) * 100}{Wet\ sample\ (g)}$$

4.3.7 Determination of Yttrium and other elements in feed and faeces

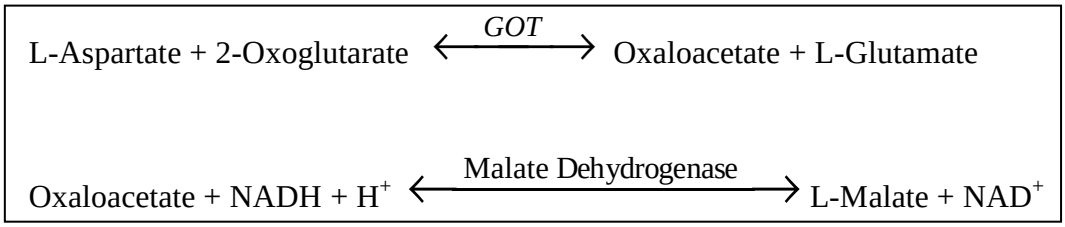
Yttrium (Y) was measured by means of an ICP-MS (Inductive Coupled Plasma Mass Spectrophotometer). This is a multi element instrument, so it was also used to analyse other elements (Y, Ag, Ba, Cd, Co, Cu, Fe, Hg, Mn, Mo, Pb, Se, Sn, Sr, V and Zn) in feed and faeces. Analyses were performed according to NMKL (Nordic committee on food analysis) method nr.186, 2007 and Julshamn *et al*. (2007). This method was not accredited at NIFES when these samples where analysed, due to recently moving of instruments. Samples were mixed with nitric acid and hydrogen peroxide and were then digested in a micro wave oven (Milestone, Microwawe digestion system, MLS-1200 Mega, Microwawe digestion Rotor, MDR 300/10). Measurements of trace elements were then quantified by the ICP-MS (Agilent 7500c with HP-computer, with Chem Station). In the ICP an argon gas stream transform to a plasma beam where the samples are atomised. This lightning ionic plasma is shot in to the MS, where ions are focused and only ions with a certain mass are able to pass the filter and reach the detector. That is how concentrations of different elements are measured. By changing properties of the filter several elements can be measured in the same sample. Concentrations of trace elements were calculated by means at a calibration curve. Additionally it was used an internal standard, to have better control over results, since an internal standard is added directly with the samples and have therefore gone through same treatment as the samples.

4.3.8 Determination of haematocritt (Hct), red blood cell count (RBC) and haemoglobin (Hb) in blood

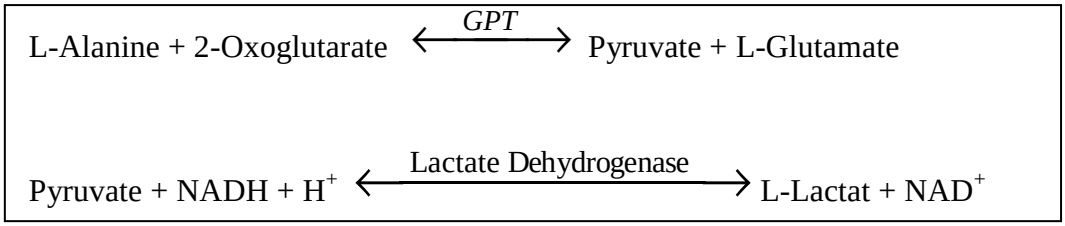
Blood chemical analyses (Hct, RBC and Hb) was performed according to Sandnes *et al*. (1988). Hct was analysed at sampling as explained earlier, and Hb and RBC one day after sampling. Hb was analysed as described by the cell counter producer (Seqoia-Turner Corporation). Diluter 771 Swelab Instrument and Cell-Dyn 400 (Seqoida Turner) was used to analyse the RBC and Hb in blood samples. These methods are not accredited at NIFES. When analysing RBC, blood was first diluted to a fitting concentration for the cell counter. Red blood cells were determined from increased resistance in an electricity field between two electrodes in the cell counter. After RBC was analysed, the same blood was used for analysing Hb. The blood samples were added a cyanid containing solution, which results in ruptures in blood cell walls and leakage of Hb. Hb is a pigment and could therefore be measured photometrical at 540 nm.

4.3.9 Determination of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), glucose and total protein in blood plasma

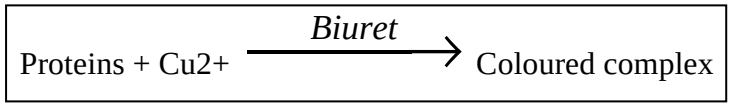
Pooled plasma were analysed for ASAT, ALAT, glucose and total protein. Analyses were performed according to methods described for the analysing instrument MAXMAT^{TM} PL (same instrument as used for determining glycogen in liver and whole body, and starch in feed). These methods are also not accredited at NIFES. ASAT was measured by the principle that NADH (reduced NAD) is oxidised to NAD^+, and this results in a decrease in absorbance at 340 nm. This decrease in absorbance is directly proportional with activity of Glutamate Oxaloacetate Transaminase (GOT) in samples, GOT is also called ASAT. The following reactions take place in MaxMat:



ALAT was measured by the same principle as ASAT. ASAT is also called Glutamate-Pyruvate Transaminase (GPT). The following reactions take place in MaxMat:



Glucose in plasma was measured by the same principle as glycogen in MaxMat. However, a "digestion process" of samples is of course not needed for plasma as glucose exists as such in plasma. Protein in plasma was measured by the principle that proteins form a coloured complex in presence of copper salt in an alkaline solution (Biuret reaction). The following reaction takes place in MaxMat:



The coloured complex is measured photometrically at 540 nm, and the intensity of the coloured complex is directly proportional to protein concentration in the sample.

4.4 Calculations

Calculations that have been performed with results from analyses of samples and growth data from the trial are as follows:

SGR (specific growth rate) = 100 % (ln W_1 – ln W_0) d^{-1} = % per day

(W_0 = initial body weight, W_1 = final body weight, d = sum experimental days)

FCR (feed conversion ratio) = $\frac{Dry\ feed\ eaten\ (g)}{Weight\ gain\ (g)}$

HSI (hepatosomatic index) = $\frac{Liver\ weight\ (g)}{Live\ weight\ (g)} * 100$

ADC (apparent digestibility coefficient) = $100\left(1 \div \frac{\frac{Nutrient\ in\ faeces\ (\%)}{Yttrium oxide in\ faeces\ (\%)}}{\frac{Nutrient\ in\ feed\ (\%)}{Yttrium\ oxide\ in\ feed\ (\%)}}\right)$

CF (condition factor) = $\frac{(Liver\ weight\ (g)) * 100}{(Length\ (cm))^3}$

MCV (mean cell volume) = $\frac{Hct}{RBC} * 10$

MCH (mean cell haematocrit) = $\frac{Hb}{RBC} * 10$

MCHC (mean cell haemoglobin concentration) = $\frac{Hb}{Hct} * 100$

PER (protein efficiency ratio) = $\frac{Live\ weight\ gain\ (g)}{Protein\ eaten\ (g)}$

PPV (protein productive value) = $\frac{Protein\ retention\ (g)}{Protein\ eaten\ (g)}$

Live weight gain (g) = *End weight* (g) – *Start weight* (g)

Protein eaten (g) = $\frac{Eaten\ feed\ (g) * Protein\ in\ feed\ (\%)}{100}$

Protein retention = Protein end biomass (g) – Protein start biomass (g) + dead fish biomass (g)

Biomass (by end and start) = Mean weight * number of fish in tank

Protein in biomass (g): $\frac{Biomass\ (g) * protein\ in\ whole\ \ fish\ (\%)}{100}$

Energy intake (kJ/fish/day) = $\frac{\text{Total energy in feed (kJ * g -1) * feed eaten (g / day / tank)}}{Number\ of\ \ fish\ in\ the\ \text{tank}}$

4.5 Statistics

Statistica (StatSoft, Inc. 2008. data analysis software system, version 8.0. www.statsoft.com) was used for statistical analyses. Linear regression was performed, on both FM and PP diets, to evaluate if there were any effects from the increasing α-cellulose, on growth parameters, digestibility parameters and analytical results. Spearman rank order correlation was performed, on both FM and PP diets, to evaluate non-parametric correlations between growth parameters, digestibility parameters and analytical results. The two different series of diets (FM and PP), were compared, when both regression lines were significant, to find if they had different slopes and elevations. Comparing equality of two regression coefficients involves student's *t* (Zar, 2005), to find if the slopes are different. A t-test was used when examining if the elevations are different. This was done in an excel spreadsheet (Microsoft Excel 2002). Most results are presented with standard deviations (SD), which are also calculated in an excel spreadsheet.

5. Results

5.1 Growth and feed utilization

5.1.1 Growth and feed utilization during week 0-7

The first 7 weeks (01.Nov.2007–18.Des.2007) with growth data from the feeding trial are listed in Table 5.1.1.

Table 5.1.1. Initial and final 7-week-weight (g), final length (mm), 0-7-week-weight gain (%), condition factor (CF; initial and 7-week), specific growth rate (SGR), feed conversion ratio (FCR), mean energy intake (kJ/cod/day) and mean feed intake (g/cod/day) for Atlantic cod fed 8 different diets in duplicate, in trial-week 0-7. Weight and length measurements are given as mean ±SD. Number of cod in each tank in week 0 was from 89 to 93 and in week 7 it was from 88 to 93 in each tank. From 0 to 2 cod died in each tank.

Trial-week	0	7	7	0-7	0	7	0-7	0-7	0-7	0-7
Diets*	**Weight** g	**Weight** g	**Length** mm	**Weight gain** %	**CF**	**CF**	**SGR**	**FCR**	**Feed intake** g	**Energy intake** kJ
FM 0	135 ± 24	222 ± 39	265 ± 20	64	1.09	1.20	1.07	0.73	1.35	30
FM 0	138 ± 23	217 ± 36	260 ± 16	57	1.12	1.17	0.98	0.77	1.30	29
FM 6	135 ± 28	221 ± 43	262 ± 27	63	1.11	1.24	1.07	0.75	1.37	29
FM 6	138 ± 27	225 ± 40	268 ± 18	63	1.14	1.20	1.06	0.76	1.41	29
FM 12	139 ± 28	223 ± 43	268 ± 23	61	1.10	1.16	1.03	0.81	1.46	29
FM 12	136 ± 30	218 ± 44	265 ± 19	60	1.15	1.18	1.03	0.80	1.39	28
FM 18	135 ± 26	228 ± 43	263 ± 20	68	1.14	1.26	1.13	0.79	1.55	29
FM 18	138 ± 29	222 ± 37	264 ± 17	60	1.18	1.23	1.02	0.84	1.49	28
PP 0	142 ± 33	229 ± 57	264 ± 21	61	1.12	1.23	1.04	0.73	1.35	30
PP 0	133 ± 30	214 ± 44	262 ± 19	61	1.16	1.18	1.04	0.72	1.27	29
PP 6	141 ± 31	224 ± 45	260 ± 21	59	1.14	1.29	1.01	0.76	1.35	28
PP 6	145 ± 32	215 ± 52	261 ± 22	48	1.16	1.12	0.85	0.87	1.28	27
PP 12	141 ± 28	213 ± 46	264 ± 22	51	1.12	1.15	0.89	0.90	1.37	26
PP 12	132 ± 30	209 ± 43	259 ± 22	58	1.14	1.17	1.00	0.88	1.43	28
PP 18	138 ± 29	214 ± 43	258 ± 17	55	1.11	1.18	0.96	0.90	1.47	26
PP 18	140 ± 29	202 ± 59	257 ± 22	44	1.11	1.17	0.80	1.02	1.35	24

*Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal, 0, 6, 12 & 18 = inclusions of α-cellulose (on a weight basis).

The initial cod weight was 138 ±3.5g, and after 7 weeks they had grown to 218 ±7.2g. There was a tendency to decreased weight with increased α-cellulose for cod fed PP diets (R^2=0.49, p=0.054), Figure 5.1.1., however, for cod fed the FM diets there were no effects on weight. The cod had good weight gain during the first period, with an average of 58 ±6.2%, which was not influenced by increased α-cellulose. Average specific growth rate (SGR) was 1.00 ±0.09, and there were no effects on SGR from increased α-cellulose.

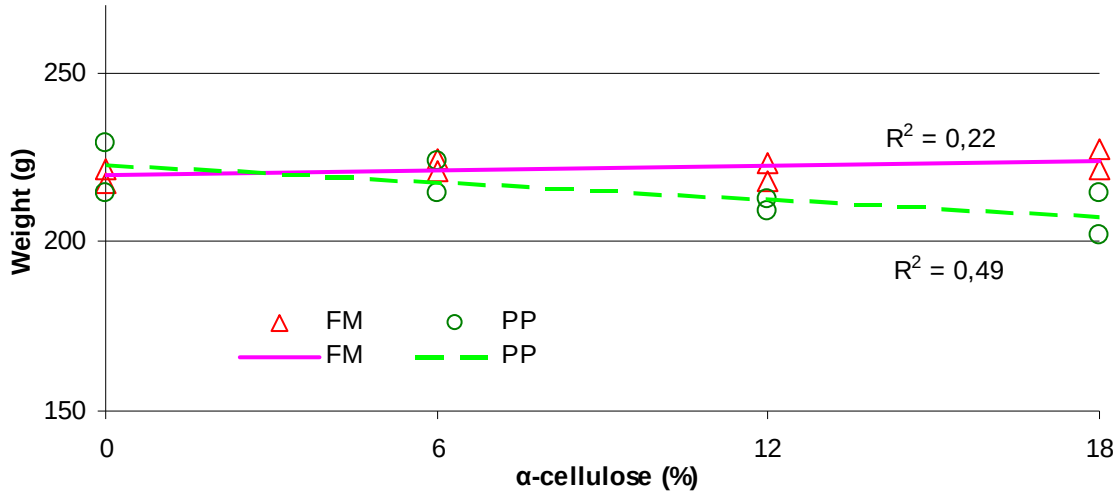


Figure 5.1.1. Regression with weight (g), after 7 weeks, for cod fed four increasing levels of α-cellulose in; four FM diets (Y=219.89 + 0.22x, R^2=0.22, p=0.25) and four PP diets (Y= 222.43 - 0.83x, R= -0.70, R^2=0.49, p=0.054). Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal.

Average cod length was initially 229 ±2.7mm and 263 ±3mm in week 7. In week 7, lengths varied from 258 to 268mm between all dietary groups. Lengths decreased slightly with increased α-cellulose in PP diet groups (R^2=0.56, p<0.05), however, not in FM diet groups. Average condition factor (CF) in week 0 was 1.13 ±0.03, and in week 7 the average CF was 1.2 ±0.04. Increased α-cellulose in diets had no effect on CF.

Feed conversion ratio (FCR) was at average 0.81 ±0.08. Increased α-cellulose led to increased FCR for cod fed both FM and PP diets (FM: R^2=0.62, p<0.05, PP: R^2=0.82, p<0.05), see Figure 5.1.2., and the two regression lines are significantly different. Average feed intake was 1.39 ±0.08g/fish/day. Feed intake increased with increased α-cellulose, for cod fed FM diets (R^2=0.85, p<0.05) and tended to increase for cod fed PP diets (R^2=0.46, p=0.067), Figure 5.1.3. Average energy intake was 28 ±2kJ/fish/day. Energy intake decreased with increased α-cellulose for cod fed PP diets (R^2=0.71, p<0.05), Figure 5.1.2., but cod fed FM diets had no effects.

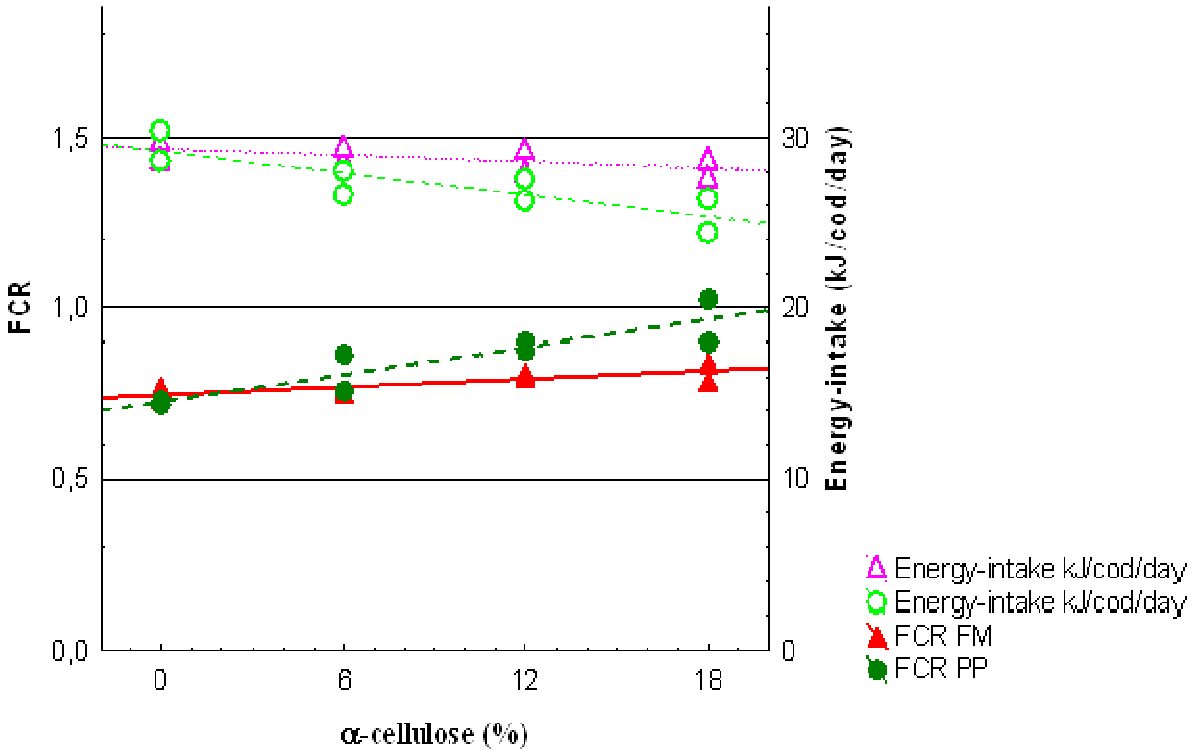


Figure 5.1.2. Regression with feed conversion ratio (FCR) and mean energy intake per day for individual cod, from week 0-7, for cod fed four increasing levels of α-cellulose in; four FM diets and four PP diets (FCR: FM: Y=0.75 + 0.004x, R=0.79, R^2=0.62, p=0.021 and PP: Y=0.73 + 0.02x, R=0.91, R^2=0.82, p=0.002), (Energy intake: FM: Y=29.30 – 0.06x, R=-0.60, R^2=0.36, p=0.12 and PP: Y=29.18 – 0.21x, R=-0.84, R^2=0.71, p=0.009). Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal

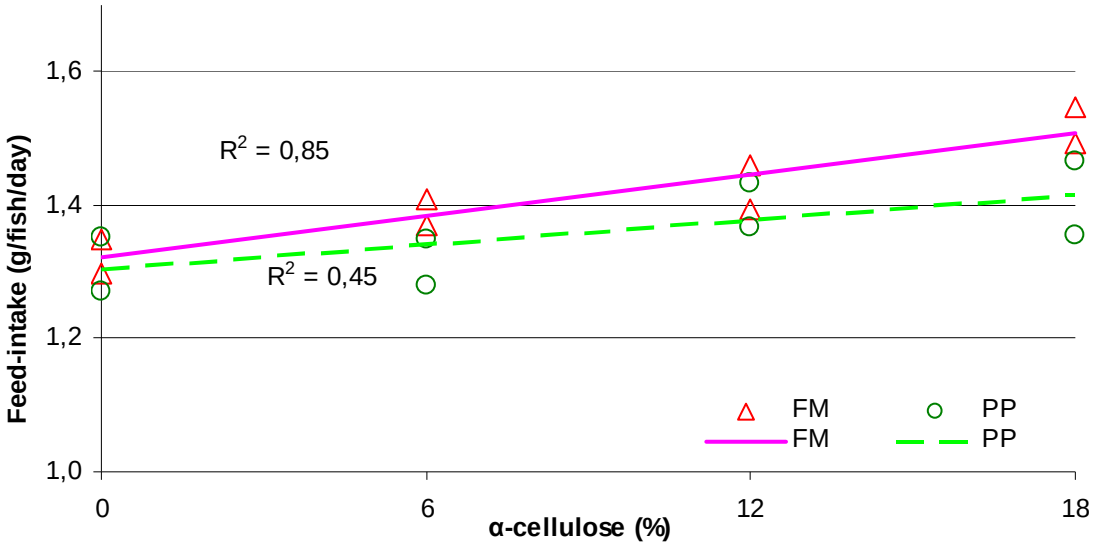


Figure 5.1.3. Regression with mean feed intake (g) per day for individual cod, from week 0-7, for cod fed four increasing levels of α-cellulose in; four FM diets (Y=1.3209 + 0.0104x, R=0.92, R^2=0.85, p=0.001) and four PP diets (Y=1.3021 + 0.0063x, R=0.67, R^2=0.45, p=0.067). Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal.

5.1.2 Growth and feed utilization during week 7-14, and compared to week 0-7

Growth data from the last 7 weeks (18.Des.2007–05.Feb.2008) are given in Table 5.1.2. At final sampling in week 14, average cod weight was 312 ±11.2g. The weight gain in the last period was at average 43 ±5.2%. Increased α-cellulose did not result in any significant effects on final weight or weight gain.

Table 5.1.2. Final weight (g), final length (mm), weight gain (%), final condition factor (CF), specific growth rate (SGR), feed conversion ratio (FCR), average energy intake (kJ/fish/day) and average feed intake (g/fish/day) for Atlantic cod fed eight different diets in duplicate from trial-week 7-14. The weight and length measurements are given as mean ±SD. There was from 88 to 93 cod/tank in week 7 was and in week 14 it was from 86 to 92 cod/tank. 0 to 5 cod died in each tank.

Trial-week	14	14	7-14	14	7-14	7-14	7-14	7-14
	Weight	**Length**	**Weight gain**	**CF**	**SGR**	**FCR**	**Feed intake**	**Energy intake**
Diets*	g	mm	%				g/fish/day	kJ/fish/day
FM 0	304 ± 76	296 ± 22	37	1.16	0.67	0.99	1.63	36
FM 0	320 ± 76	299 ± 20	48	1.18	0.83	0.82	1.74	38
FM 6	301 ± 74	293 ± 24	36	1.18	0.66	0.92	1.49	31
FM 6	330 ± 79	301 ± 20	47	1.20	0.82	0.85	1.84	38
FM 12	318 ± 80	299 ± 25	42	1.18	0.75	0.89	1.72	35
FM 12	323 ± 86	299 ± 24	48	1.19	0.84	0.83	1.78	36
FM 18	328 ± 72	299 ± 21	44	1.21	0.78	0.95	1.93	36
FM 18	301 ± 84	293 ± 24	36	1.18	0.65	1.10	1.79	33
PP 0	315 ± 103	298 ± 29	38	1.19	0.68	0.89	1.55	35
PP 0	299 ± 133	293 ± 24	40	1.17	0.71	0.95	1.58	36
PP 6	323 ± 91	298 ± 27	44	1.20	0.78	0.99	1.97	41
PP 6	323 ± 110	299 ± 29	50	1.19	0.87	0.90	1.97	41
PP 12	300 ± 91	294 ± 29	41	1.15	0.73	0.99	1.75	34
PP 12	305 ± 94	294 ± 27	46	1.17	0.80	0.95	1.85	36
PP 18	300 ± 86	294 ± 25	40	1.17	0.71	1.09	1.90	34
PP 18	308 ± 102	298 ± 29	53	1.14	0.90	0.89	1.94	35

*Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal, 0, 6, 12 & 18 = % inclusions of α-cellulose (on a weight basis).

Average weight of all diet groups, in trial week 0, 7 and 14, is shown in a growth curve in Figure 5.1.4. Weight gain from week 7-14 seemed to be higher in PP groups with α-cellulose, than the other diet groups, Figure 5.1.5. SGR during week 7-14 was at average 0.76 ±0.08m, and was not significantly influenced by increased α-cellulose. SGR was generally less in the second period (week 7-14) than in the first period (week 0-7).

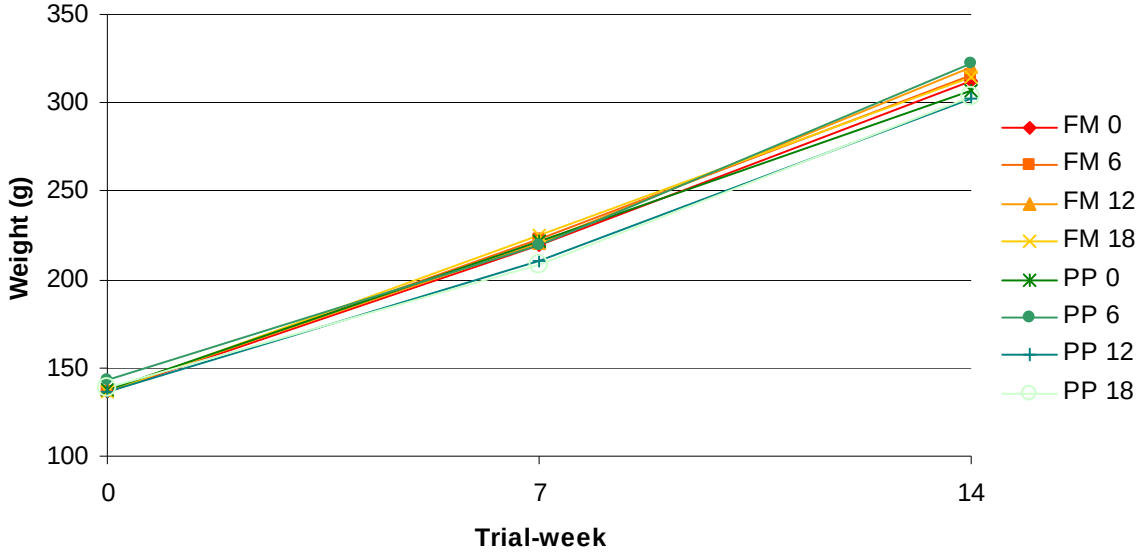


Figure 5.1.4. Growth curve with average cod weight (g) of the duplicate tanks with the same feed (n=2), in trial-week 0, 7 and 14. Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal, 0, 6, 12 & 18 = % inclusions of α-cellulose (on a weight basis).

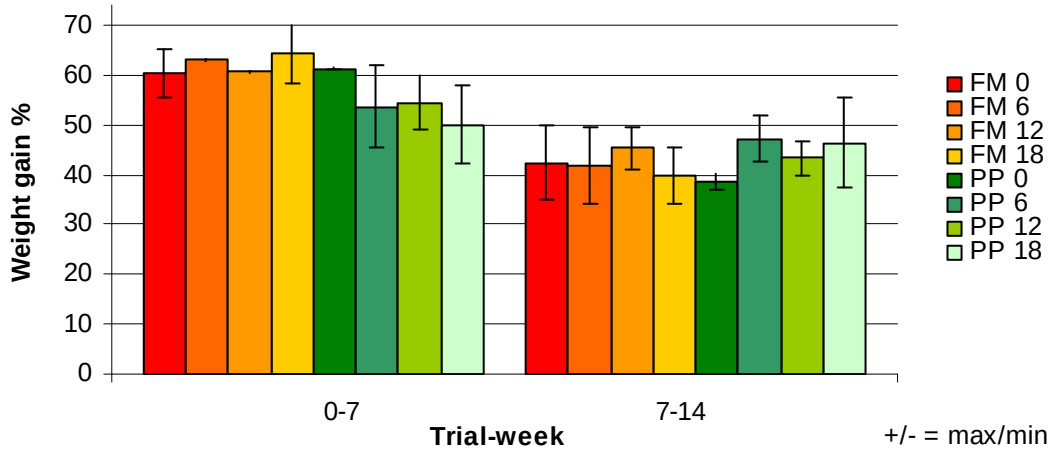


Figure 5.1.5. Bar graph with average weight gain (%) of the duplicate tanks with the same feed (n=2), in trial-week 0-7 and 7-14. Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal, 0, 6, 12 & 18 = % inclusions of α-cellulose (on a weight basis).

Final length was at average 297 ±2.8mm, and were not significantly affected by increased α-cellulose. Final average CF showed 1.18 ±0.02 among all dietary groups, and was not affected by increased α-cellulose.

Average FCR was 0.94 ±0.08 in the last 7 trial-weeks. Increased α-cellulose had no significant effect on FCR in the last period, as it had in the first period. But FCR seemed to be higher, in most diet groups, in the second period than in the first period, Figure 5.1.6. Feed intake in the last period averaged 1.78 ±0.15g/fish/day, and tended to increase with increased α-cellulose, for cod fed PP diets (R^2=0.39, p=0.10), however, not for cod fed FM diets. Feed intake for week 0-7 and week 7-14 is illustrated in Figure 5.1.7. Energy intake in the last period was at average 36 ±3kJ/fish/day, and was not affected by increased α-cellulose. Energy intake for week 0-7 and week 7-14 is illustrated in Figure 5.1.8.

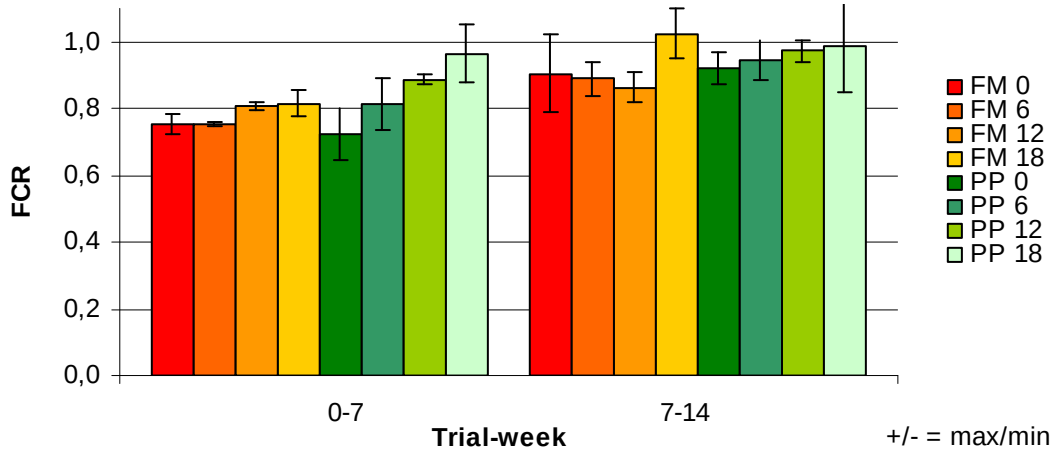


Figure 5.1.6. Bar graph with average feed conversion ratio (FCR) of duplicate tanks with the same feed (n=2), in trial-week 0-7 and 7-14. Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal, 0, 6, 12 & 18 = % inclusions of α-cellulose (on weight basis).

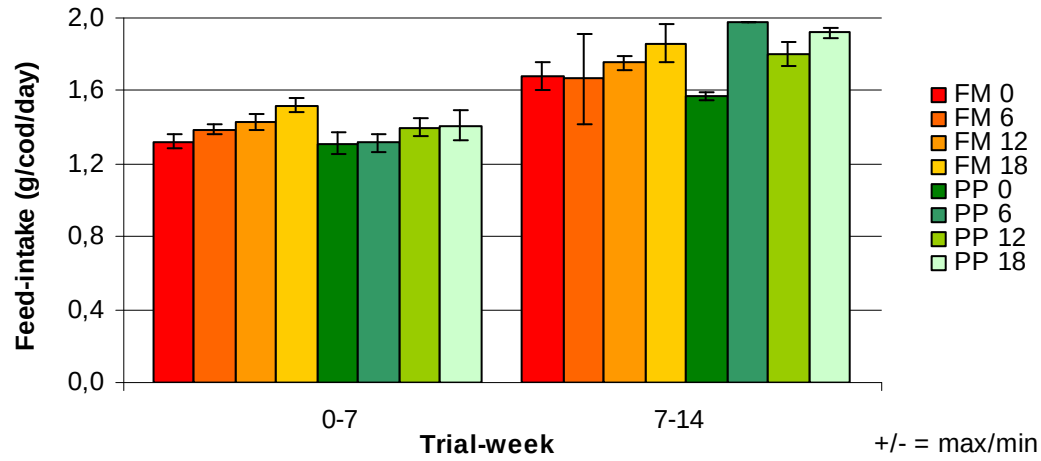


Figure 5.1.7. Bar graph with average feed intake (g/cod/day) of duplicate tanks with the same feed (n=2), in trial-week 0-7 and 7-14. Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal, 0, 6, 12 & 18 = % inclusions of α-cellulose (on weight basis).

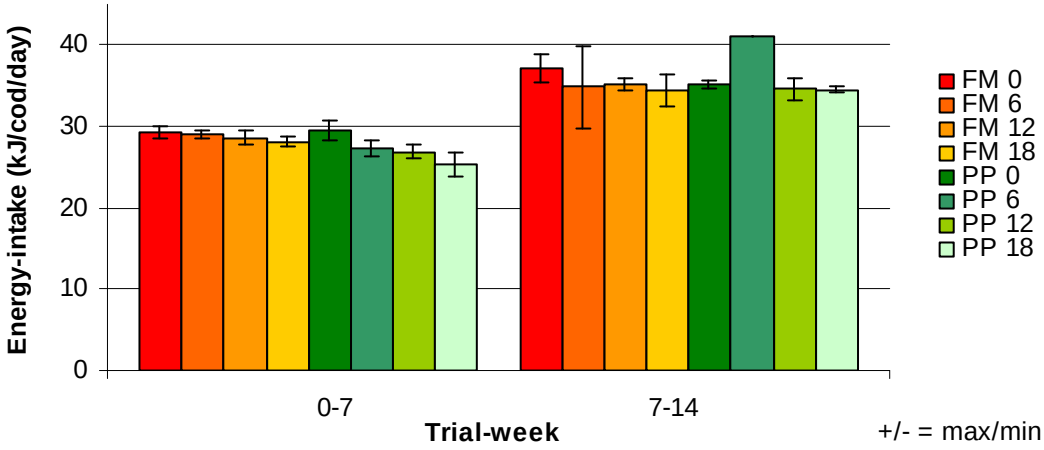


Figure 5.1.8. Bar graph with average energy intake (kJ/cod/day) of duplicate tanks with the same feed (n=2), in trial-week 0-7 and 7-14. Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal, 0, 6, 12 & 18 = % inclusions of α-cellulose (on weight basis).

5.1.3 Growth and feed utilization during total period (week 0-14)

Growth data from the total period (01.Nov.2007–05.Feb.2008) are listed in Table 5.1.3. and Table 5.1.4. Weight gain average was 126 ±8.5%, and varied from 112-142% between the different dietary groups. Weight gain was not affected by increased α-cellulose. Average SGR was 0.88 ±0.04. There were no influence from increased α-cellulose on SGR.

Table 5.1.3. Weight gain (%), specific growth rate (SGR), feed conversion ratio (FCR), average daily energy intake (kJ/fish/day), average daily feed intake (g/fish/day) from the total feeding trial (week 0-14), gutted weight (g), total gutted yield of whole body (%), one fillet side (g), total fillet yield of whole body (%) and liver index (HSI) from final sampling for Atlantic cod fed eight different diets in duplicate of each. There was from 89 to 93 cod/tank in week 0 and in week 14 it was from 86 to 92 cod/tank. From 0 to 5 cod died in each tank.

Trial-week	0-14	0-14	0-14	0-14	0-14	14	14	14	14	14
	Weight gain	**SGR**	**FCR**	**Feed intake**	**Energy intake**	**Gutted***		**Fillet**		**HSI**
Diets**	%			g/cod/day	kJ/cod/day	g	%	g	%	
FM 0	124	0.87	0.86	1.50	33	270	82	75	42	10.5
FM 0	132	0.90	0.80	1.52	34	294	82	87	46	10.8
FM 6	123	0.86	0.84	1.44	30	267	83	79	44	9.5
FM 6	140	0.94	0.80	1.63	34	287	82	89	48	10.4
FM 12	129	0.89	0.85	1.59	32	263	83	76	44	9.6
FM 12	138	0.93	0.81	1.59	32	302	81	103	47	10.6
FM 18	142	0.95	0.87	1.75	32	290	81	87	47	10.8
FM 18	117	0.83	0.97	1.64	30	264	84	80	46	9.4
PP 0	121	0.85	0.81	1.46	33	284	84	84	45	11.4
PP 0	125	0.87	0.84	1.45	32	258	82	83	45	10.8
PP 6	130	0.89	0.87	1.68	35	283	82	86	44	10.5
PP 6	122	0.86	0.88	1.64	34	279	81	92	46	10.9
PP 12	112	0.81	0.95	1.60	31	238	82	73	43	10.1
PP 12	131	0.90	0.91	1.65	32	267	82	76	45	10.8
PP 18	117	0.84	0.99	1.70	31	252	82	71	45	10.5
PP 18	120	0.85	0.96	1.65	30	297	81	104	47	10.7

*Gutted weight is total body weight without internal organs in abdomen and pericardium.
**Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal, 0, 6, 12 & 18 = % inclusions of α-cellulose (on a weight basis).

Average FCR was 0.88 ±0.06, and FCR increased significantly with increased α-cellulose for cod fed PP diets (R^2=0.94, $p<0.05$), but not for cod fed FM diets. Feed intake showed an average of 1.59 ±0.09g/fish/day. Feed intake increased with increased α-cellulose for cod fed both FM and PP diets (FM: R^2=0.58, $p<0.05$, PP: R^2=0.78, $p<0.05$), Figure 5.1.9., but the

regression lines were not significantly different. Energy intake was at average 32 ± 2 kJ/fish/day. Energy intake decreased with increased α-cellulose for cod fed PP diets (R^2=0.51, p<0.05), Figure 5.1.10, but not in FM based treatments.

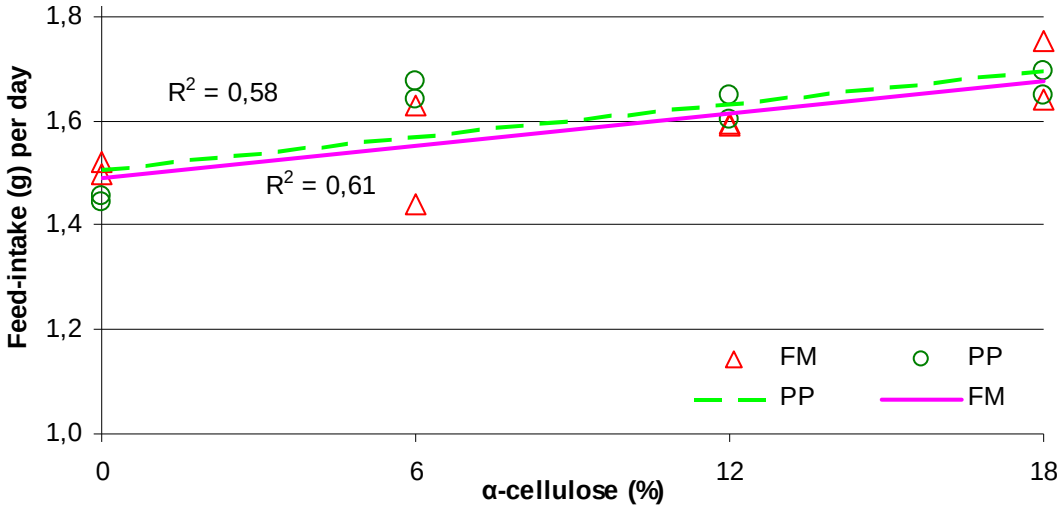


Figure 5.1.9. Regression with feed intake, from week 0-14, for cod fed 4 increasing levels of α-cellulose in; four FM diets (Y=1.49 + 0.01x, R=0.76, R^2=0.58, p=0.03) and four PP diets (Y=1.51 + 0.01x, R=0.78, R^2=0.61, p=0.02). Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal.

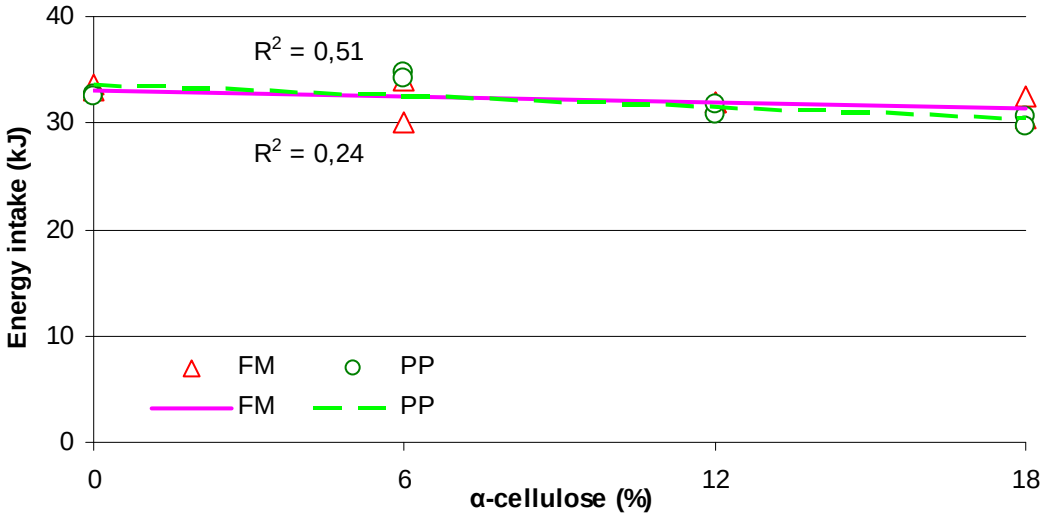


Figure 5.1.10. Regression with energy intake, from week 0-14, for cod fed 4 increasing levels of α-cellulose in; four FM diets (Y=33.07 – 0.10x, R=-0.49, R^2=0.24, p=0.21) and four PP diets (Y=33.68 – 0.18x, R=-0.72, R^2=0.51, p=0.046). Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal.

At trial end the average gutted weight was 275 ±18g, total gutted yield of whole body was at average 82 ±1%, average one side fillet weight was 84 ±10g and total fillet as part of whole body weight was at average 45 ± 1.5%. None of these parameters were significantly affected by increased α-cellulose. Gutted weight, fillet weight and liver index (HSI) are calculated from weight of sampled cod and not total average weight of all cod in the trial. Three weeks before the feeding trial started, when all cod were fed equal feed, the HSI averaged 11.9 ±0.8% and at the end of the trial HSI averaged 10.5 ±0.6%. All diet groups seemed to have lower final HSI compared to initial, Figure 5.1.11. HSI was not significantly affected by increased α-cellulose in any of the diet groups.

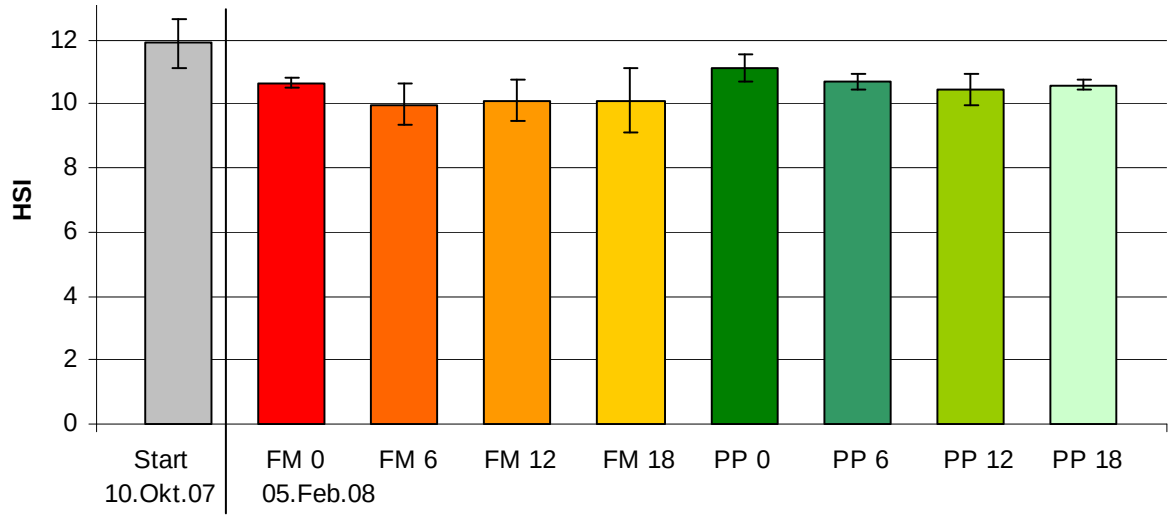


Figure 5.1.11. Average liver index (HSI) from first sampling (10.okt.2007) (n=20) ± SD and average HSI from duplicate cod tanks fed same feed from last sampling (5.feb.2008) (n=2) ± = max/min. Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal, 0, 6, 12 & 18 = inclusions of α-cellulose.

Efficiency ratios and productive values for protein and lipid in the total period (01.Nov.2007–05.Feb.2008) are listed in Table 5.1.4. Efficiency ratios and productive values for starch and ash in the total period are listed in Appendix 3, Table 9.1., and will not be discussed. Average protein efficiency ratio (PER) was 2.37 ±0.11 among dietary groups and was not influenced by increased α-cellulose. Protein productive value (PPV) was averagely 0.37 ±0.02 among diet groups and α-cellulose did not affect PPV. Lipid efficiency ratio (LER) averaged 6.52 ±0.64. LER increased with increased α-cellulose for cod fed PP diets (R^2=0.63, $p<0.05$), and tended to increase also for cod fed FM diets (R^2=0.39, p=0.098), Figure 5.1.12. Average lipid productive value (LPV) was 0.42 ±0.06 and α-cellulose did not affect LPV.

Table 5.1.4. Protein efficiency ratio (PER), protein productive value (PPV), lipid efficiency ratio (LER), lipid productive value (LPV), starch efficiency ratio (SER), starch productive value (SPV), ash efficiency ratio (AER) and ash productive value (APV) for Atlantic cod fed eight different diets in duplicate of each, from the total feeding trial (week 0 to 14).

Diets*	PER	PPV	LER	LPV
FM 0	2.21	0.35	6.33	0.42
FM 0	2.38	0.36	6.81	0.50
FM 6	2.37	0.36	7.03	0.38
FM 6	2.41	0.40	7.15	0.43
FM 12	2.43	0.38	6.97	0.37
FM 12	2.57	0.40	7.38	0.46
FM 18	2.53	0.41	7.53	0.51
FM 18	2.30	0.34	6.84	0.27
PP 0	2.42	0.38	5.76	0.51
PP 0	2.28	0.37	5.44	0.36
PP 6	2.31	0.35	5.96	0.40
PP 6	2.32	0.35	5.97	0.44
PP 12	2.19	0.34	5.62	0.35
PP 12	2.45	0.37	6.28	0.39
PP 18	2.28	0.35	6.37	0.43
PP 18	2.47	0.36	6.88	0.45

*Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal, 0, 6, 12 & 18 = % inclusions of α-cellulose (on a weight basis).

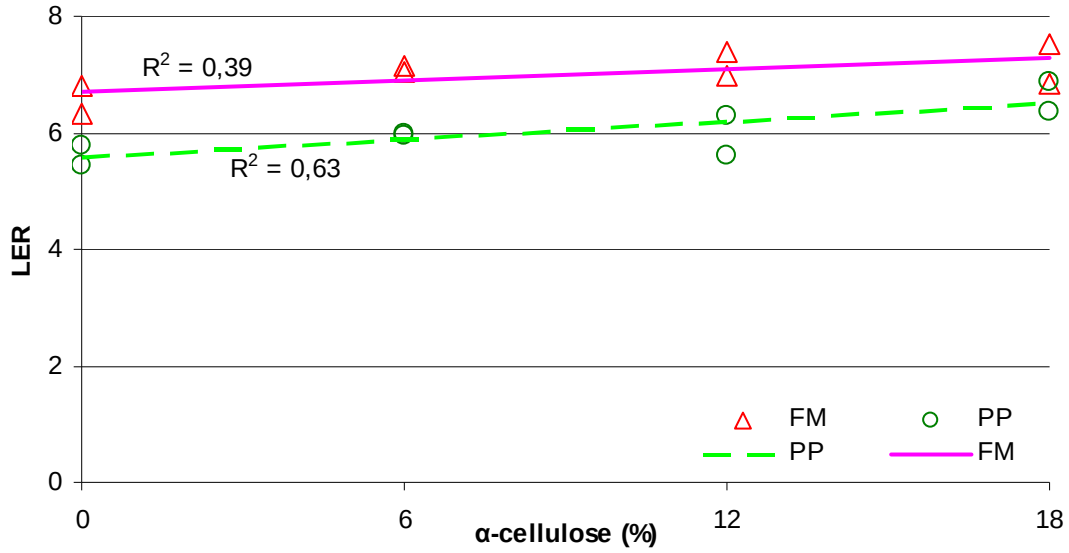


Figure 5.1.12. Regression with lipid efficiency ratio (LER), in week 14, for cod fed four increasing levels of α-cellulose in; four FM diets (Y=6.72 - 0.03x, R=0.63, R^2=0.39, p=0.098) and four PP diets (Y=5.58 - 0.05x, R=0.79, R^2=0.63, p=0.02). Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal.

5.1.4 Correlations between growth parameters

Some spear man rank order correlations between growth parameters from the first period, second period and total period in the feeding trial (week 0-7, 7-14 & 0-14), are presented.

SGR did not correlate with feed intake in the total period (0-14), or in the first period (0-7), but there was a significant positive correlation between SGR and feed intake in the second period (7-14) for cod fed PP diets ($R=0.74$, $p<0.05$), but not for cod fed FM diets. Weight gain, consequently, also correlates in the same way as SGR. Increased SGR did correlate with increased energy intake in the first period for cod fed PP diets ($R=0.91$, $p<0.05$), but not for cod fed FM diets. Increased SGR tended to correlate with increased energy intake in the second period, for cod fed FM diets ($R=0.62$, $p=0.10$), but not with PP diets. Increased SGR slightly tended to correlate with increased energy intake in the total period for cod fed FM and PP diets (FM: $R=0.60$, $p=0.12$, PP: $R=0.60$, $p=0.12$), Figure 5.1.13. Feed intake did not correlate with energy intake in any of the periods. Lower FCR correlated with a high energy intake in the total period for cod fed PP diets ($R=-0.74$, $p<0.05$), but not FM diets. FCR decreased with increased energy intake in the first period for cod fed PP diets ($R=-0.91$, $p<0.05$), but not FM diets. FCR did not significantly correlate with energy intake in the second period, for cod fed either FM or PP diets.

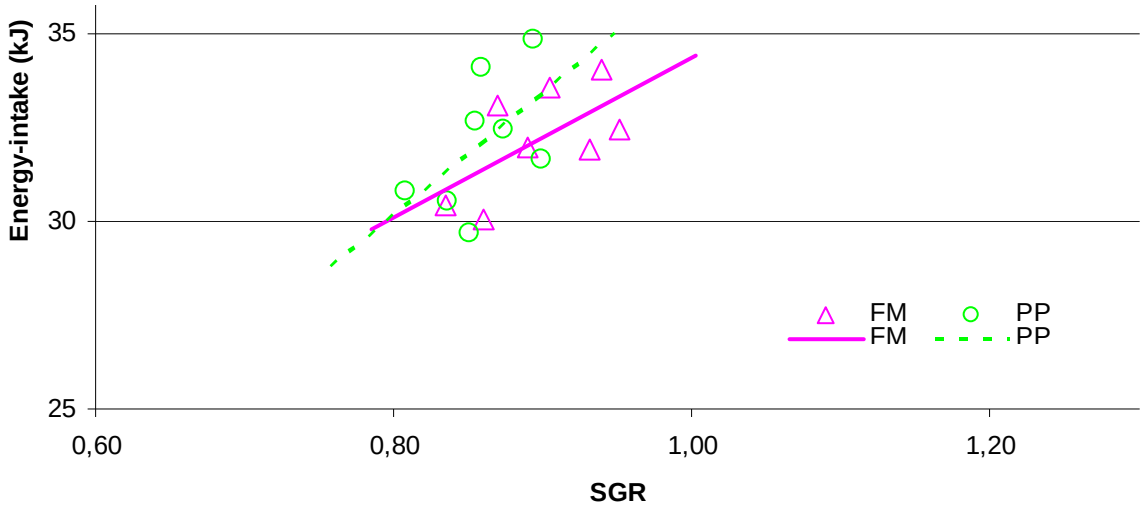


Figure 5.1.13. Spearman rank order correlation between energy intake (kJ) and SGR, from week 0 to 14, for cod fed four increasing levels of α-cellulose in; four FM diets ($R=0.60$, $p=0.12$) and four PP diets ($R=0.60$, $p=0.12$). Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal.

Energy intake and feed intake did not correlate with PER or PPV. Increased SGR correlated with increased PPV and PER for cod fed FM diets (PPV: $R=0.93$, $p<0.05$, PER: $R=0.76$, $p<0.05$), but not PP diets. FCR did not correlate with PPV or PER for cod fed PP diets or FM diets, although, there was a tendency for increasing FCR with decreasing PPV for cod fed PP diets ($R=-0.69$, $p=0.058$).

Increased HSI correlated with increased PPV for cod fed FM and PP diets (FM: $R=0.67$, $p<0.05$, PP: $R=0.86$, $p<0.05$). HSI did not correlate with PER in any diet groups. Larger total fillet yield in cod correlated with increased PPV, for cod fed FM diets ($R=0.71$, $p<0.05$), but not PP diets. Larger total fillet yield in cod tended to correlate with increased PER, for cod fed FM diets ($R=0.69$, $p=0.058$), but not PP diets. High PPV correlated with high PER for cod fed FM diets ($R=0.93$, $p<0.05$ and), but not PP diets.

Increased LER correlated with increased PER for cod fed FM diets and tended to for PP diets (FM: $R=0.81$, $p<0.05$, PP: $R=0.67$, $p=0.07$). LER and LPV did not correlate with energy intake. Increased LER correlated with increased feed intake for cod fed PP diets ($R=0.79$, $p<0.05$), but not FM diets. LPV did not correlate with feed intake. LER did not correlate with SGR, FCR or HSI. LPV increased with increased SGR for cod fed FM diets ($R=0.83$, $p<0.05$), but not PP diets. LPV increased with increased HSI for cod fed FM diets ($R=0.95$, $p<0.05$), but not PP diets. LPV did not correlate with FCR.

Increased SGR (week 7-14) correlated with a high HSI, for cod fed FM diets, ($R=0.74$, $p<0.05$), but not PP diets. Higher HSI correlated with a higher live weight in week 14 for cod fed FM diets ($R=0.86$, $p<0.05$), but not PP diets. Higher HSI tended to correlate with higher energy intake for cod fed FM diets ($R=0.62$, $p=0.10$), but not PP diets. FCR (week 7-14) did correlate with a decreased HSI for cod fed PP diets ($R=-0.83$, $p<0.05$), however, not FM diets. Increased HSI did not correlate with feed intake.

5.2 Digestion

5.2.1 Digestibility of fat, protein and dry matter (week 14)

Digestibility of fat, protein and dry matter from the final sampling (05.Feb.02) are listed in Table 5.2.1. The apparent digestibility coefficient (ADC) for fat was averagely 93.7 ±2.4%. Digestibility of fat decreased with increased α-cellulose for cod fed FM and PP diets (FM: R^2=0.65, p<0.05, PP: R^2=0.72, p<0.05), Figure 5.2.1., but the two regression lines are not significantly different. Average ADC for protein was 85.3 ±2.1%. There were no significant differences in protein ADC caused by increased α-cellulose. The average ADC for dry matter was 69.1 ±5.5%. Dry matter ADC decreased with increased α-cellulose for cod fed FM and PP diets (FM: R^2=0.83, p<0.05, PP: R^2=0.78, p<0.05), Figure 5.2.2., and the two regression lines are not significantly different.

Table 5.2.1. Apparent digestibility coefficients (ADC) for protein, fat and dry matter.

Diets*	ADC (fat)	ADC (protein)	ADC (dry matter)
FM 0	96.7	85.5	79,0
FM 0	94.2	80.6	73.6
FM 6	95.1	87.1	76.0
FM 6	94.8	83.3	71.4
FM 12	94.3	86.2	70.8
FM 12	94.9	85.7	69.5
FM 18	90.1	81.6	61.3
FM 18	91.5	86.4	63.3
PP 0	97.5	87.2	76.7
PP 0	95.0	83.4	69.2
PP 6	93.4	85.6	67.8
PP 6	95.1	86.7	70.5
PP 12	94.1	86.7	66.8
PP 12	92.4	87.2	66.8
PP 18	88.3	86.9	62.9
PP 18	92.0	85.4	60.6

*Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal, 0, 6, 12 & 18 = inclusions of α-cellulose (on a weight basis).

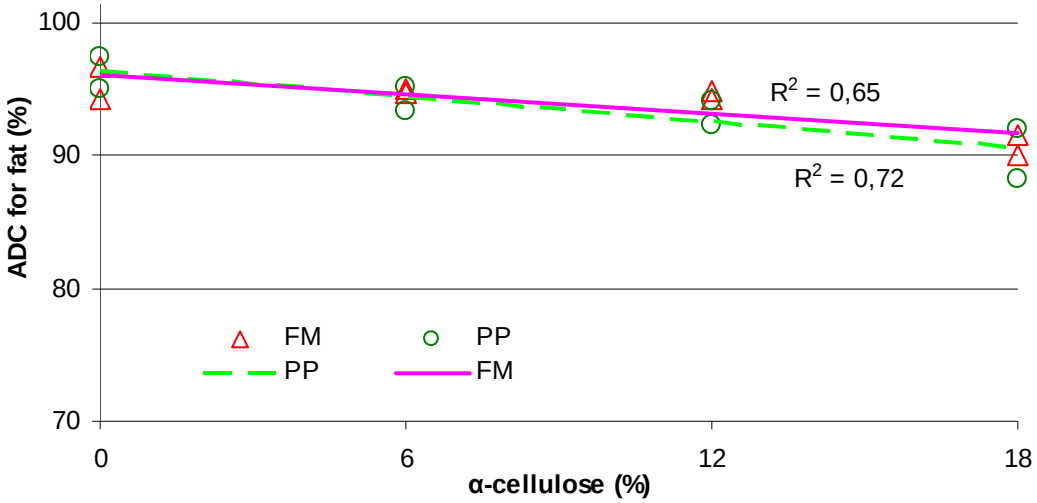


Figure 5.2.1. Regression with apparent digestibility coefficient (ADC) for fat, in week 14, for cod fed four increasing levels of α-cellulose in; four FM diets (Y=96.0712 - 0.2373x, R= -0.81, R^2= 0.65, p= 0.016) and four PP diets (Y= 96.38 - 0.32x, R=-0.85, R^2= 0.72, p=0.008). Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal.

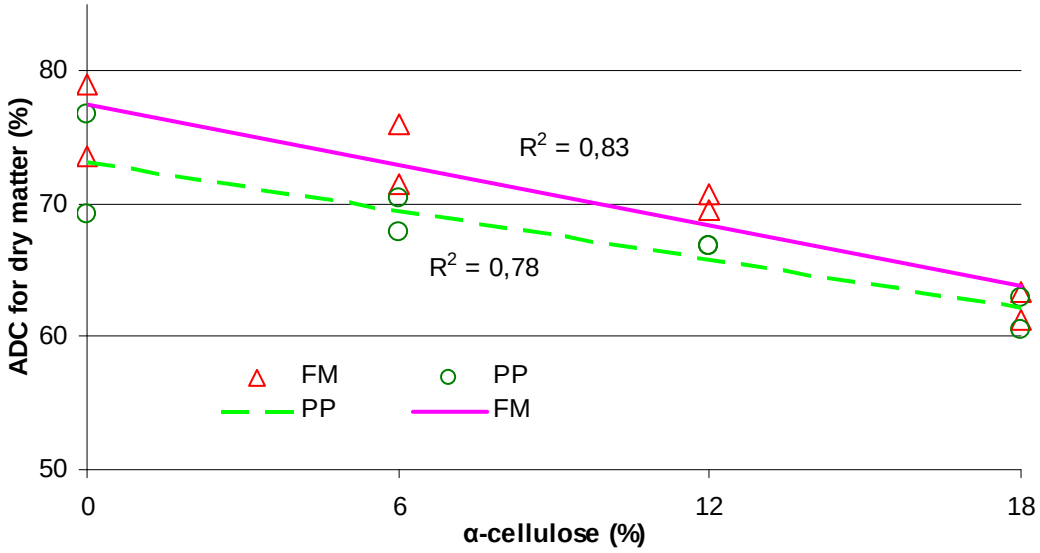


Figure 5.2.2. Regression with apparent digestibility coefficient (ADC) for dry matter, in week 14, for cod fed four increasing levels of α-cellulose in; four FM diets (Y=77.45 – 0.76x, R=-0.91, R^2=0.83, p=0.002) and four PP diets (Y=73.07 – 0.60x, R=-0.88, R^2=0.78, p=0.004). Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal.

5.2.2 Digestibility of essential elements (week 14)

The apparent digestibility coefficients for essential elements analysed, from the final sampling (05.Feb.2008), are presented in table 5.2.2.

Table 5.2.2. Apparent digestibility coefficients (ADC) (%) for the essential elements analysed; Cobalt (Co), Copper (Cu), Iron (Fe), Manganese (Mn), Molybdenum (Mo), Selenium (Se), Tin (Sn), Vanadium (V) and Zinc (Zn).

Diets***	Co	Cu	Fe	Mn	Mo	Se	Sn	V	Zn
FM 0	24	14	**	54	**	*	*	**	25
FM 0	21	13	24	71	47	*	*	**	43
FM 6	50	32	13	56	32	*	*	20	38
FM 6	53	21	26	79	7	*	*	31	55
FM 12	**	11	6	49	**	*	**	**	28
FM 12	**	32	19	81	**	*	**	**	58
FM 18	**	14	37	86	**	*	**	2	60
FM 18	22	26	38	79	**	*	**	**	51
PP 0	3	24	25	71	65	*	*	11	47
PP 0	**	**	19	63	67	*	*	**	29
PP 6	**	**	3	67	71	*	**	**	33
PP 6	**	8	10	76	72	*	2	10	42
PP 12	1	14	19	75	64	*	**	7	46
PP 12	**	9	33	77	71	*	**	13	35
PP 18	**	3	23	83	67	*	**	0	48
PP 18	**	0	29	89	66	*	**	7	49

*Element was below LOQ (limit of quantification) in faeces or/and feed. More details about LOQ and parallel measurements in Appendix 4, Table 9.2, 9.3. and 9.4.
**ADC result below 0%. More details in Appendix 4, Table 9.5.
***Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal, 0, 6, 12 & 18 = inclusions of α-cellulose (on a weight basis).

Cobalt (Co) ADC was mostly below 0%; therefore it was not possible to test if there were any effects from the increased α-cellulose. Copper (Cu) ADC was below 0% in two of the PP diet groups, but the average in all other diet groups was 16 ±10%. Cu ADC was not significantly influenced by increased α-cellulose. Iron (Fe) ADC was below 0% in one FM0 replicate; however, average Fe ADC for the rest of the diet groups showed 22 ±10%. Fe ADC was not significantly influenced by increased α-cellulose.

Average ADC for manganese (Mn) was 72 ±12% among all dietary groups. Mn ADC increased with increased α-cellulose, for cod fed PP diets (R=0.88, p<0.05), Figure 5.2.3., although, not FM diets. ADC for molybdenum (Mo) was below 0%, for most cod fed FM diets, however, Mo ADC for cod fed PP diets was at average 68 ±3%, and was not significantly affected by increased α-cellulose. Selenium (Se) was below limit of quantification in both feed and faeces, therefore could Se ADC not be calculated in any diet groups. Tin (Sn) was below limit of quantification in FM0, FM6 and PP0 in feed and in one PP0 replicate in faeces, consequently Sn ADC could not be calculated in those. Sn ADC was below 0% in the rest of the diet groups, except from one PP6 replicate where ADC for Sn was 2%. Vanadium (V) ADC was below 0% in FM0, FM12, one FM18 replicate, one PP0 replicate and one PP6 replicate. In the rest of the diets V ADC was low and ranged from 0-31%. Average ADC for zinc (Zn) was 43 ±11% among all diet groups, and was not significantly affected by increased α-cellulose.

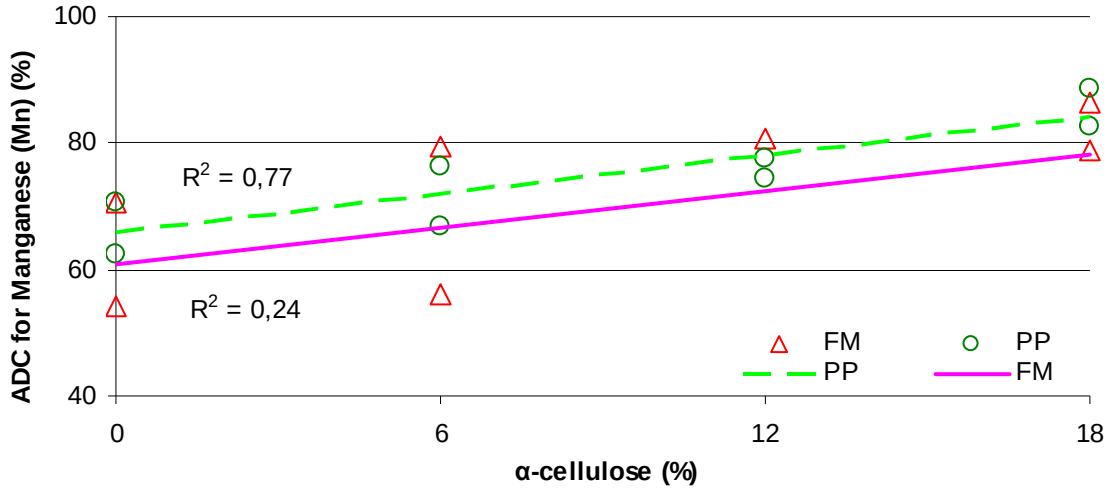


Figure 5.2.3. Regression with apparent digestibility coefficient (ADC) for Manganese (Mn), in week 14, for cod fed four increasing levels of α-cellulose in; 4 FM diets (Y=60.75 + 0.97x, R=0.49, R^2=0.24, p=0.22) and four PP diets (Y=65.75 + 1.02x, R=0.88, R^2=0.77, p=0.004). Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal.

5.2.3 Digestibility of undesirable elements (week 14)

The apparent digestibility coefficients for undesirable elements analysed, from the final sampling (05.Feb.2008), are shown in table 5.2.3.

Table 5.2.3. Apparent digestibility coefficients (ADC) (%) for the undesirable elements; Silver (Ag), Barium (Ba), Arsen (As), Cadmium (Cd), Mercury (Hg), Lead (Pb) and Strontium (Sr).

Diets***	Ag	Ba	As	Cd	Hg	Pb	Sr
FM 0	*	24	86	15	72	*	**
FM 0	*	36	81	**	67	*	**
FM 6	*	6	88	31	75	**	**
FM 6	*	54	84	7	70	69	**
FM 12	*	11	85	16	73	*	**
FM 12	*	**	86	32	63	*	**
FM 18	*	64	79	16	*	*	**
FM 18	*	40	85	21	*	*	**
PP 0	*	41	82	15	*	*	**
PP 0	*	15	75	**	*	*	**
PP 6	*	32	82	**	*	*	**
PP 6	*	40	82	**	*	*	**
PP 12	*	50	83	7	*	*	**
PP 12	*	38	82	**	*	*	**
PP 18	*	57	81	**	*	*	**
PP 18	*	56	81	**	*	*	**

* Element was below LOQ (limit of quantification) in faeces or/and feed. More details about LOQ and parallel measurements in Appendix 4, Table 9.2, 9.3. and 9.4.
** ADC result below 0%. Details in Appendix 4, Table 9.5.
***Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal, 0, 6, 12 & 18 = inclusions of α-cellulose (on a weight basis).

Silver (Ag) was below the quantification limit in both feed and faeces. ADC for total arsenic (As) was at average 82 ±3%, and was not affected by increased α-cellulose. Average ADC for barium (Ba) was 38 ±18%. ADC for Ba increased with increased α-cellulose for cod fed PP diets ($R=0.81$, $p<0.05$), Figure 5.2.4., but not FM diets. Cadmium (Cd) ADC was mostly below 0% for cod fed PP diets, and therefore not included, but Cd ADC for cod fed FM diets had an average at 20 ±9%. Cd ADC for cod fed FM diets was not affected by increased α-cellulose. Mercury (Hg) ADC was below limit of quantification for cod fed PP and FM18 diets. Hg ADC for cod fed FM0, FM6 and FM12 diets averaged 70 ±4%, and was not significantly influenced by increased α-cellulose for cod fed FM0, FM6 and FM12 diets. Lead (Pb) was mostly below limit of quantification in all diet groups, except for cod fed FM6 diets, where Pb ADC was below 0% in one replicate and was 69% in the other. Strontium (Sr) ADC was below 0% in all diet groups.

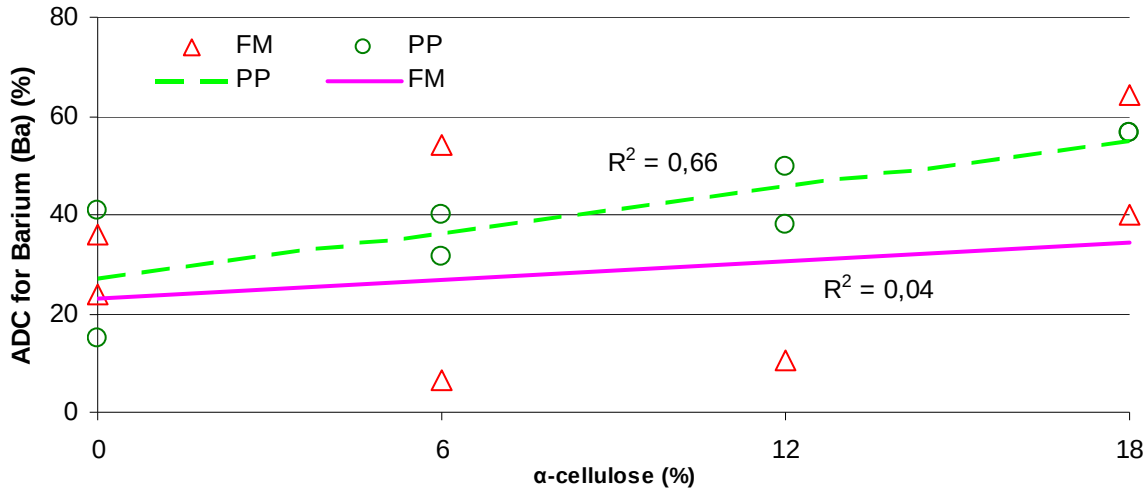


Figure 5.2.4. Regression with apparent digestibility coefficient (ADC) for Barium (Ba), in week 14, for cod fed four increasing levels of α-cellulose in; four FM diets ($Y=25.04 + 1.01x$, $R=0.36$, $R^2=0.13$, $p=0.43$) and four PP diets ($Y=26.88 + 1.57x$, $R=0.81$, $R^2=0.66$, $p=0.014$). Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal.

5.2.4 Faeces composition (week 14)

Protein, fat and dry matter in faeces, from final sampling (05.Feb.2008), are presented in Table 5.2.4. Average protein in faeces was 4.4 ±1.0%. Protein in faeces decreased with increased α-cellulose for cod fed FM and PP diets (FM: R^2=0.91, p<0.05, PP: R^2=0.92, p<0.05), Figure 5.2.5., and the two regression lines were significantly different. Average fat in faeces was 0.6 ±0.1%, and was not significantly influenced by increased α-cellulose. Average dry matter in faeces was 17.4 ±1.6%. Dry matter in faeces increased with increased α-cellulose for cod fed FM and PP diets (FM: R^2=0.64, p<0.05, PP: R^2=0.66, p<0.05), Figure 5.2.6., however, the two regression lines were not significantly different. Faeces colour after freeze drying was darker in FM0, FM6 and PP0 than in FM12, FM18, PP6, PP12 and PP18.

Table 5.2.4. Faeces compositions at trial end; protein, fat and dry matter, week 14. Nutrient values are given in wet weight.

	Protein	**Fat**	**Dry matter**	**Colour****
Diets*	%	%	%	description
FM 0	6.1	0.5	15.4	dark
FM 0	6.0	0.6	14.1	dark
FM 6	5.4	0.7	18.4	dark
FM 6	5.9	0.6	18.4	dark
FM 12	4.7	0.7	19.2	light
FM 12	4.5	0.6	18.5	light
FM 18	4.2	0.8	18.2	light
FM 18	3.5	0.7	19.4	light
PP 0	4.8	0.4	15.9	dark
PP 0	4.4	0.6	15.0	dark
PP 6	3.7	0.7	15.9	light
PP 6	4.0	0.6	17.2	light
PP 12	3.5	0.6	17.9	light
PP 12	3.6	0.8	18.9	light
PP 18	2.9	0.9	17.7	light
PP 18	3.1	0.6	18.0	light

*Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal, 0, 6, 12 & 18 = inclusions of α-cellulose (on a weight basis).
**The colour of faeces is described with eyesight after freeze drying.

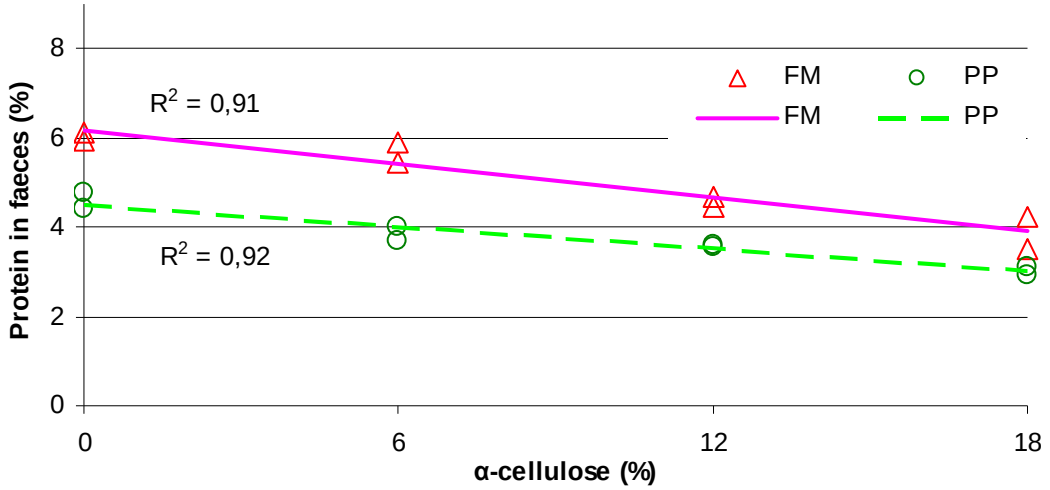


Figure 5.2.5. Regression with protein in faeces (%), in week 14, for cod fed 4 increasing levels of α-cellulose in; four FM diets (Y=6.18 - 0.13x, R=-0.95, R^2=0.91, p=0.0002 and four PP diets (Y=4.5025 - 0.0833x, R=-0.96, R^2=0.92, p=0.0002). Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal.

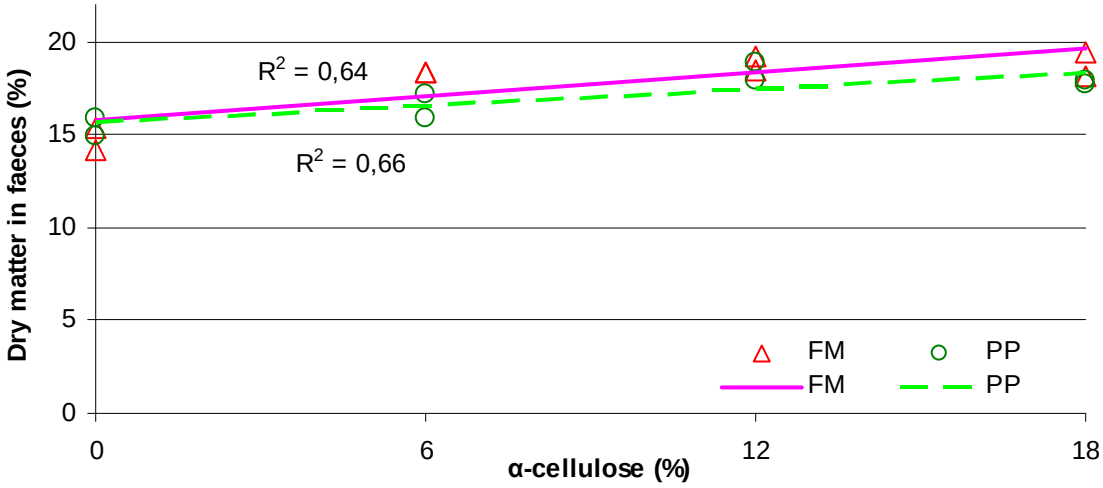


Figure 5.2.6. Regression with dry matter in faeces (%), in week 14, for cod fed 4 increasing levels of α-cellulose in; four FM diets (Y=15.79 + 0.21x, R=0.80, R^2=0.64, p=0.018) and four PP diets (Y=15.70 + 0.15x, R=0.81, R^2=0.66, p=0.014). Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal.

5.2.5 Correlations between digestibility coefficients and growth parameters

Some spear man rank order correlations for digestibility values and growth parameters from the feeding trial are presented**.**

HSI did not correlate with ADC for fat, for cod fed FM and PP diets. Increased HSI correlated with decreased protein ADC in FM diet groups ($R=-0.86$, $p<0.05$), but not PP diets. A high feed intake resulted in higher ADC for Mn and Zn, for cod fed FM diets ($R=0.86$, $p<0.05$), but not PP diets. A high PPV tended to be found simultaneously as Zn ADC increased, for cod fed FM diets ($R=0.69$, $p=0.058$), but not PP diets. Increased LER correlated with increased ADC for Mn for cod fed FM and PP diets (FM: $R=0.74$, $p<0.05$, PP: $R=0.91$, $p<0.05$). Increased ADC for Mn correlated with increased ADC for Zn for cod fed FM and PP diets (FM: $R=0.98$, $p<0.05$, PP: $R=0.76$, $p<0.05$).

SGR and weight gain decreased coincided with higher Hg ADC for cod fed FM diets ($R=-0.94$, $p<0.05$). Increased ADC of protein correlated with increased ADC for Cd, for cod fed FM and PP diets (FM: $R=0.79$, $p<0.05$, PP: $R=0.79$, $p<0.05$). Increased ADC for protein correlated with increased ADC for total As, for cod fed FM diets ($R=0.76$, $p<0.05$), but not PP diets. High total As ADC was found simultaneously with high Cd ADC in all diet groups (FM: $R=0.69$, $p=0.058$, PP: $R=0.86$, $p<0.05$). High total As ADC also correlated with high Cu ADC, for cod fed PP diets ($R=0.76$, $p<0.05$), but not FM diets.

As a result of lower ADC for fat, faeces values showed higher residue fat levels, for cod fed FM diets ($R=-0.78$, $p<0.05$), and there was a tendency of correlation for cod fed PP diets ($R=-0.68$, $p=0.062$).

5.3. Fish health

5.3.1 Haematological values (week 14)

Haematological values from the final sampling (05.Feb.2008) are given in Table 5.3.1. Haematocrit (Hct) varied from 24-33% between all diet groups. Red Blood Cell count (RBC) varied from 1.51-2.36 10^{12}/L among all diet groups. Haemoglobin (Hb) ranged from 3.93-4.85g/100ml in all diet groups. Mean cell haemoglobin concentration (MCHC) ranged from 13-18g/100ml. Mean cell volume (MCV) varied from 119-194 10^{-15}L among all dietary groups. Mean cell haemoglobin (MCH) ranged from 19-30 10^{-6}g in all diet groups. None of the haematological values were significantly influenced by increased α-cellulose.

Table 5.3.1. Haematological values in Atlantic cod fed eight different diets in 14 weeks.

Diets*	**Hct*** (%)	**RBC*** *10^{12} /L	**Hb*** g/100 ml	**MCHC*** g/100 ml	**MCV*** 10^{-15} L	**MCH*** 10^{-6} g
FM 0	28	1.71	4.65	17	162	27
FM 0	28	1.65	4.90	18	170	30
FM 6	28	2.05	4.82	17	138	24
FM 6	28	1.65	4.55	16	168	28
FM 12	35	1.79	4.85	14	194	27
FM 12	27	1.78	4.82	18	154	27
FM 18	28	2.36	4.42	16	119	19
FM 18	25	1.51	3.93	16	164	26
PP 0	27	2.01	4.55	17	132	23
PP 0	28	1.63	4.47	16	169	27
PP 6	24	1.61	4.36	18	149	27
PP 6	30	1.74	4.68	16	170	27
PP 12	33	1.69	4.37	13	194	26
PP 12	28	1.79	4.80	17	158	27
PP 18	26	1.74	4.47	17	149	26
PP 18	27	1.63	4.27	16	168	26

*Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal, 0, 6, 12 & 18 = inclusions of α-cellulose (on a weight basis), Hct = Haematocrit, RBC = Red blood cell count, Hb = Haemoglobin, MCHC = Mean cell haemoglobin concentration, MCV = Mean cell volume and MCH = Mean cell haemoglobin.

5.3.2 Clinical and nutritional values (week 14)

Clinical and nutritional values in blood plasma are listed in Table 5.3.2. Aspartate aminotransferase (ASAT) ranged from 1-49 U/L and Alanine aminotransferase (ALAT) from 0-18 U/L among all diet groups. Plasma glucose ranged from 3.5-7.5mmol/L among all diet groups. Total protein ranged from 23.9-40.1g/L among all dietary groups. None of the clinical or nutritional values were significantly influenced by increased α-cellulose.

Table 5.3.2. Clinical and nutritional values in blood plasma in Atlantic cod fed eight different diets for 14 weeks.

Diets*	**ASAT*** (U/L)	**ALAT*** (U/L)	**Glucose*** (mmol/L)	**Total protein*** (g/L)
FM 0	20	5	4.5	32.2
FM 0	17	0	5.5	33.3
FM 6	16	18	4.9	38.0
FM 6	17	3	4.4	28.6
FM 12	20	10	4.4	31.3
FM 12	18	7	4.6	34.7
FM 18	19	0	4.8	36.0
FM 18	8	0	3.5	23.9
PP 0	17	13	4.9	34.2
PP 0	7	10	4.6	38.1
PP 6	16	0	4.1	30.9
PP 6	4	0	5.2	38.0
PP 12	1	6	7.5	40.1
PP 12	49	12	5.5	28.8
PP 18	40	16	6.1	34.6
PP 18	21	6	3.9	32.5

*Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal, 0, 6, 12 & 18 = inclusions of α-cellulose (on a weight basis), ASAT = Aspartate aminotransferase and ALAT = Alanine aminotransferase.

5.4. Whole body and liver compositions

5.4.1 First sampling (3 weeks before feeding trial started)

Whole body composition of protein, fat, glycogen, dry matter and ash, from the first sampling (10.Oct.2007), three weeks before the feeding trial started, are listed in Table 5.4.1. Nutritional values are given in wet weight. Protein concentration was 14.9%, fat 8.7%, glycogen 0.07%, dry matter 27.1% and ash 2.1%.

Table 5.4.1. Average whole body composition of protein (%), fat (%), glycogen (mg/g), dry matter (%) and ash (%), from the first sampling (10.Oct.2007), three weeks before fish distribution and feeding trial onset. Nutrient values are given in wet weight.

	Protein %	**Fat** %	**Glycogen** %	**Dry matter** %	**Ash** %
Start*	14.9	8.7	0.07	27.1	2.1

*Average from cod before feeding trial started, when all cod were fed equal feed.

5.4.2 Final sampling (week 14)

Whole body composition from the last sampling (05.Feb.2008) are given in Table 5.4.2. Values here are all given on a wet weight basis. Protein in whole body was at average 15.3 ±0.5%. Protein in whole body decreased with increased α-cellulose for cod fed PP diets (R^2=0.51, p<0.05), Figure 5.4.1., but not for cod fed FM diets. Whole body fat averaged 7.4 ±0.6%, average glycogen level was 0.05 ±0.01% and dry matter averaged 26.4 ±0.5%, and either of these three parameters were influenced by increased α-cellulose for cod fed FM or PP diets. Whole body ash averaged 2.3 ±0.3%. Ash in whole body tended to decrease with increased α-cellulose for cod fed PP diets (R^2=-0.66, p=0.074), however, not for cod fed FM diets.

Table 5.4.2. Whole body composition at trial end (05.Feb.2008). Nutritional values are given in wet weight.

	Protein	**Fat**	**Glycogen**	**Dry matter**	**Ash**
Diets*	%	%	%	%	%
FM 0	15.6	7.5	0.04	26.4	2.5
FM 0	15.3	7.9	0.07	27.0	2.2
FM 6	14.9	6.9	0.04	25.4	2.2
FM 6	16.2	7.1	0.05	26.5	2.3
FM 12	15.4	6.8	0.03	25.8	2.6
FM 12	15.6	7.3	0.05	26.1	2.1
FM 18	15.8	7.5	0.05	26.9	2.4
FM 18	14.9	6.1	0.03	25.5	2.6
PP 0	15.5	8.7	0.05	26.4	2.4
PP 0	15.7	7.4	0.04	26.8	2.6
PP 6	14.8	7.5	0.04	26.2	2.0
PP 6	15.1	7.9	0.05	27.2	2.8
PP 12	14.5	7.2	0.05	26.1	2.2
PP 12	15.3	7.3	0.05	26.5	2.1
PP 18	14.9	7.6	0.05	26.7	2.1
PP 18	14.8	7.5	0.06	26.6	1.9

*Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal, 0, 6, 12 & 18 = inclusions of α-cellulose (on a weight basis).

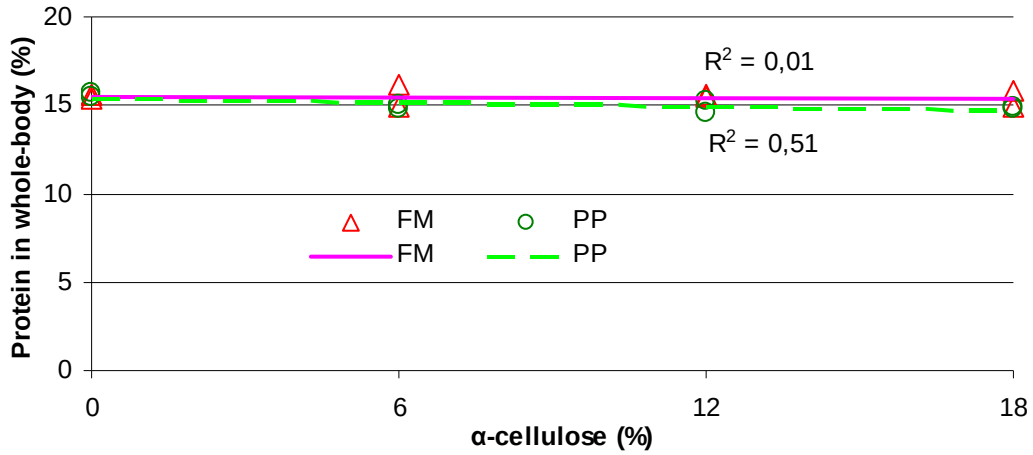


Figure 5.4.1. Regression with protein in whole body (%), in week 14, for cod fed four increasing levels of α-cellulose in; four FM diets (Y=15.512 – 0.005x, R^2=0.01, p=0.84) and four PP diets (Y=15.42 – 0.040x, R^2=0.51, p=0.047). Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal.

Liver composition at final sampling is presented in Table 5.4.3. Among diet groups the average liver protein was 4.2 ±0.6%, liver fat 67.8 ±1.3%, liver glycogen 0.36 ±0.06% and dry matter 74.5 ±1.0%, and neither protein, fat, glycogen nor dry matter in liver were significantly affected by increased α-cellulose, in any of the diet groups.

Table 5.4.3. Liver composition at trial end (05.Feb.2008). Nutrient values are given in wet weight.

Diets*	Protein %	Fat %	Glycogen %	Dry matter %
FM 0	5.5	68.4	0.24	74.2
FM 0	4.0	67.1	0.42	73.3
FM 6	4.6	67.6	0.37	73.2
FM 6	4.0	67.7	0.33	75.5
FM 12	4.2	69.5	0.33	73.7
FM 12	3.9	66.4	0.42	73.7
FM 18	4.2	66.2	0.38	73.0
FM 18	4.3	66.0	0.26	73.9
PP 0	3.8	68.2	0.34	75.0
PP 0	4.0	69.5	0.35	74.9
PP 6	3.8	67.8	0.32	76.1
PP 6	3.8	69.6	0.39	74.9
PP 12	5.1	66.6	0.41	75.0
PP 12	3.7	67.9	0.42	75.0
PP 18	5.5	66.7	0.40	74.1
PP 18	3.6	69.6	0.33	76.5

*Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal, 0, 6, 12 & 18 = inclusions of α-cellulose (on a weight basis).

6 Discussion

6.1 Discussion of materials and methods

6.1.1 Statistical design

This trial had a simple linear regression design. When the true relationships between variables are not known, regression is the most chosen alternative (Shearer, 2000). To establish a good dose response relationship it is important to allocate 1/3 of nutrient inputs at the low end, 1/3 at the high end and 1/3 in the middle. The high end in this experiment was unknown for cod, however, 18% was chosen due to results from cod and other fish species given fibre or other indigestible matters (Al-Ogaily, 1996; Dias *et al.*, 1998; Turano *et al.*, 2002; Toppe *et al.*, 2006; Hansen and Storebakken, 2007). Increasing number of replicates result in increased accuracy of the different levels. This is possible under ideal conditions and with unlimited resources. However, when using regression, replicates are not absolute necessary (Shearer, 2000). If total observations are greater than 20 (levels x replicates), then more observations give little improvement. In this experiment there were 4 levels (0%, 6%, 12% and 18% α-cellulose) and 2 replicates at each level giving 8 observations. This might not be ideal, but the 8 points in the graph will give a good indication if there were any signs of a dose response relationship. In addition the total 16 observations from both fish meal (FM) and plant protein (PP) diets will also give a good indication, even though FM and PP might interact differently with α-cellulose. Most models are not intrinsically linear, and if the cellulose response in reality was a curved line, it probably would not have been discovered in the linear regression design. Furthermore, 2 replicates are not good enough for analysing differences between means, such as ANOVA (analysis of variance), because the power of the test would be too small, therefore this was not done. However, tendencies to different responses between different diet groups were most likely indicated in the regression design graph. In this trial there were two different basic diet formulas (FM and PP), both with similar increasing α-cellulose. When both regression lines were significant, these were compared to find if they had different slopes and elevations (Zar, 2005). This analysis was influenced by the same uncertainties as linear regression.

6.1.2 Feeding trial

To be able to compare different cod groups fed different diets, the different diet groups had to be treated as similar as possible. However, since it is an experiment with animals, there are many uncertain factors that can affect the results besides the targeted independent variable such as individual differences, stress, diseases, hierarchy systems and temperature changes. The feed was produced by Skretting ARC (Stavanger, Norway). All analysed macronutrients (fat, protein and starch) in feed met cod requirements as described by Rosenlund *et al*. (2004), analysed desirable elements with requirements (Co, Cu, Fe, Mn, Se and Zn) met requirement ranges as described by Watanbe *et al*. (1997), analysed essential elements with upper limits (Cu, Zn, Mn, Co, V and Se) were below upper limits set by EU (Julshamn *et al.*, 2003), and undesirable elements (Hg, Pb, Cd and As) were below upper limits set by EU (Julshamn *et al.*, 2003). Requirements for Sn, V and Mo, and limits for Ba, Ag and Sr, are not known, at least not to my knowledge. Cellulose inclusions in cod feed has not been tried earlier. Therefore we did not know if these cellulose inclusions would work well in combination with the other feed ingredients and if cellulose was the right insoluble fibre for energy dilution in cod diets. The cod approximately doubled its weight during the feeding trial, which means that any adverse effects from the cellulose most likely would have come forward, at least indications of these.

6.1.3 Sampling methods

There can be large individual differences within one tank, even though the fish are fed and treated in a similar manner. All cod from one tank counted as one independent sample in the statistical treatments, since the fish in the one tank can not be regarded as independent and are influenced by each other. Further individual feed intake could not be measured, which could have masked any regression effects. To get a good average from each tank several random samples were taken, which were thereafter pooled. In addition, organ samples were taken from the same spot in the organ, independent on individual, and performed by the same person. Whole body/organ samples were homogenized, which is also an uncertainty factor. Although, this would most likely be detected, since there were always at least two chemical parallels of each sample, and a standard deviation at the chemical level above 10% would lead to reanalyses. To obtain homogenous and representative samples it is important to take large

enough samples, but this is not always possible. Faeces samples were small, due to small cod, even if these were pooled from 10 fish per tank. Therefore, faeces did not have large enough sample size to perform parallel chemical measurements of fat. In addition, starch analysis of faeces could not be performed at all. Another uncertainty factor is the random sampling performed when netting, as there might be individuals, e.g. the smaller fish in a tank, that are more able to escape than the larger individuals. This might explain why sampled cod used for HSI measurements in last sampling (05.feb.08) seemed to have higher mean weight (335 ±22g) than the rest of the cod (312 ±11g). Perhaps it is easier to catch bigger fish when we use a landing net. This should be considered in future samplings, since random sampling is the preferred and most used method in international published data from fish experiments.

6.1.4 Analytical methods

There are many possible sources of error during analytical processes; weighing, pipette measurements, dilution, extraction, time, steaming, filtrating, temperature, etc. However, error sources are controlled by control samples, reference material, parallel measurements and accreditation of methods (Appendix 2). In addition all samples in the same analysis are analysed as equally as possible, which means that they are comparable to each other, even though all results might not be the absolute true values.

The total nitrogen analysis to measure protein is based on the factor 6.25, assuming that protein contains 16% nitrogen, which is not totally correct, since amino acids contain different amounts of nitrogen and different protein sources can have different amino acid profiles. In addition protein is not the only substance containing nitrogen in fish (also nucleic acids, phospholipids (phosphatidylethanolamine), nucleotides, etc. contain nitrogen). But still, protein is the main nitrogen source. Anyhow, as said, the results are comparable to each other. Determination of fat in faeces was performed with acid hydrolysis. There were not enough faeces for duplicate analyses, but the results are reliable because of control and reference material. One whole body sample (FM6) had 11.4% in difference between analysed parallels, which is above the accepted 10%. This could be due to low glucose values in whole body, because the analysis is more accurate when the values are higher. However, control and reference material were within normal values, which means that the analysis is good. The

difference between parallels is probably due to not good enough homogenization, since whole fish is hard to get totally homogenized, due to skin and bones. Parallels were re measured, with the same result, and the mean of all four results was therefore used. In glycogen analyses the calibration curve have only two points, and this could be an error source. Anyhow, since reference and control materials are used, wrong results most likely would have been detected. Yttrium oxide and other elements were analysed with ICP-MS (Inductive Coupled Plasma Mass Spectrophotometer). Some parallel measurements had a variance above the accepted 10% (Appendix 4, Table 9.2 and 9.3), indicating that the results are not totally reliable.

To determine digestibility an indirect method is used, by means of an inert indicator, here yttrium-oxide. Digestion can be affected by temperature, salinity (Storebakken *et al.,* 1998a) and other environmental factors; therefore is a stable environment important. Another uncertainty factor in measurement of digestibility is that some feed could have been digested by micro-organisms (Nortvedt and Krogdahl, 2001). It is important to know how much the fish has eaten; therefore waste feed was collected and subtracted from amount feed given. This is hard to do precisely; however, equal procedures were performed each day, after feeding, before the feed dissolves too much. To get politely faeces samples, faeces were stripped directly from the intestine, as described by Hemre *et al*. (2003). Faeces collecting might include fragments from the mucus membrane in the intestine. Anyway, this method is better than collecting faeces from the water column where water soluble components might be lost. Bioavailability of nutrients and minerals can differ in different feed sources (Lorentzen *et al.*, 2001), depending on chemical state of minerals, and interaction with other feed components.

Methods for aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), glucose and protein in plasma are not accredited at NIFES, neither are haematocrit (Hct), haemoglobin (Hb) or red blood cell count (RBC). These are however standardized methods used in clinics (for humans and animals), and the level of certainty is expected to be high. Blood samples are important to mix with an anti-clotting reagent, e.g. heparin, when analysing RBC, since blood cells sediment fast. Blood coagulate fast; therefore blood analyses have to be done right after the sampling, but heparinized syringes delayed coagulation efficiently.

6.1 Discussion of results

6.2.1 Growth and feed utilization

Growth

Growth can be affected by environmental factors like water temperature (Howell, 1984), light (Hemre *et al.*, 2004), fish size (Braaten, 1984; Jobling, 1992), sexual maturation (Braaten, 1984; Hansen *et al.*, 2001; Hemre *et al.*, 2002), health, nutrition (Morais *et al.*, 2001; Rosenlund *et al.*, 2004) and heritage (Gjerde *et al.*, 2004). Specific growth rate (SGR) was higher in the first 7 weeks (1.00 ±0.09) than in the last 7 weeks (0.76 ±0.08), which was expected, since smaller cod have higher feed intake per weight unit than larger cod (Braaten, 1984). SGR in this trial was higher than predicted by growth models for cod at the same size and held at 8°C (Björnsson and Steinarsson, 2002). In addition, growth in this trial was good compared to previous studies with good growth for cod juveniles (Lie *et al.*, 1988; Hemre *et al.*, 1989; Morais *et al.*, 2001; Rosenlund *et al.*, 2004; Hansen *et al.*, 2007b). Growth was not significantly influenced by the increased α-cellulose, in either FM or PP diets, in the total experiment. There was a tendency to decreased weight with increased α-cellulose for cod fed PP diets, in the first 7 weeks. However, in the last 7 weeks there were no such tendencies for either SGR, weight gain, weight or condition factor (CF) in either PP or FM diets. This might be due to cod being adapted physiologically to both high PP and α-cellulose during the first period. Therefore, in conclusion, there was great growth in all diet groups. Good growth with partly plant protein inclusions in diets is in accordance with previous studies on cod (Albrektsen *et al.*, 2006; Refstie *et al.*, 2006a; Hansen *et al.*, 2007a; Hansen *et al.*, 2007b). Increasing cellulose inclusions have not previously been investigated in cod diets. While, good growth is found in other fish species with cellulose inclusions in feeds; rainbow trout with cellulose inclusion levels up to 30% (Bromley and Adkins, 1984) and 15% (Hansen and Storebakken, 2007), seabass diets up to 20% (Dias *et al.*, 1998), tilapia diets up to 9% (Al-Ogaily, 1996) and red drum diets up to 20% (Turano *et al.*, 2002). Although, other studies report growth depression with increasing cellulose in diets for tilapia and rainbow trout, with inclusion levels up to 10% and 20% (Hilton *et al.*, 1983; Dioundick and Stom, 1990). The

gutted weight and fillet weights being equal in all diet groups are in our trial are in agreement with Hansen *et al*. (2007a), who found equal gut and fillet weight when plant protein in diets increased.

Feed utilization

Feed conversion ratio (FCR) and feed intake were in accordance with other juvenile cod trials (Lie *et al.*, 1988; Morais *et al.*, 2001; Hansen *et al.*, 2006; Hansen *et al.*, 2007a). In our trial, FCR during the first 7 weeks increased significantly with increased α-cellulose inclusions, in both FM and PP diet groups. In addition, the two regression lines for FM and PP diets were significantly different, which indicates that the two diets (FM and PP) combined with α-cellulose, affected cod differently. In the last 7 weeks, no influence from increased α-cellulose on FCR was revealed, but FCR seemed higher than during the first 7 weeks. In addition, FCR still seemed to be most elevated in the diet groups with high α-cellulose inclusions. Also feed intake seemed to be higher in the groups with α-cellulose inclusions compared to those without, indicating that α-cellulose was the cause of the increased feed intake. These results are in accordance with cellulose inclusion results on seabass (Dias *et al.*, 1998) and rainbow trout (Bromley and Adkins, 1984). Although feed intake in some experiments was not affected, e.g. in tilapia and rainbow trout reported by Al-Ogaily (1996) and Hansen and Storebakken (2007). However, those data were based on studies with very low growth rate, e.g. did the study reported by Hansen and Storebakken (2007) not result in doubling of fish weight. Some studies even reported reduction in feed intake, combined with growth depression; tilapia and rainbow trout (Hilton *et al.*, 1983; Dioundick and Stom, 1990). In the first 7 weeks of our trial, feed intake and growth were lower for cod fed PP diets plus α-cellulose, compared to cod fed FM diets with α-cellulose. Though, during the last 7 weeks, cod fed PP diets plus α-cellulose managed to increase its feed intake, tending to a higher weight gain than the other groups, again resulting in final weight being similar to the other diet groups. All this indicate that cod fed PP based diets added α-cellulose needed more time to adjust to the feed. Probably since PP6, PP12 and PP18 had more variable composition, compared to the pre experiment feed, than PP0 and FM diets. Furthermore, the tendency to lower feed intake and growth in the first 7 weeks in the PP diets plus α-cellulose, compared to FM diets, might have induced a compensatory growth in the last 7 weeks, when the cod got used to the diet. Anyway, this increased feed intake and weight gain in the last 7 week period would most likely level off when the cod catches up with its “normal growth rate”.

In our trial, cod fed PP0 had similar growth and feed utilization as cod fed FM0, which indicate that the plant protein used was equally well utilized as the fish meal. In other studies there have been found increased feed intake with increasing plant ingredients in diets for cod, especially when plant inclusion exceeded 25% (Hansen *et al.*, 2006; Refstie *et al.*, 2006a). However, utilization of plant ingredients depend on source, amount and processing treatment (Francis *et al.*, 2001). The use of soy protein concentrate (SPC), compared to full fat soybean meal, is known to eliminate negative effects expected in salmon, especially since SPC have a lower concentration of oligosaccharides and anti nutritional factors than extracted soybean meal (Refstie *et al.*, 1998). SPC plus wheat gluten in cod diets is found to result in same feed utilization, macronutrient digestibility and growth as fish meal (Hansen *et al.*, 2006; 2007a), and that is one explanation why these ingredients were used in the present trial.

Energy intake is not often reported in cod trials, to my knowledge. Anyway, the protein to energy ratio (PE) in our diets was similar to the PE ratio in cod diets recommended by Rosenlund *et al*. (2004). Although Rosenlund *et al*. (2004) reported digestible energy in diets, and in our trial the total energy was calculated. The independent and similar energy intake in the FM based diets, but not in the PP based diets only during the first feeding period, can be explained by cod adjusting to the new feed, especially when fed a PP based diet added α-cellulose. The similar energy intake, at the end of the trial, even though the diets had different energy concentrations, might indicate that cod adjusted its feed intake in accordance to the energy concentrations, to maintain great growth. Increased feed intake to compensate low energy feed is in accordance with cod juveniles fed marine ash inclusions (Toppe *et al.*, 2006). In addition, energy intake tended to correlate with SGR, in our trial. However, the similar energy intake could instead be due to that the digestible macronutrients (protein, fat and carbohydrates) and PE had the same ratio in all diets. Then the cod increased feed intake to get enough of other nutrients, as protein, independently on energy intake. Most likely the cod depends on both energy and protein concentration.

Liver index (HSI)

In accordance with previous observations, HSI in our trial mostly seemed to be larger than normally found in wild cod (Jobling, 1988; Grant *et al.*, 1998; Gildberg, 2004; Mørkøre, 2005), and was within ranges of farmed cod juveniles (Lie *et al.*, 1986; Morais *et al.*, 2001; Rosenlund *et al.*, 2004; Toppe *et al.*, 2006; Hansen *et al.*, 2007a). The reduction in HSI, from initial (11.9 ±0.8%) to final values (10.5 ±0.6%) was one of the main objectives of the study,

however, hard to explain, since fat and protein levels were equal in the pre experimental feed and in the experimental feed. There might be environmental or individual variations that we do not know about. It is known that protein growth in animals is most likely strongly regulated through control of food intake (Webster, 1993; Jobling *et al.*, 1994), foreseen that both protein and amino acid requirements are met. Thus, energy may be consumed in excess, resulting in excess fat deposition and thereby increased liver sizes in cod. High lipid diets are known to increase cod liver size (Lie *et al.*, 1986; Jobling *et al.*, 1991; Jobling *et al.*, 1994; Morais *et al.*, 2001; Rosenlund *et al.*, 2004; Karlsen *et al.*, 2006). Therefore our experimental diets had protein/lipid/starch ratios according to Rosenlund *et al*. (2004) to maintain good growth and still keep liver size to a minimum. Bulk inclusions of cellulose have tended to reduce HSI in seabass (Dias *et al.*, 1998) and give a low fat deposition in red drum (Turano *et al.*, 2002). In broiler chicken, increasing raw fibre in feed reduced metabolic energy per metabolic weight unit, and chickens retained more energy as body protein and consequently less as body fat (Jorgensen *et al.*, 1996). However, HSI in the present trial was not significantly affected by increased α-cellulose in any of the diet groups at final sampling. Hence, it seems hard to manipulate the energy deposition in cod more than previously done by means of dietary fat content in feeds. Another theory for that α-cellulose did not affect energy deposition in cod liver, could be due to that the heritability for HSI in cod is found to be high (Refstie, 2009). Then heritage for HSI perhaps conceal or overrule the possible effects from energy dilution with α-cellulose.

Protein and fat utilization

Protein efficiency ratio (PER) and protein productive value (PPV) in the present trial were good, when compared to earlier reports for cod juveniles (Lie *et al.*, 1988; Hemre *et al.*, 1989; Morais *et al.*, 2001; Hemre *et al.*, 2002; Rosenlund *et al.*, 2004; Hansen *et al.*, 2007a). PER and PPV were not influenced by increased α-cellulose. Rosenlund *et al*. (2004) reported that PER was positively correlated with dietary lipid and negatively with starch and protein. However, in our trial, the dietary digestible nutrient ratio (protein/lipid/carbohydrates), dietary protein to energy ratio (PE) and energy intake were similar in all our diet groups. This was probably the reason for protein utilization being unaffected, even though the indigestible part of the diets increased and reduced the energy contents in feeds.

Lipid efficiency ratio (LER) (average 6.5 ±0.6) increased with increased α-cellulose for cod fed PP diets, and tended to increase also for cod fed FM diets. Although average lipid productive value (LPV) being equal between diet groups, did however not further confirm this finding. However, the increase in LER was minor. In salmon it is found that PER correlated positively with LER (Sagstad *et al.*, 2008), in agreement with the present study. Which of the two factors, LPV or LER, give the more accurate answer depends on several factors; LPV depends on more steps in analytical procedures and is calculated from the fat amount in the biomass increase, while LER is calculated from the total biomass increase, also discussed by Lie *et al*. (1988). This is the case for PPV and PER too, PPV is found to be more accurate than PER in cod studies (Lie *et al.*, 1988). Reminding on cod storing most of its fat in the liver and very small amounts in muscle. In our study, LER did not correlate with HSI, while LPV increased with increased HSI for cod fed the FM based diets, but not for cod fed PP diets. This might indicate that LPV is more accurate than LER, in addition this also indicates that FM and PP diets might have different effects.

6.2.2 Digestion of fat, protein and dry matter

Digestion of fat

In our trial the apparent digestibility coefficient (ADC) for fat was averagely 94 ±2%. The high ADC results are in accordance with previous cod studies, which have reported ADC of fat from 80% to above 90% (Lie *et al.*, 1988; Hemre *et al.*, 1989; Dos Santos *et al.*, 1993; Hemre *et al.*, 2003; Førde-Skjærvik *et al.*, 2006; Hansen *et al.*, 2006; Toppe *et al.*, 2006; Hansen *et al.*, 2007b). In our study digestibility of fat decreased with increased α-cellulose, for cod fed both FM and PP diets, and the two regression lines were identical. This indicates that it was the α-cellulose that affected ADC negatively, independent if the dietary protein came from PP or FM. The equal fat digestibility between FM and PP is in accordance with previous digestibility studies in salmon and cod (Refstie *et al.*, 1998; Hansen *et al.*, 2006) with the same plant protein ingredient (soybean protein concentrate) as used in our study. Reduced ADC for fat with increasing plant ingredients has previously been found in cod experiments, but, when using other PP types and mixtures (Førde-Skjærvik *et al.*, 2006; Hansen *et al.*, 2006; Refstie *et al.*, 2006a).

High starch levels have been reported to decrease digestion of fat in salmon (Hemre *et al.*, 1995), however, there were no differences in protein/fat/carbohydrate ratios in diets in our experiment. Starch is water soluble and available to intestinal enzymes, while cellulose is not, meaning that cellulose will not affect digestion in a similar manner as starch. Cellulose inclusions up to 15% did not affect ADC for fat in diets for rainbow trout (Hansen and Storebakken, 2007), similar results were found in tilapia (Amirkolaie *et al.*, 2005). The decrease in fat ADC with increasing cellulose inclusions in our cod trial was in accordance with salmon fed diets with cellulose inclusions (Aslaksen *et al.*, 2007), where fat ADC ranged from 96-87%. Hansen and Storebakken (2007) gave two plausible reasons for how cellulose affected fat ADC differently; dietary lipid content was higher and water temperature lower in the trials performed by Aslaksen et al. (2007), probably challenging capacity for lipid digestion. Further, the trout (Hansen and Storebakken, 2007) and tilapia (Amirkolaie *et al.*, 2005) were reared in freshwater, an environment that favours lipid digestion compared to saltwater (Storebakken *et al.*, 1998a). Aslaksen *et al*. (2007) experiments with salmon were carried out in close to same water temperature (8°C) in seawater, as the cod in our trial. Therefore the cellulose inclusions in our trial could have affected fat digestion in same direction as Aslaksen *et al.* (2007). Furthermore, cellulose is an indigestible fibre, and indigestible fibres are known to increase feed flow rate trough the GIT (Hemre, 2001), which may have led to decreased time to digest nutrients, e.g. fat. There could also have been a combination of these factors that resulted in the decreased fat digestion. However, in our experiment it was not possible to examine whether the lower fat digestibility was caused by reduced lipid hydrolysis, reduced intestinal fatty acids uptake, feed flow rate or other factors.

Reduced fat ADC would expectedly result in LER and LPV also being negatively affected by α-cellulose. Still, this was not the case, actually LER increased with increased α-cellulose in PP diets and tended to in FM diets, which is hard to explain. If we speculate, the reason might be that the bacterial load in the distal intestine increased due to increased levels of α-cellulose (bacterial load was not measured). Significant numbers of bacteria are previously observed in the lower intestinal tract of cod (Hansen *et al.*, 2006; Seppola *et al.*, 2006). The increased α-cellulose could have resulted in α-cellulose being an available energy source for the bacteria, which again may have increased bacteria production of short chain fatty acids. Mammals are reported to use these short chain fatty acids as a source of energy (Burkitt, 1979; Coultate, 2002). There might also be other unknown sources for increased fat in faeces, causing a fake

decreased ADC for fat. However, this is not likely since the decrease in fat digestion caused by α-cellulose is supported by literature and previous experiments, as discussed. Also the increased LER in PP diets with α-cellulose was minor compared to PP0 results. A reason for cod tolerating cellulose could be due to that cellulose is similar to chitin (Campbell and Farrel, 2006), they have equal bindings (β-1,4-glycosidic) between monomers, which may be hydrolyzed with similar enzymes. Chitin is a part of the natural diet for wild cod, which feeds on a variety of fish and invertebrates (Link and Almeida, 2000; Orlova *et al.*, 2005), and e.g. crustaceans contain large amounts of carbohydrates, like chitin. It is also speculated that this is why farmed cod show a high tolerance to plant protein (Hansen *et al.*, 2006; Refstie *et al.*, 2006a), in agreement with our trial.

Digestion of protein

Average protein apparent digestibility (85 ±2%) in this study was in accordance with previous cod studies, where protein ADC was reported from 80 to above 90% (Lie *et al.*, 1988; Dos Santos *et al.*, 1993; Hemre *et al.*, 2003; Hansen *et al.*, 2006; Refstie *et al.*, 2006a; Toppe *et al.*, 2006; Hansen *et al.*, 2007b). In our study, there were no significant differences in protein ADC caused by increased α-cellulose. This is in agreement with other fish species, where dietary cellulose inclusions did not affect protein ADC; seabass (Dias *et al.*, 1998), salmon (Aslaksen *et al.*, 2007), tilapia (Amirkolaie *et al.*, 2005) and rainbow trout (Hansen and Storebakken, 2007). In addition, in our trial, there seemed to be no differences between FM and PP diet groups. Other cod studies reported reduction in protein ADC in diets with plant ingredients (Førde-Skjærvik *et al.*, 2006; Refstie *et al.*, 2006a), however these were not the same plant ingredients as used in our trial. Hansen *et al*. (2006) reported highest protein ADC in diets with soybean protein concentrate (SPC) and wheat gluten, which were the same plant ingredients as used in our trial. Except from that Hansen *et al*. (2006) found a higher protein ADC with SPC and wheat gluten than with pure fish meal diets, in our trial there seemed to be no such differences. Furthermore, the reason for no reduction in protein ADC might be due to low amounts of anti nutrients in SPC and wheat gluten, compared to other plant ingredients (Refstie *et al.*, 1998).

Digestion of dry matter

Average dry matter ADC (69.1 ±5.5%) of all diet groups in our trial was lower than previous cod trials ranging from 71 to 84% (Hemre *et al.*, 2003; Toppe *et al.*, 2006). This is likely due

to the high α-cellulose inclusions in our trial, since dry matter ADC decreased with increased α-cellulose, in both FM and PP diets. This is in agreement with Toppe *et al*. (2006), where ADC for dry matter decreased for cod fed increased marine ash inclusions in feed. Further, this was also in agreement with other fish species, where dietary cellulose inclusions have been found to negatively affect organic matter ADC, in rainbow trout (Hansen and Storebakken, 2007) and dry matter and organic matter ADC for tilapia (Amirkolaie *et al.*, 2005). Low ADC for dry matter have been found in cod fed diets with 100% PP (Hansen *et al.*, 2007b), but in our trial cod was fed 50% PP, and PP0 seemed to be similar to FM0. The two regression lines for FM and PP diets are equal, which confirm that α-cellulose, not PP, negatively affected ADC for dry matter.

6.2.3 Digestion of elements

Information still lacks about essential minerals, especially in fish. Anyway, essential minerals described for other animals may also be relevant for fish (Davis and Gatlin, 1996). The problem is that most trace elements are required only in very small amounts and it is difficult to control such small amounts in formulated diets. In experiments with salmon different amounts of yttrium were recollected in faeces depending on collection time (Ward *et al.*, 2005), and greatest concentrations were observed between 6 and 12 hours after last feeding. In our trial, samplings were performed 12 hours after last feeding. Ward *et al*. (2005) found biggest difference in Fe, Mg and Mn, and concluded that faeces should be collected over at least 18 hours after the last feeding for determination of true apparent digestibility (AD) for these minerals. However, our samples were all collected at the same time after last feeding; therefore they are at least comparable.

Some element digestibility results provided negative (below 0%) in our trial, in some or all diet groups; essential: Co, Cu, Fe, Mo, Sn, V, and undesirable: Ba, Cd, Pb, Sr, details in Appendix 4 Table 9.5. This might be due to presence of elements in water, which is in agreement with that fish may absorb elements from water (Lall and Bishop, 1977). Furthermore, experiments with salmon have reported negative AD for some elements (e.g. V, Mo, Mn, Cd, Ba and Fe), depending on sampling time (Ward *et al.*, 2005). Storebakken *et al*.

(1998b) also found that some elements were influenced by water uptake. Water uptake gives digestibility studies uncertainties. However, the diet is the main source of elements in fish (Lall and Bishop, 1977), and element absorption can differ among fish species (Sugiura *et al.*, 1998).

Elements in our trial were measured in feed and faeces, therefore we can not know how they are deposited in the cod (the cods element status), and if there are any differences in element deposition between cod fed PP or FM plus/minus α-cellulose inclusions. This would have been interesting to know, since there were some differences in element concentrations in the different diets, especially between FM and PP diets.

6.2.3 Digestion of essential elements

Selenium (Se), tin (Sn), cobalt (Co) and vanadium (V)

The elements Se and Sn were mostly below LOQ in feed and/or faeces in our trial, therefore ADC could not be calculated. There are no trials on digestibility of Se and Sn in cod, at least not to my knowledge. The Co and V ADC were mostly below 0%, therefore it was hard to test if there were any effects from the increased α-cellulose. Experiments with salmon also reported negative AD for V (Ward *et al.*, 2005), but Co AD above 0%. Se, Sn, Co and V are required in small amounts, which are probably why they were hard to measure.

Copper (Cu), iron (Fe), molybdenum (Mo) and zinc (Zn)

Cu ADC in cod has not been previously reported to my knowledge. However, Cu AD have been reported ranging from 45-50% in salmon (Ward *et al.*, 2005), which is higher than results from our trial. This could be due to different amounts of Cu in diets (Sugiura *et al.*, 1998), since absorption of Cu is thought to be well regulated in fish (Berntssen *et al.*, 2000). In addition availability of Cu depends on the physiological state of the animal and amounts of antagonists (e.g. Zn, Fe, Cd and Mo), competing for binding sites in absorption (Watanbe *et al.*, 1997), but details in fish are little known.

Fe ADC was below 0% in one FM0 replicate; though, average Fe ADC for the rest of the diet groups showed 22 ± 10%. Fe ADC in cod has not previously been examined. Anyhow, Fe ADC for salmon have been reported lower than in our trial (Ward *et al.*, 2005) and higher in trials with coho salmon and rainbow trout (Sugiura *et al.*, 1998). However, our results are in accordance with earlier reported low bioavailability of iron, in salmon, non-haem being lower than haem iron (Andersen *et al.*, 1997; Lorentzen and Maage, 1999).

Mo ADC was below 0% for most diet groups fed FM diets, but Mo ADC for cod fed PP diets was at average 68 ±3%. Perhaps partly due to Mo concentrations in PP diets (0.77 ±0.08mg/kg) being higher than in FM diets (0.14 ±0.07mg/kg). Mo ADC for cod has not been previously studied, at least not to my knowledge. Though, Mo ADC in salmon has been reported from below 0% to 12% (Ward *et al.*, 2005), which is lower than PP diet results in our trial, but in accordance with our FM diet results. Little is known about Mo functions in fish.

The average Zn ADC (43 ±11%) in our trial, was higher than found in earlier cod trials (8-16%) (Førde-Skjærvik *et al.*, 2006). Zn ADC for salmon has been reported within the same range as the cod in our trial, 16-55% (Storebakken *et al.*, 1998b; Ward *et al.*, 2005). Plant ingredients like soybean meal can reduce zinc absorption. Phytic acid in soy concentrate (SC), in addition to lower concentrations of Zn in SC, have been reported to reduce Zn absorption, in salmon diets (Storebakken *et al.*, 2000b; Denstadli *et al.*, 2006). Also a high content of bone minerals (calcium and phosphorous) might inhibit zinc absorption (Watanbe *et al.*, 1997; Lorentzen and Maage, 1999). Therefore adding zinc over the minimum requirement seems to be necessary in fish meal based diets, which was done in our experimental diets. Anyway, in our trial, Zn ADC seemed to be similar among the different diets, meaning that there were probably no considerable anti nutrient activity or nutrient interactions affecting Zn ADC.

The no effects from increased α-cellulose on ADC for Co, Fe, Mo and Zn, in our trial, indicate that α-cellulose did not affect digestibility of these elements.

Manganese (Mn)

Concentrations of Mn in PP diets (48 ±4mg/kg) were higher than in FM diets (26 ±1mg/kg), in accordance with Storebakken *et al*. (2000a) who reported that wheat gluten contained more Mn than fish meal, since our PP diets contained wheat gluten. The difference between FM and PP is also in accordance with some fish meal having low Mn content (Lorentzen *et al.*, 1996).

However, bioavailability of Mn in fish meal is uncertain. In our trial, Mn ADC seemed similar between PP and FM fed cod, and was high (72 ±12%). In accordance with Sugiura *et al*. (1998), who found high apparent availability of Mn in diets with wheat gluten (68%) and in control diets (54%) for rainbow trout. Mn ADC in cod experiments have been found ranging from 19 to 42% in different diets (Førde-Skjærvik *et al.*, 2006), but as in our trial, there seemed to be no difference between Mn digestibility for cod fed plant and fish based diets. Mn ADC and AAC (apparent absorption coefficient) in salmon have been reported between 0 and 20% (Storebakken *et al.*, 2000a; Ward *et al.*, 2005), also lower than in our trial. Absorption of Mn from water is not likely, since the Mn content in seawater is very low (Davis and Gatlin, 1996). Anyway, absorption of Mn is hard to determine, considering that some Mn in faeces is likely to be endogenic Mn from bile (Sugiura *et al.*, 1998), and high dietary calcium and phosphorous can reduce absorption of Mn (Watanbe *et al.*, 1997). Mn ADC increased with increased α-cellulose, for cod fed PP diets, but not FM diets. This could indicate that α-cellulose in combination with PP increases Mn digestion.

6.2.4 Digestion of undesirable elements

Silver (Ag), lead (Pb) and strontium (Sr)

Information about digestibility of undesirable elements for fish is scarce. In our trial, Ag and Pb were mostly below LOQ in feed or/and faeces. The Sr ADC was below 0% in all diet groups. The negative Sr ADC was in accordance with salmon in Storebakken *et al*. (1998b) and coho salmon in Sugiura *et al*. (1998) who found Sr ADC and availability below 0%, and just above 0%. Sr and Ag are not found vital for fish, therefore assumed to be toxic or at least non essential. Pb is less toxic to marine organisms compared to other heavy metals (As, Hg and Cd) and fish contain low amounts of Pb (Lorentzen *et al.*, 2001; NIFES, 2009). In this study Pb was mostly below LOQ in feed, but was above LOQ in most faeces samples, only below LOQ in faeces from cod fed diets with high α-cellulose inclusions. The Pb below LOQ amount in feed and not in faeces could be due to feed being analysed “wet” and faeces freeze dried. The below LOQ amount of Pb in faeces with high α-cellulose indicate that high α-cellulose conceal Pb in faeces. Previous studies have found that absorption of Pb increases when the calcium concentration in feed is low, this could either be confirmed or disconfirmed in our study. However, the α-cellulose inclusions did not seem to stimulate Pb digestion.

Arsenic (As)

ADC for total As was high (82 ±3%). As is found in most marine organisms and toxicity of As depends on its chemical state and valence. In literature As is listed as both a heavy metal and an essential element; in our trial we listed it as an undesirable, since only total As was determined, and we did not know which state the digested As was in. Anyhow, marine organisms mostly contain a stable and less toxic As (arsenobetaine), which is the possible essential As form (Amlund *et al.*, 2006). ADC for total As was not affected by increased α-cellulose in diets. However, the concentration of As in FM diets (3.5 ±0.3mg/kg) seemed to be higher than in PP diets (2.4 ±0.2mg/kg), confirming high levels of As in marine organisms.

Cadmium (Cd)

The Cd ADC was mostly below 0% for cod fed PP diets, but Cd ADC for cod fed FM diets had an average at 20 ±9%. Concentrations of Cd seemed to be higher in FM feed (0.18 ±0.01mg/kg) than in PP feed (0.12 ±0.01mg/kg). This could be the reason for higher Cd ADC for cod fed FM diets than PP diets. Though, this is in contrast to that Cd absorption is found to increase when the dietary concentration is low (Harrison and Jefferson, 1992). Cd ADC for cod fed FM diets were not affected by increased α-cellulose.

Mercury (Hg)

Different Hg forms have different toxicity and metabolism (Lorentzen *et al.*, 2001; Coultate, 2002), however, in this trial only total Hg was measured. Pollution is not always the blame for high Hg levels in seafood; marine predatory fish accumulate Hg as they age (Morel *et al.*, 1998). The concentration of Hg seemed to be higher in FM diets (0.08 ±0.01mg/kg) than PP diets (0.04 ±0.01mg/kg), this is in accordance with Hg being below LOQ in all PP and FM18 faeces samples and just above in the other faeces samples. In addition α-cellulose inclusions probably diluted Hg concentrations in faeces. Hence, Hg ADC for cod fed PP diets and FM18 could not be calculated. Hg ADC for cod fed FM0, FM6 and FM12 diets were not influenced by increased α-cellulose. Anyhow, the high Hg ADC for these FM diets (70 ±4%), were in accordance with that cod is found to readily absorb dietary mercury (Amlund *et al.*, 2007).

Barium (Ba)

Average ADC for Ba was 38 ±18%. Ba is assumed to be undesirable or at least non essential for fish, although reports for this are not found, therefore Ba can not be excluded as an

essential mineral in small amounts. Concentrations of Ba in otoliths are one of more element concentrations used as identification to locate cod nursery areas (Gibb *et al.*, 2007), meaning that Ba is at least known to be absorbed in fish. ADC for Ba increased with increased α-cellulose for cod fed PP diets, but not FM diets. In addition there were higher concentrations of Ba in PP diets (3.3 ±0.1mg/kg) than in FM diets (1.1 ±0.1mg/kg). This could be the reason for the noticeable effects on Ba ADC for cod fed PP diets. Whether the increased digestion of Ba was favourable or not is unknown, since requirements of Ba is unknown.

6.2.5 Faeces composition

Fat, protein (total nitrogen) and elements in faeces were measured as percents, which mean that increased α-cellulose will influence the percent of these in faeces. The nutrients (fat and protein) varied in diets dependent on α-cellulose inclusion, therefore only water content in faeces is discussed here, due to its relevance in describing diarrhoea like conditions, the other nutrients (protein and fat) are discussed together with ADC results.

Dry matter in faeces increased with increased α-cellulose for cod fed FM and PP diets. The two regression lines were not different. Thus the increase in dry matter came most likely from α-cellulose inclusions, and further indicating that α-cellulose was not utilized. Faeces colour after freeze drying was darker in faeces from FM0, FM6 and PP0 than all other diets, this also indicates that there was more α-cellulose in faeces from α-cellulose diets, since α-cellulose is white. In addition, the increased dry matter in faeces was in accordance with decreased ADC for dry matter. There have been observed lower dry matter in faeces for cod fed soybean products (Førde-Skjærvik *et al.*, 2006; Olsen *et al.*, 2007). Olsen *et al*. (2007) observed 10.8% dry matter in faeces for cod fed 100% plant as protein in diets, which indicated diarrhoea. SPC and wheat gluten were used in our trial too, but not soya bean meal, and we only used 50% PP as protein. The lowest amount of dry matter (14.1%) observed in our trial was in one FM0 replicate, a fish meal control group without α-cellulose. Therefore, due to high dry matter in our trial, the cod did not have diarrhoea. Although, since the α-cellulose inclusions were probably the source for the higher dry matter percent, it might have concealed a

diarrhoea like condition in those. However, no histological changes (Olsen, RE, 2009, pers.com.) confirm that there were no diarrhoea diseases. There will be more faecal waste from cod fed large inclusions of α-cellulose, which may cause local sedimentation, and might not be environmentally friendly. Smaller inclusions of α-cellulose probably do not have considerable effects. Anyway, to fully evaluate cellulose usage in feed, aspects about solids in aquaculture effluent water would need further investigation.

Soybean meal did not affect viscosity of cod digesta (Førde-Skjærvik *et al.*, 2006), so viscosity could not explain different digestibility between diets, in agreement with salmon (Refstie *et al.*, 1999; Aslaksen *et al.*, 2007). Refstie *et al*. (1999) reported that soybean with high dietary fibre content increased water in intestinal chime of salmon, without affecting viscosity, like in broiler chickens. However, viscosity depends on type and amount of NSP (Jozefiak *et al.*, 2004), with soluble fibre giving higher viscosity than insoluble. No measurements of viscosity were performed in our study, but the water content of undigested matter decreased as α-cellulose increased. This indicates that at least there were no increased water in the intestines of the cod in our trial, and probably not increased viscosity either.

6.2.6 Fish health

Haematological parameters

Haematocrit (Hct) and haemoglobin (Hb) were within previous reported values for cod (Lie *et al.*, 1990; Hemre *et al.*, 2002; Rosenlund *et al.*, 2004; Olsen *et al.*, 2007). Red Blood Cell count (RBC) for most of the diet groups (1.51-1.79 10^{12}/L) ranged within previous reported values for cod. Although RBC in one FM6 replicate, one FM18 replicate and one PP0 replicate (2.05-2.36 10^{12}/L) were somewhat above earlier published values, however, this was probably negligible. Mean cell haemoglobin concentrations (MCHC) were within previous reported values. Mean cell volumes (MCV) were lower than previously reported values for cod in one FM6 replicate, one FM18 replicate and one PP0 replicate (119-138 10^{-15}L), all other dietary groups (149-194 10^{-15}L) ranged within previous reported values. Olsen *et al*. (2007) found significant reduction of MCV as plant protein increased. The low MCV that Olsen *et al*. (2007) found was similar to MCV in our trial. However, the reason for the low

MCV in this trial seemed to have no correlation with plant protein or α-cellulose. Mean cell haemoglobin (MCH) was in the lower range of previous reported values. None haematological values were significantly influenced by increasing α-cellulose. Furthermore, there were no significant difference between control diets and experimental diets, suggesting that the cod tolerated α-cellulose inclusions, in both FM and PP based diets. Haematological parameters in our trial indicate that the cod had good health, especially in combination with the good growth and almost no mortality.

Clinical and nutritional parameters

Aspartate aminotransferase (ASAT) and Alanine aminotransferase (ALAT) were both low, and within previous reported ranges for cod (Rosenlund *et al.*, 2004; Olsen *et al.*, 2007). Low ASAT and ALAT indicate that there were no organ damage, because ASAT and ALAT normally occur in low levels in blood plasma (Racicot *et al.*, 1975; Sandnes *et al.*, 1988) and determination of these enzymes, in high amounts in blood plasma, is efficient in diagnosis of liver and kidney diseases in fish. Plasma glucose was within previous reported normal cod ranges (Hemre *et al.*, 2002; Rosenlund *et al.*, 2004). Total protein was somewhat low in one FM18 replicate (23.9g/L), although, probably not of importance, as the rest of the dietary groups (28.6-40.1g/L) were similar to previous reported total protein in cod plasma. Anyhow, total protein in blood plasma increases when the fish is dehydrated, but low total protein is a less specific diagnosis of a disease (Sandnes *et al.*, 1986). None of the clinical and nutritional parameters were significantly influenced by increasing α-cellulose. In addition to the normal haematological values, this further suggests that cod were in good health in our feeding trial.

6.2.7 Whole body and liver composition

Whole body

Whole body composition of fat, protein, dry matter and ash were measured three weeks before and at the end of our feeding trial, and were within ranges from earlier findings in cod (Lie *et al.*, 1988; Hemre *et al.*, 2002; Rosenlund *et al.*, 2004; Toppe *et al.*, 2006). Whole body composition have been reported being affected by diets (Lie *et al.*, 1988; Rosenlund *et al.*, 2004), but then due to different protein and fat ratios, which was similar in all our diets. Increased fat in cod diets have previously shown to increase whole body fat (Lie *et al.*, 1988),

mainly due to increased liver size and fat content of liver. PP in cod feed is reported to not affect whole body composition of macronutrients (Hansen *et al.*, 2007a; Hansen *et al.*, 2007b), in agreement with our trial. Cod diets with 100% PP have shown to decrease dry matter in whole body (Hansen *et al.*, 2007b), however our trial used 50% PP. Increased protein in whole body have been observed with increased protein in cod diets (Lie *et al.*, 1988). In our trial, protein in whole body (5.3 ±0.5%) decreased slightly with increased α-cellulose, for cod fed PP diets, not FM diets. Anyway, this was probably insignificant, since protein utilization was not affected by α-cellulose, for either FM or PP based diets. Fat, glycogen and dry matter in whole body were not influenced by increased α-cellulose. Ash in whole body (2.3 ±0.3%) tended to decrease with increased α-cellulose for cod fed PP diets, but not FM diets. Tilapia fed up to 12% cellulose in diets also had slightly reduced protein content in whole body with increasing cellulose (Al-Ogaily, 1996), but they also had decreased fat content, similar to red drum fed 20% cellulose (Turano *et al.*, 2002) and trout fed 40-50% cellulose (Bromley and Adkins, 1984). Cellulose inclusions up to 20% in European seabass feed did not affect whole body composition (Dias *et al.*, 1998). Our results combined with other fish species indicate that cellulose inclusions have no remarkable effects on whole body composition of macronutrients, at least not in low amounts.

Liver

Liver composition of protein (4.2 ±0.6%), lipid (67.8 ±1.3%), glycogen (36 ±6mg/g) and dry matter (74.5 ±1.0%) were in accordance with earlier findings in cod (Lie *et al.*, 1988; Rosenlund *et al.*, 2004; Hansen *et al.*, 2007a). Although, liver fat levels in our trial were higher when compared to Morais *et al*. (2001). Lipid liver content is known to increase with increasing fat in the diet (Lie *et al.*, 1986; Jobling *et al.*, 1991; Jobling *et al.*, 1994; Morais *et al.*, 2001; Rosenlund *et al.*, 2004; Karlsen *et al.*, 2006). However, the ratios of dietary digestible macronutrients (protein/lipids/carbohydrates) were equal in our trial, which is probably the reason for no differences in the liver composition. Increased corn gluten in cod feed increased liver glycogen (Hansen *et al.*, 2007a), probably due to increased starch. Though, this plant ingredient was not used in our trial. Cod fed PP diets seemed to have equal liver macro composition as FM fed cod. In addition, increased dietary α-cellulose had no influence on macronutrient composition of the liver.

7 Conclusion

All cod showed good growth. Weight gain, total weight, SGR and CF were not influenced by increased α-cellulose in either PP or FM based diets. Cod fed PP0 had similar growth and feed utilization as cod fed FM0, which confirms that 50% PP plus 50% FM as dietary protein source is equally well utilized as 100% FM based diets, in agreement with earlier results (Hansen *et al.*, 2006; 2007a).

Feed intake seemed to be increased by α-cellulose. Cod fed PP based diets plus α-cellulose probably needed more time to adjust to the feed, which might have induced a compensatory growth, making the cod catch up with its "normal growth rate". The similar energy intake, even though the diets had different energy concentrations, and the dietary digestible macro nutrients (protein, fat and carbohydrates) and PE showing similar ratios, indicate that the cod adjusted feed intake in accordance to both energy and protein amount.

Liver index, gutted weight and fillet weight were not affected by increased α-cellulose. Protein utilization was not influenced by increased α-cellulose. Digestibility of fat decreased with increased α-cellulose, equal in both FM and PP diet groups. In disagreement with the increase in LER with increased α-cellulose, however, LPV did not vary between diet groups. Digestibility of protein was not affected by increased α-cellulose. Digestibility of dry matter decreased with increased α-cellulose, in accordance with increased dry matter in faeces.

Some of the element digestibility results were negative in our trial, in some or all diet groups, both essential: Co, Cu, Fe, Mo, Sn and V, and undesirable elements: Ba, Cd, Pb and Sr. Digestibility of the essential elements Co, Fe, Mo and Zn and the undesirable elements Pb, total As, Cd and Hg were not influenced by increased α-cellulose. Mn and Ba ADC increased with increased α-cellulose, for cod fed PP diets, not FM diets. Concentrations of Mo, Mn, V and Ba seemed to be higher in PP than in FM diets. Concentrations of Sr, As, Cd and Hg seemed to be higher in FM than in PP diets. Pb and Ag were below LOQ in feed.

Cod health was good, based on haematology, clinical and nutritional blood parameters, which all ranged within previous reported values for healthy cod. Good health was further confirmed by the almost no mortality and no diarrheic conditions. Composition of macronutrients in whole body and liver had no remarkable variation from the increased dietary α-cellulose.

Our study reports that it seemed hard to manipulate energy deposition in cod by diluting diets with cellulose, and cod fed 18% α-cellulose had more faecal waste, which may be a local environmental challenge. Our results also show that cod tolerated up to 18% α-cellulose inclusions, both in combination with FM and PP based diets. In addition the cod compensated by higher feed intake to satisfy its need for energy and protein for optimal growth.

8 References

Agbayani, E. (2001) Atlantic cod (*Gadus morhua*), Fishbase: http://www.fishbase.org/Summary/speciesSummary.php?ID=69&genusname=Gadus&speciesname=morhua, still active in April 2009.

Al-Ogaily, S.M. (1996) Effect of feeding different levels of cellulose on the growth performance and body composition of *Oreochromis niloticus*. *Arab Gulf Journal of Scientific Research,* **14**, 731-745.

Albrektsen, S., Mundheim, H. & Aksnes, A. (2006) Growth, feed efficiency, digestibility and nutrient distribution in Atlantic cod (*Gadus morhua*) fed two different fish meal qualities at three dietary levels of vegetable protein sources. *Aquaculture,* **261**, 626-640.

Amirkolaie, A.K., Leenhouwers, J.I., Verreth, J.A.J. & Schrama, J.W. (2005) Type of dietary fibre (soluble versus insoluble) influences digestion, faeces characteristics and faecal waste production in Nile tilapia (*Oreochromis niloticus* L.). *Aquaculture Research,* **36**, 1157-1166.

Amlund, H., Ingebrigtsen, K., Hylland, K., Ruus, A., Eriksen, D.Ø. & Berntssen, M.H.G. (2006) Disposition of arsenobetaine in two marine fish species following administration of a single oral dose of [^{14}C]arsenobetaine. *Comparative Biochemistry and Physiology, Part C,* **143**, 171-148.

Amlund, H., Lundebye, A.K. & Berntssen, M.H.G. (2007) Accumulation and elimination of methylmercury in Atlantic cod (*Gadus morhua* L.) following dietary exposure. *Aquatic Toxicology,* **83**, 323-330.

Andersen, F., Lorentzen, M., Waagbø, R. & Maage, A. (1997) Bioavailability and interactions with other micronutrients of three dietary iron sources in Atlantic salmon, *Salmo salar*, smolts. *Aquaculture Nutrition,* **3**, 239-246.

Aslaksen, M.A., Kraugerud, O.F., Penn, M., Svihus, B., Denstadli, V., Jørgensen, H.Y., Hillestad, M., Krogdahl, Å. & Storebakken, T. (2007) Screening of nutrient digestibilities and intestinal pathologies in Atlantic salmon, *Salmo salar*, fed diets with legumes, oilseeds, or cereals. *Aquaculture,* **272**, 541-555.

Baldisseretto, B., Chowdhury, M.J. & Wood, C.M. (2005) Effects of dietary calcium and cadmium on cadmium accumulation, calcium and cadmium uptake from the water, and their interactions in juvenile rainbow trout. *Aquatic Toxicology,* **72**, 99-117.

Berntssen, M.H.G., Lundebye, A.K. & Hamre, K. (2000) Tissue lipid peroxidative response in Atlantic salmon (*Salmo salar* L.) parr fed high levels of dietary copper and cadmium. *Fish Physiology and Biochemistry,* **23**, 35-48.

Bishop, C. & Odense, P.H. (1966) Morphology of the digestive tract of the cod, *Gadus morhua*. *FIsheries Research Board of Canada,* **23**, 1607-1630.

Björnsson, B. & Steinarsson, A. (2002) The food-unlimitied growth rate of Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences,* **59**, 494-502.

Bromley, P.J. & Adkins, T.C. (1984) The influence of cellulose filler on feeding, growth and utilization of protein and energy in rainbow trout, *Salmo gairdnerii* Richardson. *Journal of Fish Biology,* **24**, 235-244.

Braaten, B. (1984) Growth of cod in relation to fish size and ration level In *The Propagation of cod Gadus morhua L. Fløde*vigen *rapportserie, 1* (Dahl, E., *et al.* eds.), pp. 677-710. Institute of Marine Research, Austevoll, Norway.

Buddington, R.K., Krogdahl, Å. & Bakke-McKellep, A.M. (1997) The intestines of carnivorous fish: structure and functions and the relations with diet. *Acta Physiologica Scandinavica,* **161**, 67-80.

Burkitt, D.P. (1979) *Don't forget fibre in your diet,* Maritin Dunitz Limited, pp. 119, London, UK.

Campbell, M.K. & Farrel, S.O. (2006) Carbohydrates In *Biochemistry* (Campbell, J. ed.), 5 ed, pp. 435-462. Physical Sciences, Belmont, USA.

Coultate, T.P. (2002) *Food, the chemistry of its components,* 4 ed, The Royal Society of Chemistry, pp. 432, Cambridge, UK.

Das, A.K. (2000) Trace metal status in marine biological samples: a review. *International Journal of Environment and Pollution,* **13**, 208-225.

Davis, D.A. & Gatlin, D.M. (1996) Dietary mineral requirement of fish and marine crustaceans. *Reviews in Fisheries Science,* **4**, 75-99.

Denstadli, V., Skrede, A., Krogdahl, Å., Sahlstrøm, S. & Storebakken, T. (2006) Feed intake, growth, feed conversion, digestibility, enzyme activities and intestinal structure in Atlantic salmon (*Salmo salar* L.) fed graded levels of phytic acid. *Aquaculture,* **256**, 365-376.

Dersjant-Li, Y. (2002) The use of soy protein in aquafeeds In *Avances en Nutricon Acuicola VI. Memorias del VI Simposium Internacional de Nutricon Acuicola* (Cruz-Suarez, L.E., *et al.* eds.), pp. 541-558 Quintana Roo, Mexico.

Dias, J., Huelvan, C., Dinis, M.T. & Mètailler, R. (1998) Influence of dietary bulk agents (silica, cellulose and a natural zeolite) on protein digestibility, growth, feed intake and feed transit time in European seabass (*Dicentrarchus labrax*) juveniles. *Aquatic Living Resources,* **11**, 219-226.

Dioundick, O.B. & Stom, D.I. (1990) Effects of dietary α-cellulose levels on the juvenile tilapia, *Oreochromis mossambicus* (Peters). *Aquaculture,* **91**, 311-315.

Dos Santos, J., Burkow, I.C. & Jobling, M. (1993) Patterns of growth and lipid deposition in cod (*Gadus morhua* L.) fed natural prey and fish-based feeds. *Aquaculture,* **110**, 173-189.

Einen, O. (2001) Vekst og fôrutnyttelse In *Fiskeernæring* (Waagbø, R., *et al.* eds.), pp. 205-217. Kystnæringen Forlag og Bokklubb AS, Bergen, Norway.

FAO (2006) Food and agriculture organization of the United Nations, State of world aquaculture In *FAO Fisheries Technical Paper,* Vol. 500, pp. 134. FAO Fisheries Department, Rome, Italy.

Francis, G., Makkar, H.P.S. & Becker, K. (2001) Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture,* **199**, 197-227.

Førde-Skjærvik, O., Refstie, S., Aslaksen, M.A. & Skrede, A. (2006) Digestibility of diets containing different soybean meals in Atlantic cod (*Gadus morhua*); comparison of collection methods and mapping of digestibility in different sections of the gastrointestinal tract. *Aquaculture,* **261**, 241-258.

Gibb, F.M., Gibb, I.M. & Wright, P.J. (2007) Isolation of Atlantic cod (*Gadus morhua*) nursey areas. *Marine Biology,* **151**, 1185-1194.

Gildberg, A. (2004) Digestive enzyme activities in starved pre-slaughter farmed and wild-captured, Atlantic cod (*Gadus morhua*). *Aquaculture,* **238**, 343-353.

Gjerde, B., Terjesen, B.F., Barr, Y., Lein, I. & Thorland, I. (2004) Genetic variation for juvenile growth and survival in Atlantic cod (*Gadus morhua*). *Aquaculture,* **236**, 167-177.

Grant, S.M., Brown, J.A. & Boyce, D.L. (1998) Enlarged fatty livers of small juvenile cod: a comparison of laboratory-cultured and wild juveniles. *Journal of Fish Biology,* **52**, 1105-1114.

Hansen, A.-C., Rosenlund, G., Karlsen, Ø., Olsvik, P. & Hemre, G.-I. (2006) The inclusion of plant proteins in cod diets, its effects on macronutrient digestibility, gut and liver histology and heat shock protein transcription. *Aquaculture Research,* **37**, 773-784.

Hansen, A.-C., Rosenlund, G., Karlsen, Ø., Rimbach, M. & Hemre, G.-I. (2007a) Dietary plant-protein utilisation in Atlantic cod, *Gadus morhua*. *Aquaculture Nutrition,* **13**, 200-215.

Hansen, A.C., Rosenlund, G., Karlsen, O., Koppe, W. & Hemre, G.I. (2007b) Total replacement of fish meal with plant proteins in diets for Atlantic cod (*Gadus morhua* L.) I - Effects on growth and protein retention. *Aquaculture,* **272**, 599-611.

Hansen, G.A. & Kjerstad, M. (2008) Status for utnyttelse av restråstoff fra torskeoppdrett In *Rapport nr 163. Stiftelsen Rubin,* pp. 57. Møreforsking, Ålesund, Norway.

Hansen, J.Ø. & Storebakken, T. (2007) Effects of dietary cellulose level on pellet quality and nutrient digestibilities in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture,* **272**, 458-465.

Hansen, T., Karlsen, Ø., Tananger, G.L., Hemre, G.I., Holm, J.C. & Kjesbu, O.S. (2001) Growth, gonadal develpment and spawning time of Atlantic cod (*Gadus morhua*) reard under different photoperiods. *Aquaculture,* **203**, 51-67.

Harrison, S.E. & Jefferson, P.C. (1992) Comparative accumulation efficiency of ^{109}cadmium from natural food (Hyalella azteca) and artificial diet by rainbow trout (*Oncorhynchus mykiss*). *Bulletin of Environmental Contamination and Toxicology,* **49**, 757-764.

Hemre, G.I. (2001) Karbohydrat i fiskeernæring In *Fiskeernæring* (Waagbø, R., *et al.* eds.), Vol. 1, pp. 77-91. Kystnæringen, Forlag & Bokklubb AS, Bergen, Norway.

Hemre, G.I., Karlsen, Ø., Eckhoff, K., Tveit, K., Mangor-Jensen, A. & Rosenlund, G. (2004) Effect of season, light regime and diet on muscle composition and selected quality parameters in farmed Atlantic cod, *Gadus morhua*. *Aquaculture Research,* **35**, 683-697.

Hemre, G.I., Karlsen, Ø., Mangor-Jensen, A. & Rosenlund, G. (2003) Digestibility of dry matter, protein, starch and lipid by cod, *Gadus morhua*: comparison of sampling methods. *Aquaculture,* **225**, 225-232.

Hemre, G.I., Lie, Ø., Lambertsen, G. & Sundby, A. (1990) Dietary carbohydrate utilization in cod (*Gadus morhua*). Hormonal response of insulin, glucagon and glucagon-like-peptide to diet and starvation In *Department of Physiology and Nutrition*. University of Bergen, Norway.

Hemre, G.I., Lie, Ø., Lied, E. & Lambertsen, G. (1989) Starch as an energy source in feed for cod (*Gadus morhua*): digestibility and retention. *Aquaculture,* **80**, 261-270.

Hemre, G.I., Lie, Ø. & Sundby, A. (1992) Dietary carbohydrate utilization in cod (*Gadus morhua*): metabolic response to feeding and fasting. *Fish Physiology and Biochemistry,* **10**, 455-463.

Hemre, G.I. & Sandnes, K. (1999) Effect of dietary lipid level on muscle composition in Atlantic salmon *Salmo salar*. *Aquaculture Nutrition,* **5**, 9-16.

Hemre, G.I., Sandnes, K. & Lie, Ø. (1995) Carbohydrate nutrition in Atlantic salmon, *Salmo salar* L.: growth and feed utilization. *Aquaculture Research,* **26**, 149-154.

Hemre, G.I., Taranger, G.L. & Hansen, T. (2002) Gonadal development influences nutrient utilisation in cod (*Gadus morhua*). *Aquaculture,* **214**, 201-209.

Hilton, J.W. (1989) The interaction of vitamins, minerals and diet composition in the diet of fish. *Aquaculture,* **79**, 223-244.

Hilton, J.W., Atkinson, J.L. & Slinger, S.J. (1983) Effect of increased dietary fiber on the growth of rainbow trout (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Sciences,* **40**, 81-85.

Holm, J., Björck, I., Drews, A. & Asp, N.G. (1986) A rapid method for the analysis of starch. *Starch,* **38**, 224-226.

Howell, B.R. (1984) The intentsive rearing of juvenile cod, *Gadus morhua* L In *The Propagation of cod Gadus morhua L. Flødevigen rapportserie, 1* (Dahl, E., *et al.* eds.), pp. 657-675. Ministry of Agriculture, Fisheries and Food Directorate of Fisheries Research, Lowestoft, UK.

Jobling, M. (1988) A review of the physiological and nutritional energetics of cod, *Gadus morhua* L., with particular reference to growth under farmed conditions. *Aquaculture,* **70**, 1-19.

Jobling, M. (1992) Energiomsetning og vekst In *Fiskens fysiologi* (Døving, K. & Reimers, E. eds.), pp. 258-276. John Grieg Forlag, Stavanger, Norway.

Jobling, M. & Hjelmeland, K. (1992) Ernæring og metabolisme In *Fiskens fysiologi* (Døving, K. & Reimers, E. eds.), pp. 233-257. John Grieg Forlag, Stavanger, Norway.

Jobling, M., Knudsen, R., Pedersen, P.S. & Dos-Santos, J. (1991) Effects of dietary-composition and energy content on the nutritional energetics of cod, *Gadus morhua*. *Aquaculture,* **92**, 243-257.

Jobling, M., Meløy, O.H., dos Santos, J. & Christiansen, B. (1994) The compensatory growth response of the Atlantic cod: effect of nutritional histiory. *Aquaculture International,* **2**, 75-90.

Jorgensen, H., Zhao, X.Q., Knudsen, K.E.B. & Eggum, B.O. (1996) The influence of dietary fibre source and level on the development of the gastrointestinal tract, digestibility and energy metabolism in broiler chickens. *British Journal of Nutrition,* **75**, 379-395.

Jozefiak, D., Rutkowski, A., Fratczak, M. & Boros, D. (2004) The effect of dietary fibre fractions from different cereals and microbial enzyme supplementation on performance, ileal viscosity and short-chain fatty acid concentrations in the caeca of broiler chickens. *Journal of Animal and Feed Sciences,* **13**, 487-496.

Julshamn, K., Lea, P., Lindeberg, J., Lund, L., Meyland, I., Nilsson, A., Sòlbergsdòttir, E.J., Thorlacius, A. & Wallin, H. (2005) Validation of chemical analytical methods, pp. 30. Nordic Committee on Food Analysis, NMKL.

Julshamn, K., Lunestad, B.T., Haldorsen, A.K., Berntssen, M.H.G. & Waagbø, R. (2003) Overvåkningsprogram for fiskefôr In *Årsrapport 2002*, pp. 24. Nasjonalt Institutt for Ernærings og Sjømatforskning, Bergen, Norway.

Julshamn, K., Maage, A., Norli, S., H., Grobecker, K., H., Jorhem, L. & Fecher, P. (2007) Determination of arsenic, cadmium, mercury and lead by inductively coupled plasma/mass spectrometry in food after pressure digestion: NMKL interlaboratory study. *Journal of AOAC International,* **90**, 844-856.

Karlsen, Ø., Hemre, G.-I., Magnor-Jensen, A., Tveit, K. & Rosenlund, G. (2006) Effect of varying levels of macro-nutrients and continuous light on growth, energy deposits and maturation in farmed Atlantic cod (*Gadus morhua* L.). *Aquaculture*, **255**, 242-254.

Karlsen, Ø., Holm, J.C. & Kjesbu, O.S. (1994) Effects of periodic starvation on reproductive investment in first-time spawning Atlantic cod (*Gadus morhua* L.). *Aquaculture*, **133**, 159-170.

Krogdahl, A., Hemre, G.-I. & Mommsen, T.P. (2005) Carbohydrates in fish nutrition: digestion and absorption in postlarval stages. *Aquaculture Nutrition,* **11**, 103-122.

Krogdahl, Å., Bakke-McKellep, A.M. & Baeverfjord, G. (2003) Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar* L.). *Aquaculture Nutrition*, **9**, 361-371.

Kryvi, H. & Totland, G.K. (1997) *Fiskeanatomi*, Høyskoleforlaget AS, Norwegian Academic Press, Kristiansand, Norway.

Lall, S.P. & Bishop, F.J. (1977) Studies on mineral and protein utilization by Atlantic salmon (*Salmo salar*) grown in seawater In *Technical Report Fisheries and Marine Service*, Vol. 688, pp. 16. Fisheries and Marine Service, Halifax, Canada.

Langaard, L., Jensen, N. & Esmark, M. (2008) Management and technical measures in the norwegian cod and groundfish fisheries, pp. 28. World Wildlife Foundation, Norway.

Lie, Ø. (1991) Studies on digestion, deposition and fatty acid composition of lipids in cod (*Gadus morhua*) In *Department of Fisheries and Marine Biology*, Doctor philosophiae, pp. 53. University of Bergen, Bergen.

Lie, Ø., Lied, E. & Lambertsen, G. (1986) Liver retention of fat and of fatty acids in cod (*Gadus morhua*) fed different oils. *Aquaculture*, **59**, 187-196.

Lie, Ø., Lied, E. & Lambertsen, G. (1988) Feed optimization in atlantic cod (*Gadus morhua*): Fat versus protein content in the feed. *Aquaculture*, **69**, 333-341.

Lie, Ø., Lied, E. & Lambertsen, G. (1990) Haematological values in cod (*Gadus morhua*). *Fiskeridirektoratets Særskrifter, Særskrift i Ernæring*, **3**, 11-17.

Lied, E., Julshamn, K. & Braekkan, O.R. (1982) Determination of protein digestibility in Atlantic cod (*Gadus morhua*) with internal and external indicators. *Canadian Journal of Fisheries and Aquatic Sciences*, **39**, 854-861.

Lied, E. & Solbakken, R. (1984) The course of protein digestion in Atlantic cod (*Gadus morhua*). *Comparative Biochemistry and Physiology*, **77A**, 503-506.

Link, J.S. & Almeida, F.P. (2000) An overview and history of the food web dynamics program of the northeast fisheries science center In *NOOA (National Oceanic and Atmospheric Administration) Technical Memorandum NMFS-NE-159*, pp. 60. National Marine Fisheries Service, Massachusetts, USA.

Lorentzen, M., Berntssen, M.H.G. & Måge, Å. (2001) Mineraler og sporelementer In *Fiskeernæring* (Waagbø, R., *et al.* eds.), pp. 125-139. Kystnæringen Forlag og Bokklubb AS, Bergen, Norway.

Lorentzen, M. & Maage, A. (1999) Trace element status of juvenile Atlantic salmon *Salmo salar* L. fed a fish-meal based diet with or without supplementation of zinc, iron, manganese and copper from first feeding. *Aquaculture Nutrition*, **5**, 163-171.

Lorentzen, M., Maage, A. & Julshamn, K. (1996) Manganese supplementation of a practical, fish meal based diet for Atlantic salmon parr. *Aquaculture Nutrition*, **2**, 121-125.

Mohanna, C., Carre, B. & Nys, Y. (1999) Incidence of dietary viscosity on growth performance and zinc and manganese bioavailability in broilers. *Animal Feed Science and Technology*, **77**, 255-266.

Morais, S., Bell, J.G., Robertson, D.A., Roy, W.J. & Morris, P.C. (2001) Protein/lipid ratios in extruded diets for Atlantic cod (*Gadus morhua* L.): effects on growth, feed utilisation, muscle composition and liver histology. *Aquaculture*, **203**, 101-119.

Morel, F.M.M., Kraepiel, A.M.L. & Amyot, M. (1998) The chemical cycle and bioaccumulation of mercury. *Annual Review of Ecology and Systematics*, **29**, 543-566.

Murat, J.C. & Serfaty, A. (1974) Simple enzymatic determination of polysaccharide (glycogen) content of animal tissues. *Clinical Chemistry*, **20**, 1576-1577.

Mørkøre, T. (2005) Produktkvalitet In *Oppdrett av torsk - næring med framtid* (Otterå, H., *et al.* eds.), pp. 153-162. Norsk Fiskeoppdrett AS, Bergen, Norway.

NIFES (2009) Sjømatdata: http://www.nifes.no/index.php?page_id=165, still active in April 2009. Nasjonalt Institutt for Ernærings og Sjømatforskning, Bergen, Norway.

Nortvedt, R. & Krogdahl, Å. (2001) Metoder for ernæringsstudier hos fisk In *Fiskeernæring* (Waagbø, R., *et al.* eds.), pp. 187-203. Kystnæringen Forlag og Bokklubb AS, Bergen, Norway.

NRC (1993) *Nutrient requirement of fish,* National Academy Press (Wilson, R.P. ed.), Washington D.C., USA.

Olsen, R.E., Hansen, A.-C., Myklebust, R., Mayhew, T.W., Rosenlund, G., Eroldoğan, O.T., Hemre, G.I. & Karlsen, Ø. (2007) Effects of total replacement of fish meal with plant proteins in diets for Atlantic cod (*Gadus morhua* L.) – Health aspects. *Aquaculture,* **272**, 612-624.

Orlova, E., Dolgov, A., Belikov, S. & Johannesen, E. (2005) Year-to-year dynamics of trophic links of the main commercial fishes in the barents sea as indicating the state of ecosystem In *Joint Russian-Norwegian Symposium Conference (11th) - Ecosystem dynamics and optimal long-term harvest in the barents sea fisheries*, pp. 25. Polar Research Institute of Marine Fisheries and Oceanography (PINRO) & Institute of Marine Research (IMR), Murmansk, Russia.

Racicot, J.G., Gaudet, M. & Leray, C. (1975) Blood and liver enzymes in rainbow trout (*Salmo gairdneri* Rich.) with emphasis on their diagnostic use: Study of CCl_4 toxicity and a case of *Aeromonas* infection. *Journal of Fish Biology,* **7**, 825-835.

Ratte, H.T. (1999) Bioaccumulation and toxicity of silver compounds: A review. *Environmental Toxicology and Chemistry,* **18**, 89-108.

Razdan, A., Pettersson, D. & Pettersson, J. (1997) Broiler chicken body weights, feed intakes, plasma lipid and small-intestinal bile acid concentrations in response to feeding of chitosan and pectin *British Journal of Nutrition,* **78**, 283-291.

Refstie, S., Førde-Skjærvik, O., Rosenlund, G. & Rørvik, K.-A. (2006a) Feed intake, growth, and utilisation of macronutrients and amino acids by 1- and 2-year old Atlantic cod (*Gadus morhua*) fed standard or bioprossesed soybean meal. *Aquaculture,* **255**, 279.

Refstie, S., Landsverk, T., Bakke-McKellep, A.M., Ringø, E., Sundby, A., Shearer, K.D. & Krogdahl, Å. (2006b) Digestive capacity, intestinal morphology, and microflora of 1-year and 2-year old Atlantic cod (*Gadus morhua*) fed standard or bioprocessed soybean meal. *Aquaculture,* **261**, 269-284.

Refstie, S., Storebakken, T. & Roem, A.J. (1998) Feed composition and conversion in Atlantic salmon (*Salmo salar*) fed diets with fish meal, extracted soybean meal or soybean meal with reduced content of oligosaccharides, trypsin inhibitors, lectins and soya antigens. *Aquaculture,* **162**, 301-312.

Refstie, S., Svihus, B., Shearer, K.D. & Storebakken, T. (1999) Nutrient digestibility in Atlantic salmon and broiler chickens related to viscosity and non-starch polysaccharide content in different soybean products. *Animal Feed Science Technology*, **79**, 331-345.

Refstie, T. (2009) Which traits are inherited in cod and when will we see the effect of the breeding programs? (Akvaforsk Genetic Center AS) In *Go for cod conference*. Norsk Sjømatsenter and NCE Aquaculture, Bergen, Norway.

Rosenlund, G., Karlsen, Ø., Tveit, K., Mangor-Jensen, A. & Hemre, G.-I. (2004) Effect of feed composition and feeding frequency on growth, feed utilization and nutrient retention in juvenile Atlantic cod, *Gadus morhua* L. *Aquaculture Nutrition*, **10**, 371-378.

Sagstad, A., Sanden, M., Krogdahl, Å., Bakke-McKellep, A.M., Frøystad, M. & Hemre, G.I. (2008) Organs development, gene expression and health of Atlantic salmon (*Salmo salar* L.) fed genetically modified soybeans compared to the near-isogenic non-modified parental line. *Aquaculture Nutrition*, **14**, 556-572.

Saki, A.A. & Alipana, A. (2005) Effect of Dietary Wheat Screening Diet on Broiler Performance, Intestinal Viscosity and Ileal Protein Digestibility. *International Journal of Poultry Science*, **4**, 16-20.

Sandnes, K., Lie, Ø. & Waagbø, R. (1986) Blodanalyser - et viktig hjelpemiddel i vurderingen av fiskens helse. *Norsk fiskeoppdrett*, **10**, 48-49.

Sandnes, K., Lie, Ø. & Waagbø, R. (1988) Normal ranges of some blood chemistry parameters in adult farmed Atlantic salmon, *Salmo salar*. *Journal of Fish Biology*, **32**, 129-136.

Seppola, M., Olsen, R.E., Sandaker, E., Kanapathippillai, P., Holzapfel, W. & Ringø, E. (2006) Random amplification of polymorphic DNA (RAPD) typing of carnobacteria isolated from hindgut chamber and large intestine of Atlantic cod (*Gadus morhua* L.). *Systematic and Applied Microbiology*, **29**, 131-137.

Shearer, K.D. (2000) Experimental design, statistical analysis and modelling of dietary nutrient requirement studies for fish: a critical view. *Aquaculture Nutrition*, **6**, 91-102.

Smits, C.H.M., Moughan, P.J. & Beynen, A.C. (2000) The inhibitory effect of a highly viscous carboxymethylcellulose on dietary fat digestibility in the growing chicken is dependent on the type of fat. *Journal of Animal Physiology and Animal Nutrition*, **83**, 231-238.

Storebakken, T., Shearer, K.D., Baeverfjord, G., Nielsen, B.G., Åsgård, T., Scott, T. & De Laporte, A. (2000a) Digestibility of macronutrients, energy and amino acidds, absorption of elements and abscence of intestinal enteritis in Atlantic salmon, *Salmo salar*, fed diets with wheat gluten. *Aquaculture*, **184**, 115-132.

Storebakken, T., Shearer, K.D., Refstie, S., Lagocki, S. & McCool, J. (1998a) Interactions between salinity, dietary carbohydrate source and carbohydrate concentration on the digestibility of macronutrients and energy in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **163**, 347-359.

Storebakken, T., Shearer, K.D. & Roem, A.J. (1998b) Availibility of protein, phosphorus and other elements in fish meal, soy-protein concetrate and phytase-treated soy-protein-concetrate-based diets to Atlantic salmon, *Salmo salar*. *Aquaculture*, **161**, 365-379.

Storebakken, T., Shearer, K.D. & Roem, A.J. (2000b) Growth, uptake and retention of nitrogen and phosphorus, and absorption of other minerals in Atlantic salmn *Salmo salar* fed diets with fish meal and soy-protein concetrate as the main sources of protein. *Aquaculture Nutrition*, **6**, 103-108.

Sugiura, S.H., Dong, F.M., Rathbone, C.K. & Hardy, R.W. (1998) Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feed. *Aquaculture*, **159**, 177-202.

Toppe, J., Aksnes, A., Hope, B. & Albrektsen, S. (2006) Inclusion of fish bone and crab by-products in diets for Atlantic cod, *Gadus morhua*. *Aquaculture*, **253**, 636-645.

Torrissen, O. (2008) Forskning for verdiskapning In *Rapport fra havforskningen nr.3 2008*, pp. 17. Havforskningsinstituttet, Bergen, Norway.

Turano, M.J., Davis, D.A. & Arnold, C.R. (2002) Optimization of growout diets for red drum, *Sciaenops ocellatus*. *Aquaculture Nutrition*, **8**, 95-101.

Van Den Ingh, T.S.G.A.M., Krogdahl, Å., Olli, J.J., Hendriks, H.G.C.J.M. & Koninkx, J.G.J.F. (1991) Effects of soybean-containing diets on the proximal and distal intestine in Atlantic salmon (*Salmo salar*): a morphological study. *Aquaculture*, **94**, 297-305.

Ward, D.A., Carter, C.G. & Townsend, A.T. (2005) The use of yttrium oxide and the effect of faecal collection timing for determining the apparent digestibility of minerals and trace elements in Atlantic salmon (*Salmo salar*, L.) feeds. *Aquaculture Nutrition*, **11**, 49-59.

Watanbe, T., Kiron, V. & Satoh, S. (1997) Trace minerals in fish nutrition. *Aquaculture*, **151**, 185-207.

Webster, A.J.F. (1993) Energy partitioning, tissue growth and appetite control. *Proceedings of the Nutrition Society*, **52**, 69-76.

Waagbø, R. (1999) Ernæringens betydning for fiskehelsen In *Fiskehelse og fiskesykdommer* (Poppe, T. ed.), pp. 230-239. Universitetsforlaget, Oslo, Norway.

Waagbø, R. (2001) Ernæring og helse In *Fiskeernæring* (Waagbø, R., *et al.* eds.), pp. 235-258. Kystnæringen Forlag og Bokklubb AS, Bergen, Norway.

Zar, J.H. (2005) *Biostatistical analysis*, 5 ed, Pearson Education International (Lynch, D. ed.), pp. 944, New Jersey, USA.

9 Appendix

Appendix 1

Data sheets for plant ingredients used in the experimental feed:

- Vitacel: Figure 9.1, 9.2 and 9.3.
- Soycomil: Figure 9.4 and 9.5.
- Wheat gluten: Figure 9.6 and 9.7.

Appendix 2

Description of quality insurance of analytical methods:

- Quality assurance of analytical methods.
- Quality control of precision and trueness.
- Calibration.
- Accreditation.

Appendix 3

Efficiency ratios and productive values for starch and ash: Table 9.1.

Appendix 4

Concentrations (mg/kg), limit of quantification (LOQ) and parallel measurements for elements in feed and faeces, and ADC results for elements: Table 9.2, 9.3, 9.4 and 9.5.

Data sheet **Grade R 200**

VITACEL®

Multifunctional Natural Fibre Concentrate

Basic Raw Material

Extracted and highly purified powdered cellulose

Characteristics

Powdered fibre material, free of mycotoxines

Physical and Chemical Data

Cellulose content	approx.	99.5 %
Crude fibre content	approx.	70 %
Loos on drying	max.	8 %
Bulk density		110 g/l - 145 g/l
Whiteness (absolute value at 460 nm)		86 % +/- 5 %
Ash content (850 °C, 4 h)	approx.	0.3 %
pH value		6.5 +/- 1
Microbiological state:		
Total plate count	max.	10^3 KBE / g
Mycotoxines	max.	0.001 µg / kg (ppb), detection limit

Composition

Highly pure powdered Cellulose

Product name / Declaration

Cellulose powder, E 460 ii / Cellulose content min. 99 %

Information to the manufacturing process

Raw fibre concentrate, produced by a wet process. Finished by fine grinding, sieving and classification.

Used aids and supplementary products

None

Unwanted substances within the framework of risc based own controls

None

Critical substances

None

Remarks to specific analytical problems

None

Safety remarks

Usual precautionary measures have to be observed when working with all combustible materials containing dusty particles.

Storage and Shelf Life

VITACEL® R 200 can be stored at a dry place at temperatures below 20°C over a period of at least 36 months.

Practical Advice

Recommended dosage of VITACEL® R 200 in feed mixtures ranges between 0.35 - 2.0 %. VITACEL® R 200 can be mixed either in a crushing equipment or with a blender. Addition of VITACEL® at the feed Production Plant is also possible. Dry mixing is ideal. VITACEL® R 200 is stable during traditional pelletizing processes.

General Remarks

VITACEL® R 200 is a natural feed supplement with optimal effects on intestinal flora. Visible effects found out during extensive feeding trials were - for example - better feed conversion rates, increased protein digestibility, lower mortality, less diarrhoea diseases, better vitality and healthiness of the animal populations.

Beside other application areas VITACEL® R 200 is recommended especially in early feeding states and for problem cases (stable rearrangement, changes in feeding, other stress factors).

Customs tariff no.:

4704 29 00

As with all natural products slight differences to the above given values may arise.

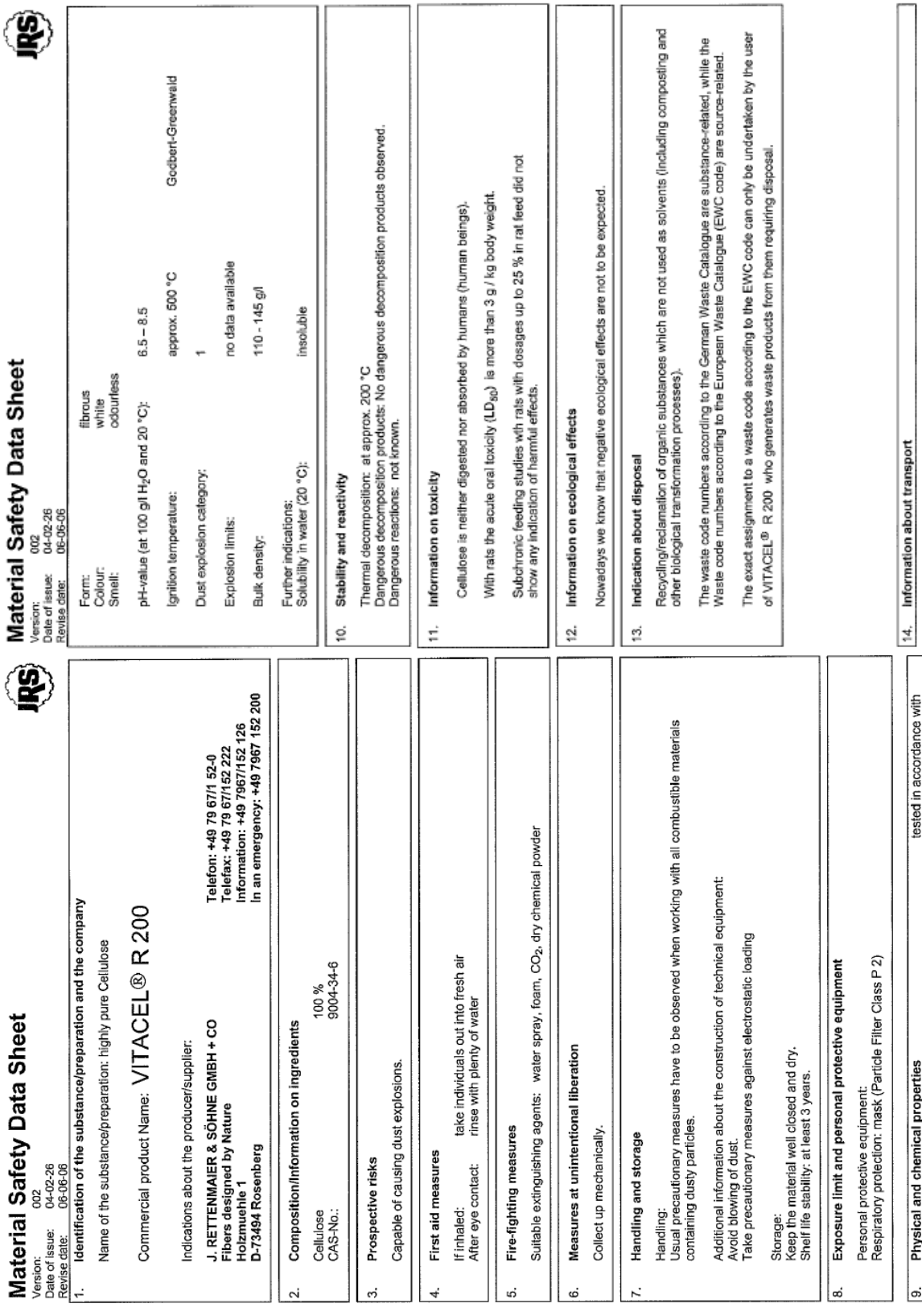
JRS

J. RETTENMAIER & SÖHNE GMBH + CO
Fibers designed by Nature
Holzmuehle 1
D-73494 Rosenberg

Telephone: ++49 / (0) 79 67 / 1 52-260
Telefax: ++49 / (0) 79 67 / 1 52-601

0604

Figure 9.1. Vitacel data sheet, part 1 of 3.



Material Safety Data Sheet

Version: 002
Date of issue: 04-02-26
Revise date: 06-06-06

1. Identification of the substance/preparation and the company

Name of the substance/preparation: highly pure Cellulose

Commercial product Name: VITACEL® R 200

Indications about the producer/supplier:

J. RETTENMAIER & SÖHNE GMBH + CO
Fibers designed by Nature
Holzmuehle 1
D-73494 Rosenberg

Telefon: +49 79 67/1 52-0
Telefax: +49 79 67/152 222
Information: +49 7967/152 126
In an emergency: +49 7967 152 200

2. Composition/Information on ingredients

Cellulose 100 %
CAS-No.: 9004-34-6

3. Prospective risks

Capable of causing dust explosions.

4. First aid measures

If inhaled: take individuals out into fresh air
After eye contact: rinse with plenty of water

5. Fire-fighting measures

Suitable extinguishing agents: water spray, foam, CO_2, dry chemical powder

6. Measures at unintentional liberation

Collect up mechanically.

7. Handling and storage

Handling:
Usual precautionary measures have to be observed when working with all combustible materials containing dusty particles.

Additional information about the construction of technical equipment:
Avoid blowing of dust.
Take precautionary measures against electrostatic loading

Storage:
Keep the material well closed and dry.
Shelf life stability: at least 3 years.

8. Exposure limit and personal protective equipment

Personal protective equipment:
Respiratory protection: mask (Particle Filter Class P 2)

9. Physical and chemical properties tested in accordance with

VITACEL R 200
page 1 of 3

Material Safety Data Sheet

Version: 002
Date of issue: 04-02-26
Revise date: 06-06-06

Form:	fibrous	
Colour:	white	
Smell:	odourless	
pH-value (at 100 g/l H_2O and 20 °C):	6.5 – 8.5	
Ignition temperature:	approx. 500 °C	Godbert-Greenwald
Dust explosion category:	1	
Explosion limits:	no data available	
Bulk density:	110 - 145 g/l	
Further indications: Solubility in water (20 °C):	insoluble	

10. Stability and reactivity

Thermal decomposition: at approx. 200 °C
Dangerous decomposition products: No dangerous decomposition products observed.
Dangerous reactions: not known.

11. Information on toxicity

Cellulose is neither digested nor absorbed by humans (human beings).

With rats the acute oral toxicity (LD_{50}) is more than 3 g / kg body weight.

Subchronic feeding studies with rats with dosages up to 25 % in rat feed did not show any indication of harmful effects.

12. Information on ecological effects

Nowadays we know that negative ecological effects are not to be expected.

13. Indication about disposal

Recycling/reclamation of organic substances which are not used as solvents (including composting and other biological transformation processes).

The waste code numbers according to the German Waste Catalogue are substance-related, while the Waste code numbers according to the European Waste Catalogue (EWC code) are source-related.

The exact assignment to a waste code according to the EWC code can only be undertaken by the user of VITACEL® R 200 who generates waste products from them requiring disposal.

14. Information about transport

VITACEL R 200
page 2 of 3

Figure 9.2. Vitacel data sheet, part 2 of 3.

Soycomil® P

Soycomil P

Soycomil P is a high quality soy protein concentrate specially designed for the animal feed industry. The unique Soycomil production process makes the soy protein highly digestible by inactivating the anti-nutritional factors and removing the soluble carbohydrates. These product properties combined with exceptional palatability make Soycomil P has no addition limitations. Soycomil P is a coarse grit.

Specification

Crude Protein (dry matter)	69%
Moisture	9%
Trypsin Inhibitor Activity	2.5 mg / g
Antigen Activity: glycinin	10 mg / kg
ß-conglycinin	10 mg / kg
Entero's	< 100 cfu / g
Salmonella	negative / 25 g

Method of Analysis

Crude Protein	NEN 3198 (72/199/EG, L179)
Moisture	NEN 3754 (71/393/EG, L279)
Trypsin Inhibitor Activity	NEN-EN-ISO 14902:2001
Antigens	TNO Sandwich ELISA
Entero's	VRBG-agar medium ISO 7402
Salmonella	ELISA

Typical composition (as is)

Crude Protein	65%
Moisture	7%
Crude Ash	6%
Crude Fat	1%
Crude Fiber	4%
Nitrogen Free Extract:	
Soluble	2%
Insoluble	15%

	100%

PDI (in water)	5%
Trypsin Inhibitor Activity	2 mg / g
Antigen Acitvity glycinin	3 mg / kg
ß-conglycinin	3 mg / kg
TPC	< 50.000 cfu / g
Iron	130 mg / kg
Particle size	> 90% 0.3 – 0.8 mm
Mean particle size	0.5 mm

Packaging: 25 kg bags / 1000 kg bags / bulk
Shelf life: 2 years after production date when stored in a cool dry place
Storage: Storage below 25°C and below 60% relative humidity promotes longer shelf life

Effective: 1st June, 2005 Replaces: Version FJ-020902 Version YDL-300505

ADM Specialty Ingredients (Europe) B.V.
P.O. Box 2 - 1540 AA Koog aan de Zaan - Netherlands - +31.75.64.64.256 (phone) - +31.75.64.64.631 (fax) - europefeedingredients@admworld.com

Figure 9.3. Vitacel data sheet, part 3 of 3.

Material Safety Data Sheet

Version: 002
Date of issue: 04-02-26
Revise date: 06-06-06

GGVSee/IMDG-Code: -- UN-NR: -- EmS: --
PG: --
MPO: --
GGVSE: KL. -- PG: -- RID/ADR: class -- PG: --
Warning sign: Hazard no.: -- Substance no. --
ADNR: Class -- PG: -- CaL -- ICAO/IATA-DGR. --

Declaration for land shipment: --
Declaration for sea shipment: --
Declaration for shipment by air: --

Other information:
No hazardous cargo.

15. **Regulations**

No hazardous material as defined by relevant regulations and decrees.

Water danger classification: not water hazardous according VwVwS, Annex 1, prefix 765

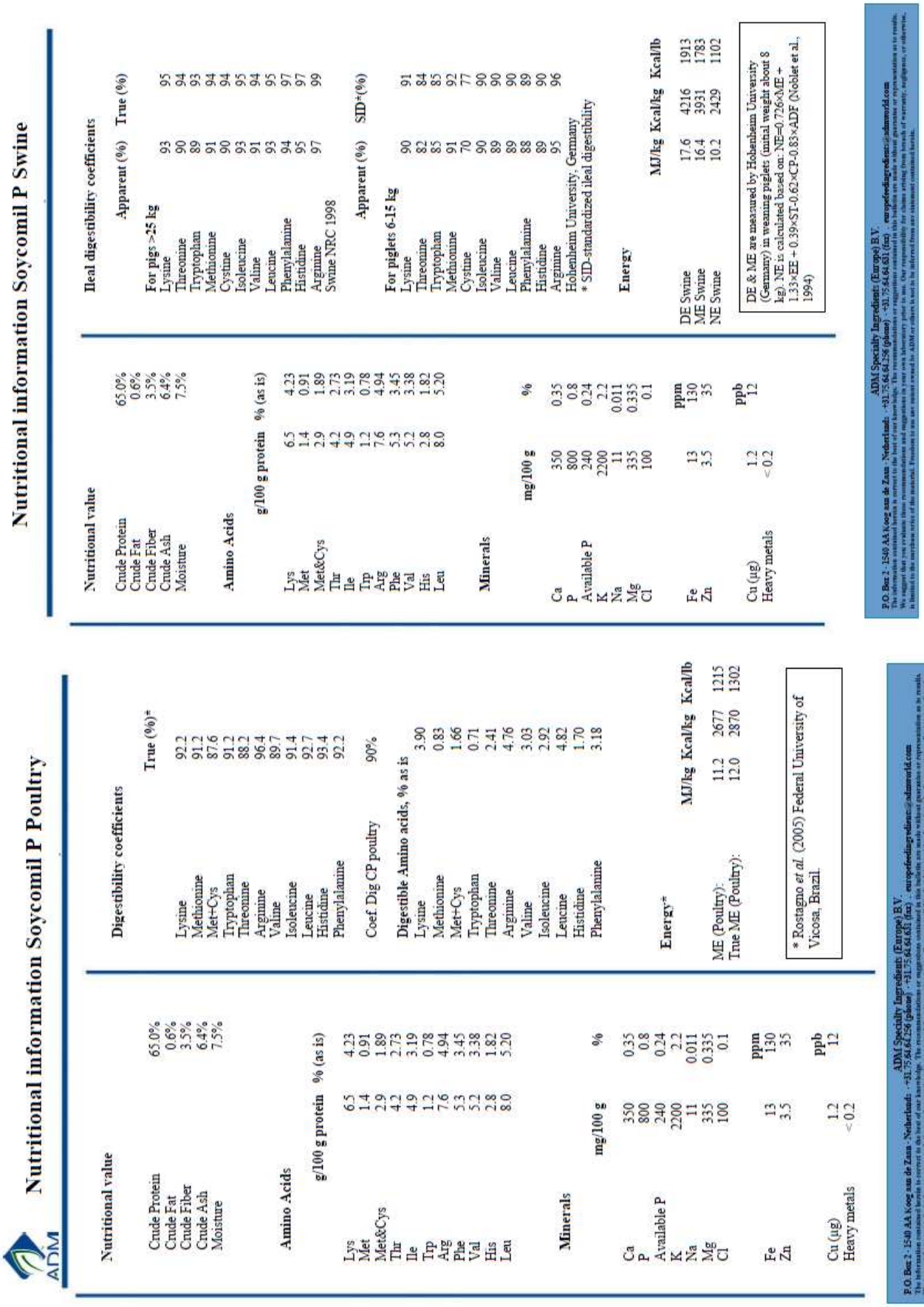
16. **Further information**

* = data have been changed, compared to the previous version

The given particulars are based on our present knowledge and experiences. However, a legally binding guarantee of certain properties cannot be derived from our statements.

VITACEL R 200
page 3 of 3

Figure 9.4. Soycomil data sheet, part 1 of 2.



Nutritional information Soycomil P Swine

Nutritional value	
Crude Protein	65.0%
Crude Fat	0.6%
Crude Fiber	3.5%
Crude Ash	6.4%
Moisture	7.5%

Amino Acids	g/100 g protein	% (as is)
Lys	6.5	4.23
Met	1.4	0.91
Met&Cys	2.9	1.89
Thr	4.2	2.73
Ile	4.9	3.19
Trp	1.2	0.78
Arg	7.6	4.94
Phe	5.3	3.45
Val	5.2	3.38
His	2.8	1.82
Leu	8.0	5.20

Minerals	mg/100 g	%
Ca	350	0.35
P	800	0.8
Available P	240	0.24
K	2200	2.2
Na	11	0.011
Mg	335	0.335
Cl	100	0.1

	ppm	ppb
Fe	13	
Zn	3.5	
Cu (µg)		12
Heavy metals		< 0.2

Ileal digestibility coefficients	Apparent (%)	True (%)
For pigs >25 kg		
Lysine	93	95
Threonine	90	94
Tryptophan	89	93
Methionine	91	94
Cystine	90	94
Isoleucine	93	95
Valine	91	94
Leucine	93	95
Phenylalanine	94	97
Histidine	95	97
Arginine	97	99
Swine NRC 1998		

	Apparent (%)	SID*(%)
For piglets 6-15 kg		
Lysine	90	91
Threonine	82	84
Tryptophan	85	85
Methionine	91	92
Cystine	70	77
Isoleucine	90	90
Valine	89	90
Leucine	89	90
Phenylalanine	88	89
Histidine	89	90
Arginine	95	96

Hohenheim University, Germany
* SID-standardized ileal digestibility

Energy	MJ/kg	Kcal/kg	Kcal/lb
DE Swine	17.6	4216	1913
ME Swine	16.4	3931	1783
NE Swine	10.2	2429	1102

DE & ME are measured by Hohenheim University (Germany) in weaning piglets (initial weight about 8 kg). NE is calculated based on: NE=0.726×ME + 1.33×EE + 0.39×ST-0.62×CP-0.83×ADF (Noblet et al., 1994)

ADM Specialty Ingredient (Europe) B.V.
P.O. Box 2 - 1540 AA Koog aan de Zaan - Netherlands - +31 75 64 64 256 (phone) - +31 75 64 64 611 (fax) - europefeedingredient@adamworld.com

Nutritional information Soycomil P Poultry

Nutritional value	
Crude Protein	65.0%
Crude Fat	0.6%
Crude Fiber	3.5%
Crude Ash	6.4%
Moisture	7.5%

Amino Acids	g/100 g protein	% (as is)
Lys	6.5	4.23
Met	1.4	0.91
Met&Cys	2.9	1.89
Thr	4.2	2.73
Ile	4.9	3.19
Trp	1.2	0.78
Arg	7.6	4.94
Phe	5.3	3.45
Val	5.2	3.38
His	2.8	1.82
Leu	8.0	5.20

Minerals	mg/100 g	%
Ca	350	0.35
P	800	0.8
Available P	240	0.24
K	2200	2.2
Na	11	0.011
Mg	335	0.335
Cl	100	0.1

	ppm	ppb
Fe	13	
Zn	3.5	
Cu (µg)		12
Heavy metals		< 0.2

Digestibility coefficients	True (%)*
Lysine	92.2
Methionine	91.2
Met+Cys	87.6
Tryptophan	91.2
Threonine	88.2
Arginine	96.4
Valine	89.7
Isoleucine	91.4
Leucine	92.7
Histidine	93.4
Phenylalanine	92.2
Coef. Dig CP poultry	90%

Digestible Amino acids, % as is	
Lysine	3.90
Methionine	0.83
Met+Cys	1.66
Tryptophan	0.71
Threonine	2.41
Arginine	4.76
Valine	3.03
Isoleucine	2.92
Leucine	4.82
Histidine	1.70
Phenylalanine	3.18

Energy*	MJ/kg	Kcal/kg	Kcal/lb
ME (Poultry):	11.2	2677	1215
True ME (Poultry):	12.0	2870	1302

* Rostagno *et al.* (2005) Federal University of Vicosa, Brazil.

ADM Specialty Ingredient (Europe) B.V.
P.O. Box 2 - 1540 AA Koog aan de Zaan - Netherlands - +31 75 64 64 256 (phone) - +31 75 64 64 611 (fax) - europefeedingredient@adamworld.com

Figure 9.5. Soycomil data sheet, part 2 of 2.

Cerestar
A CARGILL COMPANY

MATERIAL SAFETY DATA SHEET

Cerestar Scandinavia A/S
Ordrupvej 101
DK-2920 Charlottenlund
Denmark
Tel. : #45 45 46 90 45
Telefax : #45 45 46 90 40

Gluvital 21060

Issue date : 07-01-2001
Approval date : 07-06-2001
Print date : 24-05-2005

1.0 Identification of the Substance or Preparation

Vital wheat gluten

2.0 Relevant Information on Ingredients

	EINECS No	CAS No
Chemical name: Vegetable protein	2972335	93384-22-6

3.0 Health Hazards

Skin contact: Normally, not harmful to skin, though prolonged exposure should be avoided, and good personal hygiene is essential.

Eye contact: Dust in the eyes may cause physical irritation.

Inhalation: May cause sensitization by inhalation..

Ingestion: The product is a foodstuff, but ingestion should be avoided by people suffering from Coeliac Disease.

Gluvital 21040
Protein (N x 6,25) : 81,2
Fat : 4,3
Moisture : 5,7

4.0 First Aid Measures

Skin contact: Not applicable.

Eye Contact: Irrigate the eye to remove deposits and seek medical attention if discomfort persists.

Inhalation: Remove the person from the source of exposure, remove clothing contaminated with dust.

Ingestion: Not applicable.

5.0 Fire Fighting Measures

Extinguishing media: Water spray is suitable.

Extinguishing media not to be used: Not applicable.

Special precautions: In certain conditions dust/air mixtures are explosive. Avoid any ignition source.

Personal Protective Equipment (P.P.E.): Wear Self Contained Breathing Apparatus in fire conditions.

6.0 Accidental Release Measures

Personal Protective Equipment: Wear appropriate protective clothing to protect against skin contact, eye contact, and respiratory contact.

Small spillages: Use a dry cleaning method e.g. vacuum cleaner or alternatively brush up spillages and collect in suitable containers.
Large spillages: For large spillages, a vacuum cleaning system is essential, or alternatively use a vacuum truck to collect, and then dispose of material.

Dust deposits should not be allowed to accumulate on horizontal surfaces, as these may form an explosive mixture if they are suddenly released into the atmosphere.

Equipment used in dusty conditions should have a suitable electrical protection rating.

7.0 Handling and Storage

Bags: Should be stored in cool, well ventilated, dry conditions.

8.0 Personal Protective Equipment

Skin: Not required.

Eyes: Goggles or similar eye protection will prevent contact with the eyes.

Lungs: Wear a suitable dust mask when handling the material in a powder form.

Ingestion: Avoid ingestion.

Figure 9.6. Wheat gluten data sheet, part 1 of 2.

9.0 Physical and Chemical Properties

Appearance: Creamycolored, dusty.
Solubilityin water: Insoluble.
Bulk Density: Between 0.62 kg/l and 0.74 kg/l.
Moisture: approx. 7,5 %
Protein (N x 5,7): approx. 78% on d.b.

10.0 Stability and Reactivity

Starch/air mixtures form explosive mixtures in certain conditions.
Lower explosive limit: 60g/m3.
Wheat gluten is a class St1 dust at normal moisture level.
Minimum Ignition Energy (MIE): >120 mJ at normal moisture level.
P max: 8 Bar.
Kst: 100 Bar.m/s.
Layer Ignition temperature: >460 °C.

In fire, it may decompose to release carbon dioxide, water and carbon monoxide (incomplete combustion).

11.0 Toxicological Information

The product is a foodstuff and poses no toxicological risk, exceptfor the case of people suffering from Coeliac Disease, where an adverse reaction may occur.

12.0 Ecological Information

Starch does not pose an ecological risk, though it should be disposed of in a responsible manner.

No data on aquatic or vegetable toxicity .

13.0 Disposal Considerations

There are no special considerations beyond those normally applied to waste disposal (Local Authority Regulations).

14.0 Transport Information

There are no special requirements for transport.

15.0 Regulatory Information

Not applicable.

16.0 Other Information

Figure 9.7. Wheat gluten data sheet, part 2 of 2.

Appendix 2

Quality assurance of analytical methods

To get reliable results from analyses it is important to have good validations of chemical analytical methods. When validating a method, all parameters in the method have to go through examination and determination (Julshamn *et al.*, 2005). Important method parameters in a validation process are; specificity (determination of analytical component without interference), calibration, precision, trueness, ruggedness (sensitivity for changes), limit of quantification and measuring ranges. All of this has to be controlled in advance, to get reliable results from the method. Thus there are quality controls and calibrations that must be done together with analyses of samples, to make sure that analyses are done correctly.

Quality control of precision & trueness

Quality of analyses can be controlled by duplicate measurements of each sample. Variance between duplicate samples can not be over a stated number. This will control precision of results. One or more reference materials with control charts are used, and reference material will help in controlling trueness of results. Control charts have a mean value and upper and lower limit that result values from analyses have to be within to be approved. Values and limits in the control charts are made from several identical analyses on same material. Control charts are controlled by laboratory leaders.

Calibration

A calibration curve/standard curve is made from several weighing (at least two, but mostly more is better) of a control material. The control material has a known concentration and can be controlled with a control chart. Samples are quantified from the calibration curve. Therefore we have to know the measuring range where there is linearity between analytical signal and concentration. It is often important to analyse control material both before and after analysing samples, so we know that nothing have changed during analyses. A calibration curve is of course not needed in gravimetric methods.

Accreditation

Accreditation of a method means that the method is officially approved of a competent organisation. Accredited methods have to satisfy international standards, to insure trust of results internally and externally. NIFES is accredited for chemical and microbiological analyses and the management is bound to make sure that laboratories do analyses in according to general principles for good laboratory practice, control routines and quality planning. Today Nifes have over 70 accredited methods. NA (Norwegian accreditation organisation) visits NIFES yearly, and NA is evaluated by EA (European accreditation organisation) to have same requirements in different countries. Quality routines are; variance treatment, SLP (comparing analyse results with other laboratories), controlling routines and internal and external audits. Not all methods have accreditation, since this is expensive and time demanding. In addition, accreditation is not needed when results from the method are not given to the Norwegian food safety authority (Mattilsynet) and when results only are used in experiments.

Appendix 3

Table 9.1. Starch efficiency ratio (SER), starch productive value (SPV), ash efficiency ratio (AER) and ash productive value (APV) for Atlantic cod fed eight different diets in duplicate of each, from the total feeding trial (week 0 to 14). SER increased with increased α-cellulose for cod fed FM diets (R^2=0.62, $p<0.05$), but not for cod fed PP diets. SPV was not affected by α-cellulose. AER decreased with increased α-cellulose for cod fed PP diets (R^2=0.52, $p<0.05$), but not for cod fed FM diets. APV decreased with increased α-cellulose for cod fed PP diets (R^2=0.58, $p<0.05$), but not for cod fed FM diets.

Diets*	**SER**	**SPV**	**AER**	**APV**
FM 0	9.5	0.01	14.3	0.41
FM 0	10.3	0.06	15.4	0.35
FM 6	10.3	0.01	15.3	0.35
FM 6	10.5	0.03	15.5	0.39
FM 12	10.5	0.00	15.3	0.46
FM 12	11.2	0.04	16.2	0.33
FM 18	11.7	0.04	16.0	0.42
FM 18	10.6	-0.02	14.5	0.44
PP 0	12.0	0.04	16.2	0.43
PP 0	11.3	0.02	15.3	0.48
PP 6	11.2	0.02	15.6	0.30
PP 6	11.3	0.04	15.6	0.53
PP 12	10.9	0.02	14.5	0.36
PP 12	12.2	0.04	16.2	0.35
PP 18	11.9	0.04	13.3	0.28
PP 18	12.9	0.05	14.3	0.24

*Abbreviations: FM = fish meal, PP = 50% plant protein & 50% fish meal, 0, 6, 12 & 18 = % inclusions of α-cellulose (on a weight basis).

Appendix 4

Table 9.2. Analysed concentrations of elements in diets (mg/kg), included limit of quantification (LOQ) for elements below LOQ and variance (%) between parallel measurements of elements in diets.

	Elements **																
Diets**	Y	Ag	As	Ba	Cd	Co	Cu	Fe	Hg	Mn	Mo	Pb	Se	Sn	Sr	V	Zn
	Concentrations of elements in feed (mg/kg):																
FM0	65	<0.01	3.7	0.9	0.2	0.1	7	200	0.09	25	0.2	<0.04	<0.2	<0.04	26	0.1	160
FM6	68	<0.01	3.6	1.1	0.2	0.1	8	190	0.08	27	0.1	0.12	<0.2	<0.04	24	0.1	170
FM12	65	<0.01	3.4	1.0	0.2	0.1	8	170	0.07	24	0.1	<0.04	<0.2	0.05	23	0.1	170
FM18	64	<0.01	3.1	1.2	0.2	0.1	7	220	0.07	26	0.1	<0.04	<0.2	0.07	21	0.1	160
PP0	80	<0.01	2.4	3.4	0.1	0.2	10	290	0.04	54	0.9	<0.04	<0.2	<0.04	17	0.3	180
PP6	66	<0.01	2.6	3.2	0.1	0.2	9	230	0.04	47	0.8	<0.04	<0.2	0.04	16	0.3	160
PP12	63	<0.01	2.4	3.1	0.1	0.2	8	240	0.04	46	0.7	<0.04	<0.2	0.05	15	0.3	140
PP18	58	<0.01	2.1	3.3	0.1	0.2	7	230	0.03	45	0.7	<0.04	<0.2	0.07	14	0.3	130
	Variance between parallel measurements (%):																
FM0	*	*	*	24	*	33	*	14	20	17	27	*	*	48	15	*	*
FM6	12	*	*	27	*	110	*	20	*	*	25	152	*	10	*	71	*
FM12	*	*	*	*	*	*	*	*	*	*	*	29	*	*	*	24	*
FM18	*	*	*	*	*	49	*	47	*	37	*	20	*	*	*	*	*
PP0	*	*	*	*	*	11	*	33	*	14	*	*	*	10	*	*	*
PP6	*	*	*	*	*	*	*	*	12	*	*	*	*	26	*	*	*
PP12	*	*	*	*	*	*	*	29	*	*	*	11	*	*	*	*	*
PP18	*	*	*	*	*	*	*	31	11	15	*	25	*	*	*	*	*

* Variance between parallels is accepted = below the upper limit at 10%

**Abbreviations: FM = Fish Meal, PP = 50 % Plant Protein & 50 % Fish Meal, 0,6,12 &18 = % α-cellulose inclusions, Y=yttrium, Ag=silver, Ba=barium, Cd=cadmium, Co=cobalt, Cu=copper, Fe=iron, Hg=mercury, Mn=manganese, Mo=molybdenum, Pb=lead, Se=selenium, Sn=tin, Sr=strontium, V=vanadium and Zn=zinc.

Table 9.3. Analysed concentrations of elements in freeze dried faeces (mg/kg), for cod fed different diets, included limit of quantification (LOQ) for elements below LOQ and variance (%) between parallel measurements of elements.

	Elements **																
Diets**	Y	Ag	As	Ba	Cd	Co	Cu	Fe	Hg	Mn	Mo	Pb	Se	Sn	Sr	V	Zn
	Concentrations of elements in freeze dried faeces (mg/kg):																
FM0	340	<0.02	2.8	3.5	0.8	0.3	33	1100	0.13	60	1.5	0.13	<0.2	0.08	400	0.5	630
FM0	270	<0.02	2.9	2.3	0.9	0.3	26	620	0.12	30	0.5	0.11	<0.2	0.10	260	0.4	370
FM6	310	<0.02	1.9	4.7	0.6	0.3	25	750	0.09	54	0.4	1.30	<0.2	0.12	240	0.4	480
FM6	260	<0.02	2.2	1.9	0.6	0.3	24	530	0.09	21	0.4	0.14	<0.2	0.17	200	0.3	290
FM12	240	<0.02	1.9	3.3	0.5	0.2	25	590	0.07	45	6.7	0.26	<0.2	0.22	270	0.4	450
FM12	230	<0.02	1.7	3.7	0.4	0.2	18	480	0.09	16	0.3	0.09	<0.2	0.18	170	0.3	250
FM18	180	<0.02	1.8	1.2	0.4	0.2	18	390	<0.06	10	0.4	<0.08	<0.2	0.23	150	0.2	180
FM18	190	<0.02	1.4	2.1	0.4	0.2	16	400	<0.06	16	0.3	<0.08	<0.2	0.21	450	0.3	230
PP0	370	<0.02	2.0	9.3	0.5	0.9	35	1000	<0.06	73	1.4	0.12	<0.2	<0.08	310	1.4	440
PP0	280	<0.02	2.1	10.0	0.6	0.9	37	810	<0.06	70	1.0	0.11	<0.2	0.08	440	1.4	440
PP6	220	<0.02	1.6	7.3	0.5	0.8	32	740	<0.06	52	0.8	0.08	<0.2	0.14	250	1.1	360
PP6	240	<0.02	1.7	6.9	0.5	0.7	29	740	<0.06	40	0.8	0.13	<0.2	0.14	220	1.0	330
PP12	210	<0.02	1.4	5.2	0.3	0.5	22	650	<0.06	39	0.8	<0.08	<0.2	0.19	200	0.9	250
PP12	210	<0.02	1.4	6.3	0.4	0.5	23	530	<0.06	34	0.7	<0.08	<0.2	0.17	170	0.8	300
PP18	170	<0.02	1.2	4.2	0.3	0.5	21	520	<0.06	23	0.7	<0.08	<0.2	0.21	170	0.7	200
PP18	160	<0.02	1.1	3.9	0.3	0.5	20	440	<0.06	14	0.7	0.08	<0.2	0.22	140	0.6	180
	Variance between parallel measurements (%):																
FM0	*	*	*	*	*	*	*	*	*	*	80	36	*	*	*	*	*
FM0	*	*	*	15	*	*	*	*	*	*	*	*	*	35	*	*	*
FM6	*	*	*	*	*	*	*	11	*	*	*	*	*	23	*	*	*
FM6	*	*	*	*	*	*	*	*	*	*	*	41	*	11	*	*	*
FM12	14	*	*	25	*	*	*	*	*	*	21	127	*	*	*	*	*
FM12	*	*	*	107	*	*	*	*	*	*	*	*	*	14	*	*	*
FM18	*	*	11	*	10	*	*	*	12	*	*	57	*	19	*	*	*
FM18	*	*	*	*	*	*	*	*	*	*	*	*	*	13	*	*	*
PP0	*	*	*	*	*	*	*	*	*	*	*	20	14	*	*	*	*
PP0	*	*	*	23	*	*	*	*	13	*	*	40	*	16	*	*	*
PP6	*	*	*	*	*	*	*	18	*	*	*	*	*	18	*	*	*
PP6	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
PP12	*	*	*	*	*	*	*	*	*	*	*	*	*	10	*	*	*
PP12	*	*	*	*	*	*	*	*	16	*	*	*	*	*	*	*	*
PP18	*	*	*	*	*	*	*	*	30	*	*	*	*	*	*	*	*
PP18	*	*	*	*	*	*	*	*	15	*	*	13	12	*	*	*	*

* Variance between parallels is accepted = below the upper limit at 10%

**Abbreviations: FM = Fish Meal, PP = 50 % Plant Protein & 50 % Fish Meal, 0,6,12 &18 = % α-cellulose inclusions, Y=yttrium, Ag=silver, Ba=barium, Cd=cadmium, Co=cobalt, Cu=copper, Fe=iron, Hg=mercury, Mn=manganese, Mo=molybdenum, Pb=lead, Se=selenium, Sn=tin, Sr=strontium, V=vanadium and Zn=zinc.

Table 9.4. Calculated concentrations of elements in wet faeces (mg/kg), for cod fed different diets.

	Elements (mg/kg) **																
Diets**	Y	Ag	As	Ba	Cd	Co	Cu	Fe	Hg	Mn	Mo	Pb	Se	Sn	Sr	V	Zn
FM 0	52.2	*	0.43	0.5	0.13	0.05	5.1	169	0.020	9	0.23	0.02	*	0.01	61	0.08	97
FM 0	38.2	*	0.41	0.3	0.12	0.04	3.7	88	0.017	4	0.07	0.02	*	0.01	37	0.05	52
FM 6	57.0	*	0.35	0.9	0.10	0.06	4.6	138	0.017	10	0.07	0.24	*	0.02	44	0.08	88
FM 6	47.7	*	0.40	0.3	0.12	0.05	4.4	97	0.017	4	0.08	0.03	*	0.03	37	0.06	53
FM12	46.2	*	0.37	0.6	0.10	0.05	4.8	114	0.013	9	1.29	0.05	*	0.04	52	0.07	87
FM12	42.5	*	0.31	0.7	0.07	0.03	3.3	89	0.017	3	0.06	0.02	*	0.03	31	0.05	46
FM18	32.7	*	0.33	0.2	0.07	0.04	3.3	71	*	2	0.07	*	*	0.04	27	0.04	33
FM18	36.9	*	0.27	0.4	0.07	0.03	3.1	78	*	3	0.06	*	*	0.04	87	0.06	45
PP0	58.9	*	0.32	1.5	0.08	0.14	5.6	159	*	12	0.22	0.02	*	*	49	0.22	70
PP0	41.9	*	0.31	1.5	0.09	0.13	5.5	121	*	10	0.15	0.02	*	0.01	66	0.21	66
PP6	35.0	*	0.25	1.2	0.07	0.12	5.1	118	*	8	0.12	0.01	*	0.02	40	0.17	57
PP6	41.2	*	0.29	1.2	0.08	0.12	5.0	127	*	7	0.13	0.02	*	0.02	38	0.17	57
PP12	37.7	*	0.25	0.9	0.06	0.10	3.9	117	*	7	0.15	*	*	0.03	36	0.16	45
PP12	39.6	*	0.26	1.2	0.07	0.10	4.3	100	*	6	0.13	*	*	0.03	32	0.15	57
PP18	30.1	*	0.21	0.7	0.06	0.09	3.7	92	*	4	0.12	*	*	0.04	30	0.13	35
PP18	28.7	*	0.20	0.7	0.06	0.08	3.6	79	*	3	0.12	0.01	*	0.04	25	0.11	32

* Below limit of quantification (LOQ)

**Abbreviations: FM = Fish Meal, PP = 50 % Plant Protein & 50 % Fish Meal, 0,6,12 &18 = % α-cellulose inclusions, Y=yttrium, Ag=silver, Ba=barium, Cd=cadmium, Co=cobalt, Cu=copper, Fe=iron, Hg=mercury, Mn=manganese, Mo=molybdenum, Pb=lead, Se=selenium, Sn=tin, Sr=strontium, V=vanadium and Zn=zinc.

Table 9.5. Apparent digestibility coefficients (ADC) for elements (included negative results), for cod fed different diets.

	Elements (mg/kg) **															
Diets**	Ag	As	Ba	Cd	Co	Cu	Fe	Hg	Mn	Mo	Pb	Se	Sn	Sr	V	Zn
FM0	*	86	24	15	24	14	-5	72	54	-19	*	*	*	-194	-24	25
FM0	*	81	36	-11	21	13	24	67	71	47	*	*	*	-144	-10	43
FM6	*	88	6	31	50	32	13	75	56	32	-138	*	*	-119	20	38
FM6	*	84	54	7	53	21	26	70	79	7	69	*	*	-121	31	55
FM12	*	85	11	16	-30	11	6	73	49	-2168	*	*	-19	-218	-35	28
FM12	*	86	-6	32	-3	32	19	63	81	-15	*	*	-3	-112	-7	58
FM18	*	79	64	16	-2	14	37	*	86	-26	*	*	-17	-154	2	60
FM18	*	85	40	21	22	26	38	*	79	-3	*	*	-3	-633	-28	51
PP0	*	82	41	15	3	24	25	*	71	65	*	*	*	-294	11	47
PP0	*	75	15	-29	-36	-7	19	*	63	67	*	*	*	-649	-19	29
PP6	*	82	32	-18	-38	-9	3	*	67	71	*	*	-5	-369	-6	33
PP6	*	82	40	-7	-15	8	10	*	76	72	*	*	2	-284	10	42
PP12	*	83	50	7	1	14	19	*	75	64	*	*	-14	-300	7	46
PP12	*	82	38	-3	-1	9	33	*	77	71	*	*	-4	-245	13	35
PP18	*	81	57	-9	-4	3	23	*	83	67	*	*	-2	-314	0,4	48
PP18	*	81	56	-25	-6	0,3	29	*	89	66	*	*	-16	-269	7	49

* Below limit of quantification (LOQ) in feed or/and faeces

**Abbreviations: FM = Fish Meal, PP = 50 % Plant Protein & 50 % Fish Meal, 0,6,12 &18 = % α-cellulose inclusions, Y=yttrium, Ag=silver, Ba=barium, Cd=cadmium, Co=cobalt, Cu=copper, Fe=iron, Hg=mercury, Mn=manganese, Mo=molybdenum, Pb=lead, Se=selenium, Sn=tin, Sr=strontium, V=vanadium and Zn=zinc.