Effect of lipid and colorants in feed on growth and quality of Icelandic Arctic charr

Ólafur Ingi Sigurgeirsson, Holar University
Jón Árnason, Matís ohf.
Gunnar Örn Kristjánsson, Laxa Feedmill Ltd.

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Report summary

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Titill / Title	**Effect of lipid and colorants in feed on growth and quality of Icelandic Arctic charr/Samspil fitu og litarefnis í fóðri á vöxt og gæði íslenskrar bleikju**		
Höfundar/ Authors	Ólafur Ingi Sigurgeirsson, Holar University, Jón Árnason, Matís, Gunnar Örn Kristjánsson Laxa Feedmill Ltd.		
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Ágrip

Rannsóknin miðaði aðallega að því að rannsaka áhrif fóðurs á vöxt og mikilvæga gæðaþætti hjá bleikju, fitusamsetningu og holdlit. Fyrsta markmið þessarar rannsóknar var að kanna áhrif mismunandi fitugjafa og fituhlutfalls í fóðri á vöxt og efnasamsetningu í flökum. Annað markmiðið var að kanna áhrif mismunandi litarefna í fóðri og mismunandi litarefnafóðrunar á framleiðslutíma á holdlit í flökum. Tvær langtímatilraunir (1 = 250 dagar; 2 = 268 dagar) voru framkvæmdar í saltvatni (u.þ.b. 12-18 ppt) með upphafsþyngd fisks yfir 100 + g í báðum rannsóknum. Vatnshitinn var á bilinu 6-9 °C í fyrstu en 8,5-10,5 °C í annarri rannsókninni.

1. Mismunandi fituhlutfall fóðurs og ólík fituhráefni höfðu ekki áhrif á vöxt, vaxtarhraða, fóðurneyslu, FCR eða nýtingarþætti við slátrun. Fituhlutfall fóðurs endurspeglaðist í fituinnihaldi flaka. Fitusýrusamsetningin fóðurs endurspeglaðist í fitusýrusamsetningu flakanna. Mikilvægasti munurinn var á innihaldi langkeðja n-3 og n-6 fitusýra og hlutfalli n-3 / n-6 fitusýra milli hópa sem fengu lýsi eða blöndu af plöntuolíum. Ólík fituhráefni og fituhlutföll í fóðri höfðu lítil áhrif á aðra næringarefnasamsetningu í flökum. Ólík fituhráefni og fituhlutföll í fóðri höfðu lítil áhrif á sýnilegan holdlit í flökum. Meiri litabreytileiki varð greinilegri þegar aðrar greiningaraðferðir voru notaðar.

2. Greining á styrk litarefna í tilraunafóðri sýndi erfiðleika við framleiðslu fóðurs með nákvæmt litarefnainnihald, samkvæmt áætlun og fóðuruppskriftum, óháð litarefnategundum eða íbættu magni. Fóðurframleiðslan, skortur á stöðugleika viðkvæmra litarefna eða ónákvæm innihaldslýsing litarefnahráefna leiddi til breytilegra endurheimta í áætluðum litarefnastyrk tilraunafóðursins. Þess vegna þurfti að aðlaga fóðrunina með því að blanda dagskammta tilraunafóðurs með litarefnafóðri með þekktum litarefnisstyrk og fóðri án viðbættra litarefna. Magn étins litarefnis var þannig reiknað út frá litarefnisstyrk í fóðri og étins fóðurs. Vaxtarhraði, FCR og nýtingarþættir við slátrun voru sambærileg í öllum hópum. Fiskurinn var alinn í meðalstærð 1800g. Kynþroskatíðni (vísbending um kynþroska) var á bilinu 11-24%. Aðeins voru tekin sýni af ókynþroska fiski við mati á flakalit og efnasamsetningu. Lítill munur greindist í sýnilegum lit (Salmofan), en flakalitur mældur með Minolta greiningu sýndi meira samband í lit flaka og étinna litarefna. Efnagreining litarefna í flökum var í fylgni við magn étinna litarefna. Styrkur litarefna í fóðri umfram 50 mg/kg hafði ekki sýnileg áhrif í átt að rauðari holdlit í flökum.

Lykilorð: *Fitugerðir; Fituinnihald; Litarefni; Holdlitur; Bleikja; Næringarinnihald*

Summary in English:	This study mainly aimed at researching dietary effects on growth and important quality characteristics of Arctic charr, lipid composition and flesh color. The first object of this study was to investigate the effect of different lipid sources and lipid ratio in diets on growth and chemical composition in fillets. The second object was to investigate the effect of different colorant sources in diets and colorant feeding combinations on flesh color in fillets. Two long-term experimental trials (1= 250 days; 2=268 days) were run in saline water (approx. 12-18ppt) with initial weight of fish above 100+g in both trials. The water temperature was in the range of 6-9°C in the first but 8,5-10,5°C in the second trial. 1. Different dietary lipid ratio and lipid sources did not affect the fish growth, growth rate, feed consumption, FCR or harvesting output. The dietary lipid content and composition was reflected by lipid content in fillets. The fatty acid composition in the diets was reflected fatty acid composition of the fillets. The most important difference was in the content of the long chain n-3 and n-6 fatty acids and n-3/n-6 fatty acid ratio between groups fed fish oil or mix of plant oil sources. Lipid source or volume in diets only had minimal effects on other nutritional components analysed in fillets. The different dietary lipid sources and ratio was considered to have rather small effect on visual fillet color, although some existing variation related to analytical methods. 2. Analysis of carotenoids in the experimental diets showed difficulties in producing diets containing exact carotenoid content, according to plan and formulation, regardless of colorant types. The feed processing, lack of stability of the sensitive carotenoids or inaccurate content description of the colorant sources led to varying recovery of estimated concentration in the diets. Therefor the feeding and regimes needed to be adjusted by mixing daily ration with uncolored diets. Consumed colorants were calculated according to diet color content and consume. The growth rate, FCR and harvesting output was similar in all groups. The fish was grown to an average size of 1800g. Maturity ratio (indication of maturity) was in the range of 11-24% but mature fish were intentionally avoided in the estimates of fillet color and chemical composition. Minimal differences were detected in visual fillet color (Salmofan), but fillet color measured by Minolta Chromatography showed more relation in fillet color and consumed carotenoids in diets. Carotenoid analysis in fillets correlated to carotenoid consumption. Beneficial effects of increased carotenoid content in diets above 50 mg/kg were not detected in visually more reddish fillets. **The overall aim of this study was:** a) Estimate the effect of lipid content (energy) and lipid sources in diet on growth and feed efficiency of Arctic charr. b) Estimate the effect of dietary fat sources on fat composition and flesh quality of Arctic charr. c) Evaluation of economically feasible and efficient utilization of colorants in fish diets and interactions with fat content in diet and fish. d) Decrease production cost in Arctic charr culture.
English keywords:	*Lipid sources; Lipid content; Colorants; Coloring regimes; Nutrient composition; Filet colour*

Table of content

Introduction

Carnivore fishes like salmonids need protein and lipids in their diet for growth. In the feed formulation it is important to fulfil the requirement for digestible protein in the diet but also the energy demand, to gain optimal growth. The general aim is to optimize the protein retention by the fish, the feed utilization and to reduce nutrient discharges. Energy demand for metabolism can and has been met by increased lipid content in the diet, by reducing both the protein and carbohydrate content in earlier formulations. Although it is important to keep the digestible protein and digestible energy ratio (DP/DE ratio) in balance. The lipid content (energy) in fish feed seems to affect the feed conversion (FCR), defined as the ratio of kg feed eaten per kg fish gain. Increased energy content by 1 MJ/kg feed can reduce FCR by 5% in Atlantic salmon (Hillestad et al 1998; Hemre & Sandnes 1999). As a consequence, modern feed for Atlantic salmon may contain up to 40 percent crude lipid in order to affect the FCR in the production. This effect of high energy feed is however not always consistent (Bendiksen et al 2003). Einen & Roem (1997) found that optimal digestible protein/ digestible energy ratio (DP/DE ratio), or effect of increased energy in feed for salmon in seawater, was related to fish size. The optimum protein/energy ratio for maximum growth and feed utilization can be expected to decrease with fish size due to increased body fat content with increased fish weight compared to relatively stable protein content and proportionally increased energy demand for maintenance (Jobling 1994). Similar findings have been registered for rainbow trout (Cowey & Cho 1993).

Fish oil is a limited source in production of aquaculture feed, due to increased demand both in fish feed production, for direct human consumption and for other purposes. The response in salmon and salmonid feed production has been to substitute fish oil with plant oils of different sources, most commonly rapeseed oil but other plant oils may be utilized as well. Similar development occurs in feed formulation for other cultured fish species. The fish oil substitution with plant oils generally have no considerable effect on the salmonid growth, but may affect the fatty acid composition of the fish flesh (Bell et al. 2001; Ng et al. 2004). Similar results have been found for Arctic charr (Árnason et al 2008). The product yield (i.e. carcass-to-body ratio and fillet output), lipid content and composition and slaughter quality are also important criteria for evaluation of the optimum protein/energy ratio and preferable energy source and origin in salmonid diets. The fatty acid composition of the fish flesh is also an important quality and nutritive factor for the consumer.

Very limited information's are available on the usage and effect of high lipid/high energy diets on Arctic charr in culture conditions. The optimum lipid (energy) content in diets for Arctic charr has not been studied to any extent even though Sigurgeirsson (1996) found growth stimulating effect of increased lipid content in diets for that species. The composition in diets used in commercial culture of Arctic charr has generally not followed the trend in Atlantic salmon culture of very high energy diets (> 30% crude lipids) (http://www.laxa.is).

The pigmentation of the salmonid muscle depends on salmonid species, duration of pigmentation period, the dietary amount and digestibility of carotenoids, fish size and FCR (Amaya and Nickell, 2015). Flesh color is generally an important quality parameter and has a marketing value for the cultured salmonid products. Addition of colorants (carotenoid pigments, most commonly astaxanthin and to a lesser extent cantaxanthin) is necessary in formulated salmonid diets to get the preferred pink color of the flesh. According to Torrissen and Christiansen (1995), astaxanthin or canthaxanthin should be

regarded as vitamins for fish and addition at a level above 10 mg/kg dry feed is recommended to ensure the well-being of the animal.

Although the volume of colorants in the feed formulation is negligible in weight (mg/kg) it's part of the total feed cost can be significant. Therefore, it is important to know how coloration and pigmentation of fillets may vary within groups of fish in relation to pigment concentration in diets throughout the production circle. Different colorant sources or types of colorants might also be reflected differently in the fish flesh, according to their stability in the feed production process and storage. There may also be differences in digestibility and utilization of different colorant sources in diets for salmonids, both species specific but also due to the variety in raw material composition in commercial diets.

Bjerkeng et al. (1999), found a positive correlation between the concentration of idoxanthin and increased levels of mono-unsaturated and saturated fatty acids, in the flesh of salmon. They also found negative correlation between the concentration of n-3 fatty acids and flesh color. Therefor the origin of lipids and addition of colorants in fish feed formulation may affect the chemical composition and color quality appearance of the salmonid fish product.

Few studies have been done on absorption and utilisation of colorants in feed in Arctic charr culture. Most of earlier studies utilized fish of limited commercialized background which had not been through genetic selection that characterizes the stock presently used in aquaculture. Consequently, these are slower growing fish compared to the present growth intensity of farmed Arctic charr (Hatlen, B. et al. 1995; Aas et al. 1997; Metusalach, J. et al. 1996; Metusalach, J.A. et al. 1997; Christiansen, J.S., Wallace, J.C., 1988). March & MacMillan, (1996), Page, G.I. & Davies, S.J., (2006) and Bjerkeng et al. (2000) have demonstrated variability in the absorption and utilization of different types of colorants in different salmonids. Deposition of colorants in the flesh of Arctic charr seems to be different to other Salmonids as the charr transforms higher proportion (40-60%) of absorbed astaxanthin into idoxanthin (Aas et al., 1997; Hatlen et al., 1997; Hatlen et al. 1998; Bjerkeng et al. 2000; Árnason et al 2010).

In general, the retention of feed colorants is low in Salmonids (2-22%). Different factors have been found to affect the retention: Digestibility and colorant content in the consumed feed (Kiessling et al., 2003; Choubert og Storebakken 1996), lipid content of the feed (Einen & Roem, 1997; Nickell & Bromage, 1998; Torrissen, 2000), fatty acid composition of the feed (Bjerkeng et al. 1999), growth intensity, fish size, stage in life cycle (sexual maturity) (Bjerkeng et al. 1992; Hatlen et al.1996) and different binding properties of muscle proteins. In addition, considerable part of the absorbed colorants is broken down during the metabolism in the animal (Torrissen et al., 1989; Storebakken & No, 1992). The breakdown of ingested astaxanthin might be controlled by fatty acid composition in the fish as saturated fatty acids seem to affect enzymes involved in the transformation of astaxanthin to idoxanthin in Arcric charr (Aas et al., 1997). The astaxanthin to idoxanthin transformation seems to diminish with increasing weight/age until the onset of sexual maturity (Aas et al.,1997; Hatlen et al., 1997; Bjerkeng et al., 2000). Olsen and Mortensen (1997) found a dose/response effect on flesh color of diet inclusion up to 70 mg/kg but higher inclusion did not affect pigmentation. They also found significant effect of rearing temperature on pigmentation, where charr grown at 8°C was redder in flesh color than fish reared at 12°C. In addition, they found positive correlation between specific grow rate and pigmentation of fish within groups at the same rearing temperatures. Similar effects of temperature and growth rate on flesh colour, and other commercially important attributes, was found in Arctic charr grown in 15°C vs 10°C (Ginés et al., 2004), where fish grown at the lower temperature scored higher using Minolta Chromameter analysis. In recent study Imsland et al. (2020) found clear

effect of rearing temperature on color of the fillets (and other sensory quality parameters), where lower rearing temperature increased colour intensity substantially, analysed using Minolta Chromameter. Gunnarsson et al. (2012) found no effect on flesh color in Arctic charr reared under different photoperiod but a constant 12°C temperature, in a long term growth study.

Digestibility of colorants seems to be quantitatively correlated. Choubert and Storebakken (1996) tested digestibility's of astaxanthin and canthaxanthin in Rainbow trout fed different dietary colorant inclusion (12,5; 25; 50; 100 and 200 mg carotenoid/kg). Maximum digestibility was found at an inclusion level of 25 and 50 mg/kg for astaxanthin and cantaxanthin respectively. Neither relative feed intake nor salinity affected the digestibility. This is in line with the results of No, H.K. & Storebakken, T. (1992) who found no effect of water salinity on pigment digestibility in Rainbow trout. Ytrestöyl, T. et al (2006) however found an inverse relationship between relative feed intake and colorant digestibility in Atlantic salmon. There was no effect of temperature, 5 versus 15°C, on pigmentation of Rainbow trout when fed diet with 57 mg astaxanthin per kg, given that the fish was fed in equal number of day degrees (d°C) and to similar size (No, H.K & Storebakken, T., 1991). On the other hand, Ytrestøyl et al., (2005) found effect of rearing temperature (8 vs 12°C) on the digestibility of astaxanthin in Atlantic salmon.

The flesh color can be evaluated by use of color meter technology (CIE [1976] L*, a*, b*, and Hunter L,a,b systems), chemical analysis or by use of standardized color cards as DSM Salmofan®. It is a positive correlation between visual flesh color and concentration of carotenoids in flesh up to a certain level. When the concentration of carotenoids in the flesh reaches 5 mg per kg the perception of the eye becomes saturated. Above this concentration it is impossible to grade differences in flesh color visually. Assessment of carotenoids in flesh will however be imprecise. Therefore, chemical analyses gives more correct estimate of the intake, absorption and retention of the carotenoids in the flesh. For the customer in other hand, the visual perception of the flesh color is the most important criteria.

To ensure satisfactory pigmentation of arctic charr flesh, the initiation of pigment feeding and the concentration of pigments in diets must be deliberated according to harvesting size of the fish. Hatlen et al (1995) concluded that charr must reach a certain size before maximum pigmentation occurs. Theyr recommendation is that when harvesting size is planned larger than 300 gram farmers should start adding astaxanthin in the diet to fish at size of 200 -300 grams. The experience at Haukamyri fish farm in Iceland, is that using feed with 55mg/kg astaxanthin to fish of 100 grams will give reasonable pigmentation at harvesting of 600 gram live weight charr (Fannar Þorvaldsson, 2011, personal communication). The practice at Islandsbleikja ltd. in Iceland is to feed with 30 mg/ kg astaxanthin in the 3mm pellets from 100 g size, gradually increasing to 60-65 mg/kg in the finishing diets, 6-9mm pellets (Heiðdís Smáradóttir, 2011 personal communication). The recommendation from Laxa Feedmill, Iceland is to feed with 30 mg per kg for 80-200 gram charr, 50 mg per kg for 200-600 gram charr and 55 mg per kg for charr above 600 gram (http://www.laxa.is). Arnason et al (2010) fed Arctic charr diet containing 36 mg/kg astaxanthin for 19 weeks resulting in analyzed 4,6 - 6,6 mg as sum of astaxanthin and Idoxathin per kg fillet. Visible color varied from 26,7 - 27,8 on the DSM Salmofan® scale. Forsberg & Guttormsen (2006) presented a model based on published literature on coloring of Atlantic salmon for the achievement of flesh color equal to 27 on the Roche salmofan® scale. Their conclusion was that the optimal use of astaxanthin was to use feed with 40 mg Asta per kg until the salmon reached 2,5 kg and feed with 30mg Asta per kg until slaughter at 6 kg Although they consider that temperature and SGR could influence the color development significantly. No published data are available on optimal colouring regimes for Arctic charr to ensure satisfactory flesh color.

The first part of this project was to test the effect of different lipid content (%CF) and lipid sources on growth, feed utilization, flesh color and general product quality of Arctic charr.

The second part of the project was to investigate the effect of different organic pigment sources (aquasta and panaferd) and different colouring regimes on muscle pigmentation in Arctic charr.

Part I. Effect of lipid content and oil sources

Materials and methods

Fish and management

Arctic charr, (*Salvelinus alpinus*) of the commercial cultured strain in Iceland, from the Holar breeding program, was used in this experiment. 900 fishes, initial average weight of 160 grams, were distributed randomly into 15 tanks of 0,8m3 volume, 60 fish in each. Each experimental treatment (5 treatments) were in triplicate. Prior to measuring days and during handling the fish were starved for two days, then anesthetized with pehenoxyethanol (0,3ml/l) and individual weight (g) and length (mm) measured. When the fish reached approximately 550 grams in average (after 132 days), the groups were transferred to 1,6 m3 tanks and kept there throughout the experiment. The total experimental period was 281 days and the fish were weighed at ca 40 days interval (8 times in total).

The fish were fed continuously with automatic belt feeders, slightly overfed according to biomass and appetite, 6 days a week. Feed traps were fitted to the outflow of all tanks to catch uneaten pellets. The number of uneaten pellets was counted 1-2 times per day and the weight of the uneaten feed estimated from the number and the mean weight of dry pellets. Net feed intake was calculated by subtracting the weight of uneaten feed from amount of feed presented. The feed conversion ratio (FCR) was calculated for each tank as: FCR= net feed intake/increase in body mass.

Environmental conditions

The experimental tanks were in 3 separated system- lines consisting of tanks, CO2 strippers, oxygen aerators and sump tank. The water in the systems was a blend of fresh water from the local water supply and saline water pumped from drainage at the coast. The mixed water in the systems was partly recirculated (without specific bio-filtration) in the effort of keeping stable culture conditions in the tanks. The temperature, salinity and oxygen saturation were measured semi-daily. The average temperature during the trial period was 7,2˚C (ranging: 5,8 - 9,2 °C –figure 1), the salinity was on average 13,9 ‰ (range 7 -17‰, -figure 2) and the average oxygen saturation of the water, measured at the outlet was 103,3% (ranging 78,5-139%, -figure 3). The fish was reared in constant light and light period (24L: 0D), where the light intensity was above 100lux at the water surface.

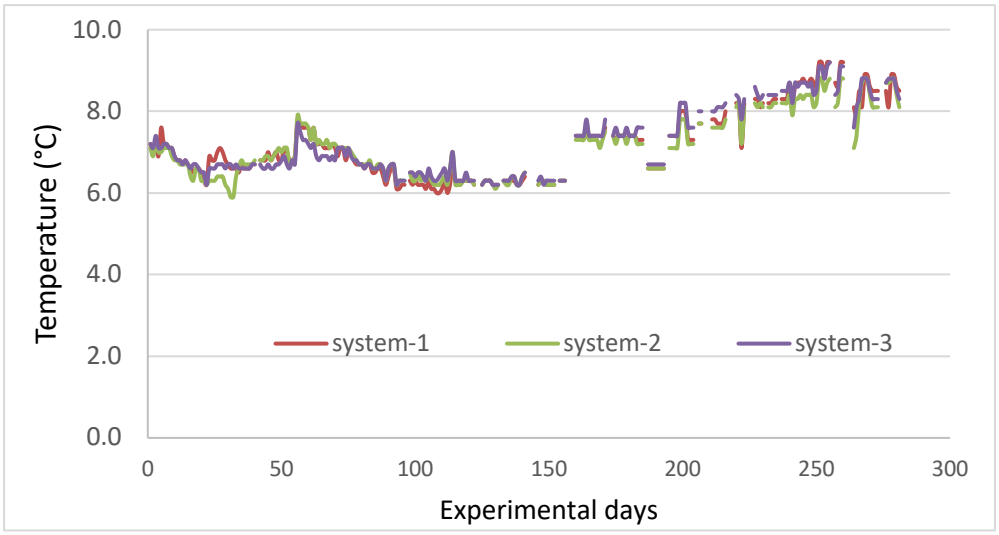

Figure 1: *Temperature (°C) in the experimental systems during the trial period.*

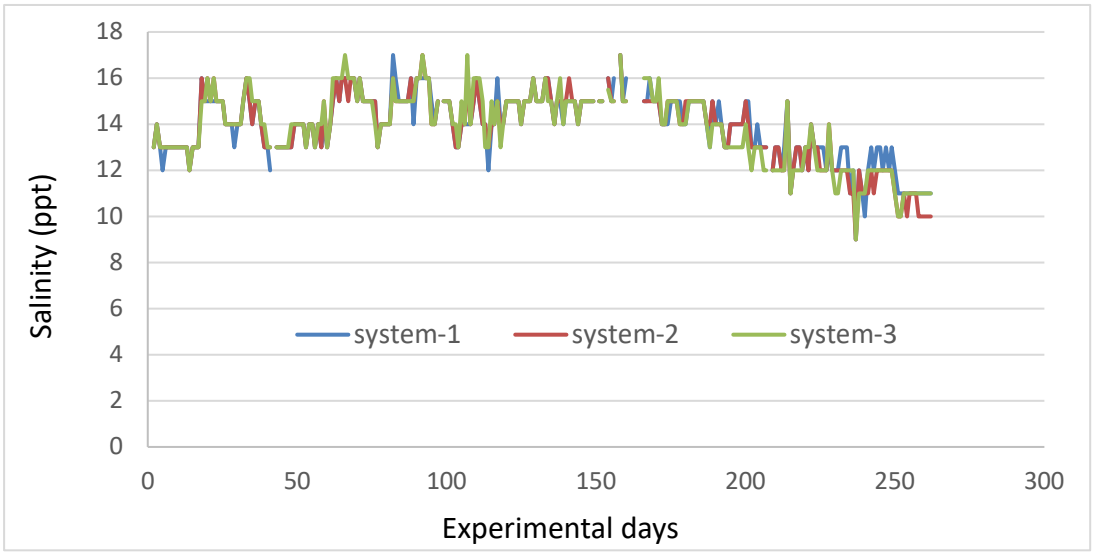

Figure 2: *Salinity (ppt) in the experimental systems during the trial period.*

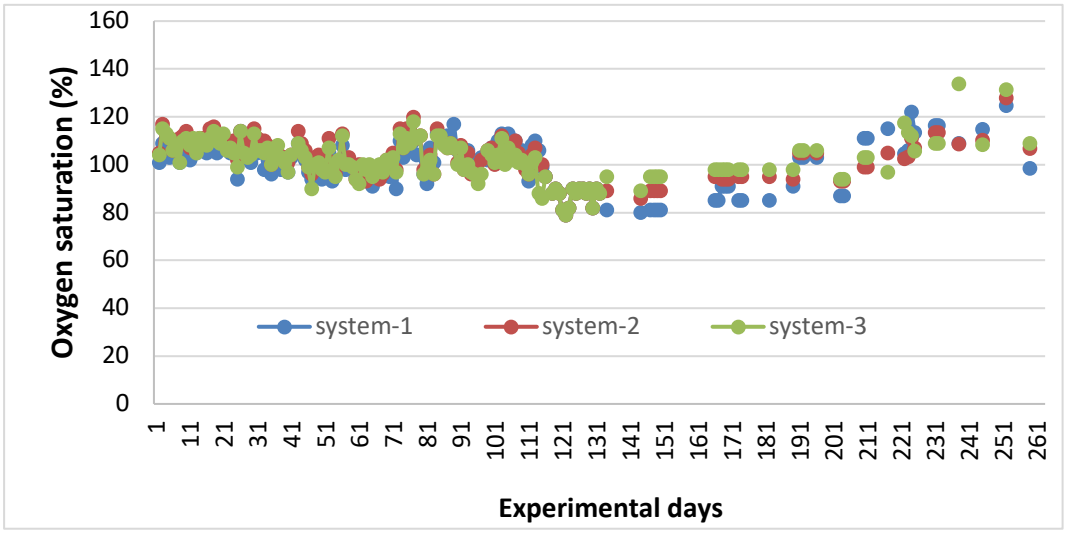

Figure 3: *Oxygen saturation (%) in the water outlet of experimental systems during the trial period.*

Feed formulation

The experimental feed was formulated attending to create feed containing 38% protein and lipid content of 23% (LO) vs 25% (HO) in period 1 and 23% (LO) vs 30% (HO) in period 2. In the first period (fish weight from 160-500 grams) 4 mm extruded feed pellets were formulated according to table 1. For the second period (fish weight from 500-1500 grams) the pellet size was 6 mm and the feed formulated according to table 2. The added lipid sources in the diets were fish oil (FO –feed 71 &72), fish oil with added rapeseed oil and palm oil (substituting fish oil in equal amounts –feed 73 & 74), a mixture of fish oil (75 %) and stearin (25%) (feed 75). Stearin is a by-product in the treatment of fish oil for concentration of the fatty acids DHA and EPA for direct human consumption. All diets were added equal amounts of the natural colorant Panaferd-AX (JXTG NipponOil &Energy) The diets for the experiment were produced by Laxa Feedmill, Krossanes, Akureyri, Iceland. The basic diets were formulated as commercial diets being equal in protein and total lipid. This resulted in different inclusion of rapeseed meal in the diets, based on earlier work on inclusion of rapeseed inclusion in diets for Arctic charr. However rapeseed meal contains 14,5% rapeseed oil and therefore the ratio between rapeseed oil and palm oil was 57/ 43 and 56/44 in feeds 73 and 74 respectively.

Table 1: *Formulation of the experimental diets (4mm) in period 1.*

Feed NR	71	72	73	74	75
Lipid type	LO/FO	HO/FO	LO/MIX	HO/MIX	HO/Stearin
Raw materials %:					
Wheat	10,0	10,0	10,0	10,0	10,0
Rape seed meal	27,1	18,2	27,5	18,8	18,4
NSM meal 709/107	25,9	29,8	25,7	29,5	29,7
MGM	20,0	20,0	20,0	20,0	20,0
Premix	1,0	1,0	1,0	1,0	1,0
Panaferd	0,2	0,2	0,2	0,2	0,2
Fish oil	15,8	20,8	3,2	4,2	15,5
Palm oil	0,0	0,0	6,1	8,0	0,0
Rape seed oil	0,0	0,0	6,3	8,3	0,0
Stearin	0,0	0,0	0,0	0,0	5,2

Table 2: *Formulation of the experimental diets (6mm) in period 2.*

Diet Nr	71	72	73	74	75
Diet type	LO/FO	HO/FO	LO/MIX	HO/MIX	HO/Sterin
Wheet %	10,0	10,0	10,0	10,0	10,0
Wheet gluten %	2,1	10,0	2,1	10,0	10,0
Rapeseed meal %	20,0	14,0	20,0	14,0	14,0
NSM 702/9 %	19,3	24,9	19,3	24,9	24,9
Corn gluten meal %	20,0	12,4	20,0	12,4	12,4
Mono cal %	0,4	0,2	0,4	0,2	0,2
Premix %	1,0	1,0	1,0	1,0	1,0
Panaferd %	0,3	0,3	0,3	0,3	0,3
Fish oil %	19,6	27,5	4,1	5,8	13,8
Palm oil %	0,0	0,0	7,7	10,8	0,0
Rapeseed oil %	0,0	0,0	7,7	10,8	0,0
Sterin %	0,0	0,0	0,0	0,0	13,8

Chemical analysis of feed and fish samples

Samples were taken for analyses of chemical composition of the experimental feeds and of composition of fish fillets. Fatty acid composition was analysed in the final feed (6 mm pellets and in fillet lipids. The moisture content of the diets was determined in a 5 g sample dried at 110°C overnight and then allowed to cool in a desiccator before the sample was reweighed (AOAC, 2000). Crude protein was calculated from total nitrogen content (0.5 g sample) determined in a Kjeldahl system following acid digestion and titration of sample distillate according to the ISO standard (ISO 5983, 2005). Crude lipid was determined gravimetrically following ethyl-ether extraction from a dried sample according to Ba 3-38 (AOCS, 1998) in a Soxhlet extractor. Ash content was determined as total inorganic matter by incineration of a 10 g sample at 550°C overnight followed by cooling in a desiccator before reweighing according to ISO standard (ISO 5984, 2002).

Fatty acid analysis

Fat extraction was based on BLIGH AND DYER method (Bligh, E.G. and Dyer, W.S. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. and Physiol. 37, 911).

Methylation was based on AOCS Official Method Ce 1b-89 with minor adjustments.

Gas chromatography was performed according to AOAC 996.06.

Harvesting output and color analysis

Samples, 4 fish pr. tank, i.e. 12 fishes for each treatment group where taken after the first period (132 days), for analysing harvesting output and flesh color. The average weight of whole fish in these samples was 620g. At the end of the trial (281 day) similar sample for measurement of liver index (HSI), gutting loss, fillet yield and flesh color was taken. The average whole fish sample weight of these samples was 1470 g.

The Hepato-somatic index (%HSI) was calculated as: 100* (liver weight (g)/body mass).

The gutting loss % was calculated as: 100%* 1- (gutted weight (g)/whole weight (g)).

The fishes were filleted by hand and the fillet yield (%) calculated from un-trimmed fillets as: 100*(fillet weight (g)/ whole weight (g)).

Fillet color, represented as CIE, L*, a* and b* values, was measured using Konica Minolta chromo meter (Minolta, Osaka, Japan) where L* value represent lightness (100= white and 0= black), a* value represent redness (a+ is red and a- is green) and b* value represent yellowness (b+ is yellow and b- is blue), were analysed. Measurements were taken at three spots on the fillet, one in the neck region, one in the centre of the fillet and one at the tail end of the fillet.

Visual fillet color was also estimated by giving score according to SalmoFan™ color card in the centre of the fillet, done in standardized light and condition.

Determination of carotenoids in diets containing Panaferd was performed according to “Analytical methods for Panaferd”, JX Nippon Oil and Energy Corporation, version 2.1, 2015. The method separates and quantifies the eight carotenoids that are present in Panaferd. The carotenoids are extracted from the sample matrix with a mixture of THF, hexane and methanol. The separation is performed by normal phase chromatography on an HPLC system by using two Si columns, connected in series. The carotenoids are detected at 470 nm.

Chemical analyses of carotenoids in fillet were performed in the following manner: Pooled homogenised muscle samples were thawed, and carotenoids were extracted from samples of accurately weighed minced muscle using a 1:1:3 mixture of distilled water, methanol (containing 500 mg l− 1 BHT), and chloroform according to Bjerkeng et al. (1997). The solvent was removed from an aliquot under reduced pressure, and re-dissolved in mobile phase (acetone/n-hexane/methanol 20:80:0.1), filtered through a 0.45 µm filter (Minisart SRP 15, Göttingen, Germany) and analysed isocratically by HPLC system II (See Bjerkeng et al. 1997).

Statistics

Data was analysed using general lineal model and one-way ANOVA to determine existing significant difference of the measured variable to the different diets as a fixed factor. The significance level was set at 5% ($p < 0,05$).

Results

Diet composition

The analysed nutrient composition of the diets fed in the two periods is shown in tables 4 and 5. In the 4 mm pellet feed, used in the former period, the protein content of the experimental diets was in the range of 38,6-40,9% and the lipid content in the range of 22-22,2% in the low lipid diets (LO) and 24,7-25,4% in the high lipid diets (HO). In the 6mm pellet feed, used in the later period, the protein content was similar, in the range of 37,8-40,2%. The difference in lipid content was from 23,1-24% in the low lipid diets (LO) to 29,4-30,7% in the higher lipid diets (HO), demonstrating more difference of low oil vs. high oil diets (max 7,6% difference) in the 6 mm feed than in the 4 mm feed (max 3,4% difference).

Table 4: *Nutrient composition of the 4mm diets used in period 1.*

Diet Nr.	71	72	73	74	75
Diet	LO/FO	HO/FO	LO/MIX O	HO/MIX O	HO/Stearin
As is:					
Water (%)	7,5	7,3	7,5	7,0	6,6
DM (%)	92,5	92,7	92,5	93,0	93,4
Protein (%)	39,6	39,0	40,9	38,6	38,7
Lipid (%)	22,0	24,8	22,2	25,4	24,7
Ash (%)	6,4	6,0	6,4	6,2	6,2
NFE (%)	24,5	22,8	23,0	22,8	23,8
GE MJ/kg*	22,4	23,0	22,5	23,2	23,1
In DM:					
Protein (%)	42,8	42,1	44,2	41,5	41,4
Lipid (%)	23,8	26,8	24,0	27,3	26,4
Ash (%)	6,9	6,5	6,9	6,7	6,7
NFE (%)	26,4	24,6	24,9	24,6	25,5

**) GE calculated from nutrients: Protein*23,7+Lipid*39,5+NFE*17,2*

Table 5: *Nutrient composition of the 6mm diets used in period 2.*

Feed NR	71	72	73	74	75
Lipid type	LO/FO	HO/FO	LO/ MIX	HO/MIX	HO/ Sterin
As is:					
Water (%)	7,2	6,6	6,4	5,5	5,1
DM (%)	92,8	93,4	93,6	94,5	94,9
Protein (%)	39,1	38,8	40,2	38	37,8
Lipid (%)	24	29,4	23,1	30,7	30,2
Ash (%)	5,8	6	5,6	6,3	6,3
NFE (%)	23,9	19,2	24,7	19,5	20,6
GE (Mj/kg)	22,9	24,2	23,0	24,5	24,5
In DM:					
Protein (%)	42,1	41,5	42,9	40,2	39,8
Lipid (%)	25,9	31,5	24,7	32,5	31,8
Ash (%)	6,3	6,4	6	6,7	6,6
NFE (%)	25,8	20,6	26,4	20,6	21,7

**) GE calculated from nutrients: Protein*23,7+Lipid*39,5+NFE*17,2*

Growth

The growth of the experimental fish is shown in figure 4. The fish multiplies its weight almost 10 times during the experimental period of 281 days, from 160g to nearly 1400 g in average. There is no significant difference in the growth response between treatment groups, i.e. no effect of oil type or lipid content in the diets on weight development at any point in the experiment.

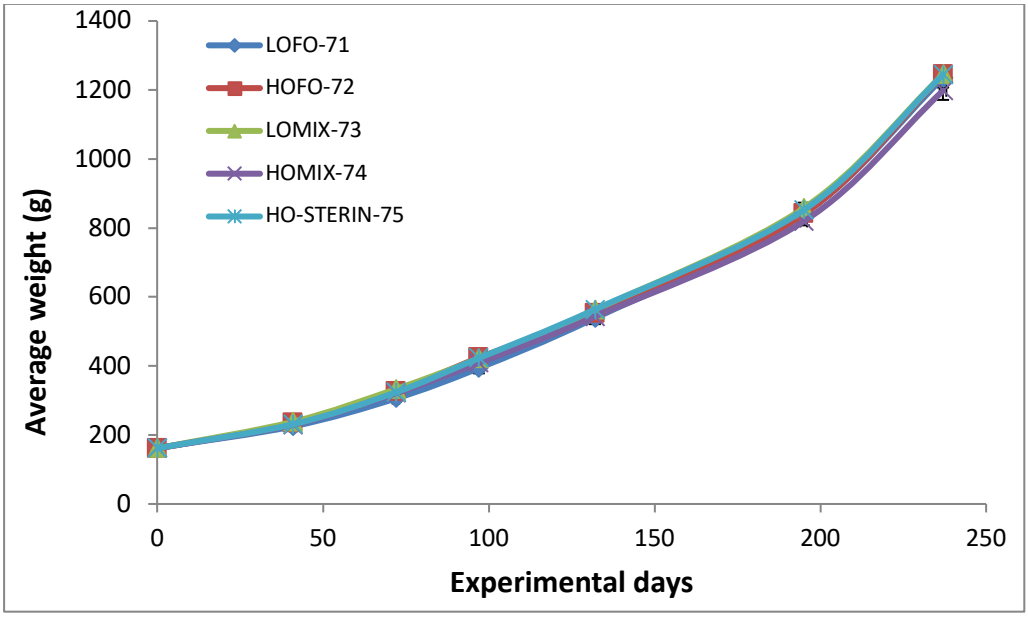

Figure 4: *Weight development of the fish over the entire experimental period.*

Growth (SGR)

The overall specific growth rate (SGR) is presented in figure 5. The average SGR, of 0,8% /day is in line with previous findings in experiments on growth of Arctic charr in similar conditions and size and is higher or equal to values found under practical conditions. There are no differences found in SGR between the different treatment groups.

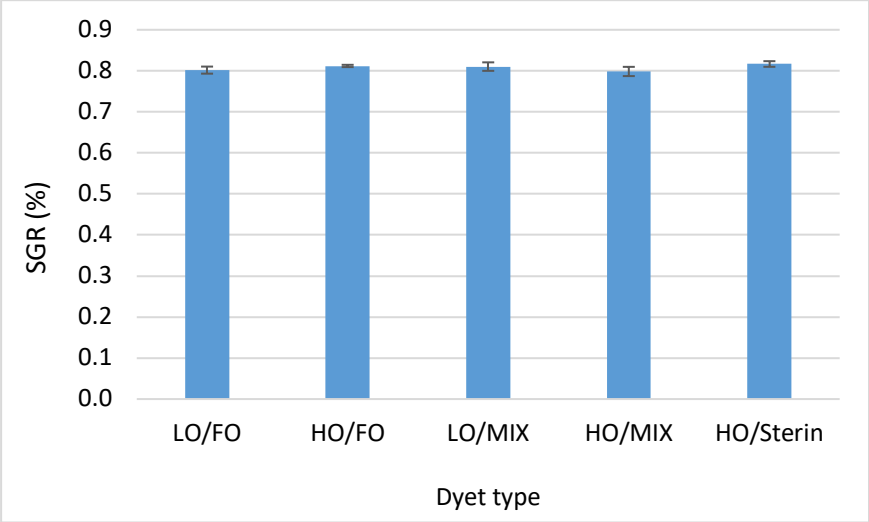

Figure 5: *Effect of different lipid source and lipid proportion in diet on average SGR (average ± SEM, n=3).*

Feed intake and feed utilization

There are only minor effects of lipid source and lipid content on average daily feed intake as percentage of average body mass (figure 6). The Feed Conversion Ratio (FCR) is around or below 1,0 in all treatment groups and similar for all feed types. This is indicating similar utilization and efficiency of the feeds (figure 7).

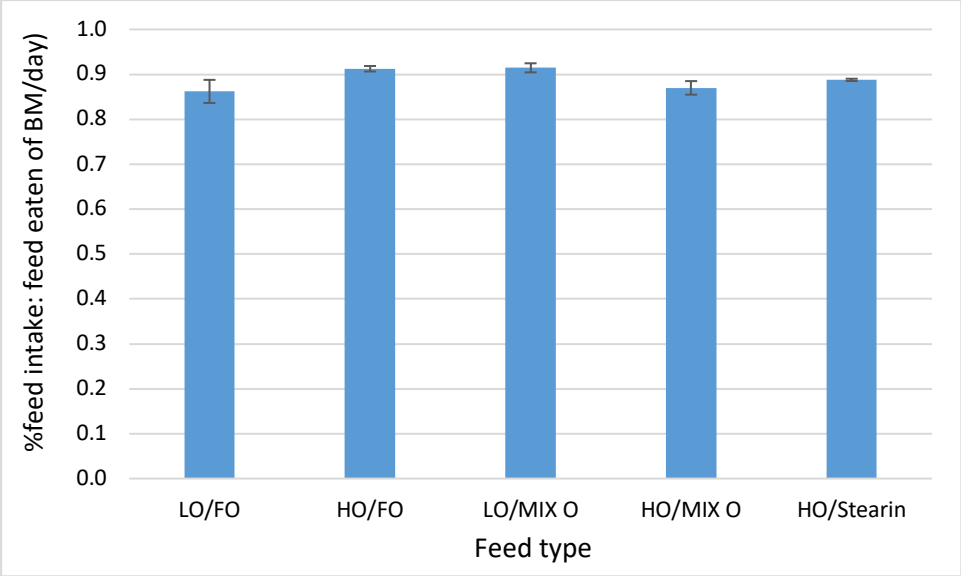

Figure 6: *Effect of different lipid source and lipid proportion in diet on feed intake, measured as average (± SEM, n=3) feed eaten of percent body mass per day.*

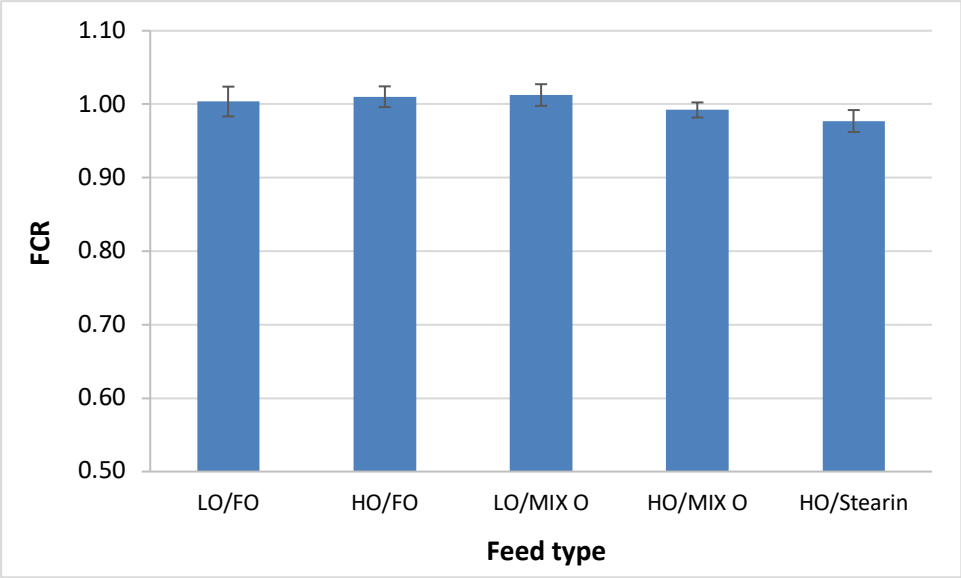

Figure 7: *Effect of lipid source and lipid content in diet on feed conversion ratio (FCR), (average ± SEM, n=3).*

Harvesting output

The results of harvesting output and HSI are shown in figures 8-10. No differences were found in gutting loss, hepatosomatic Index (HSI) or fillet yield between the different treatments. The average weight of intestines removed at gutting was around 11%, similar for all treatment groups. This indicate that the difference in lipid composition and lipid level in the diets did not have detectible effect of the amount (weight) of visceral fat in the fish.

The liver index (%HSI) was around 2,4% for all groups, showing no effect of diet type on liver size. There were no indication of fat liver or external abnormalities in the organ.

The fillet output (of untrimmed fillets) was very high, almost 70% of whole bled fish, demonstrating the excellence of this fish species for aquaculture. No effect from feed were detected on fillet output between treatment groups.

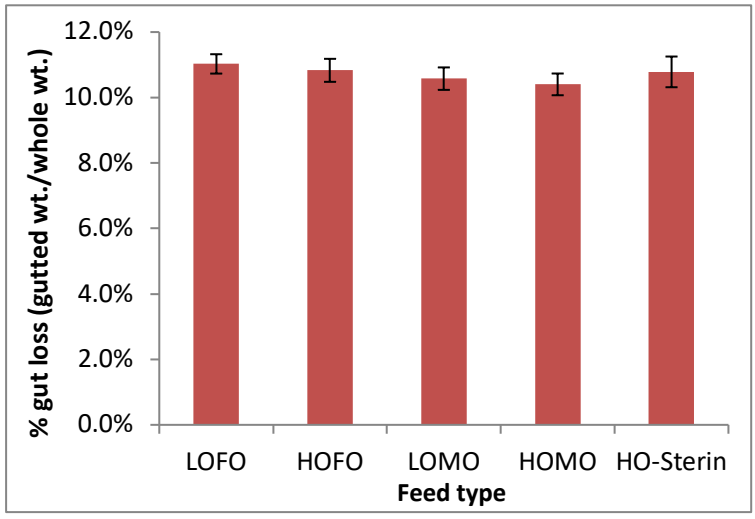

Figure 8: *Effect of lipid source and lipid amount in diet on gutting loss at final weight of the fish (average +/- SEM, n=12).*

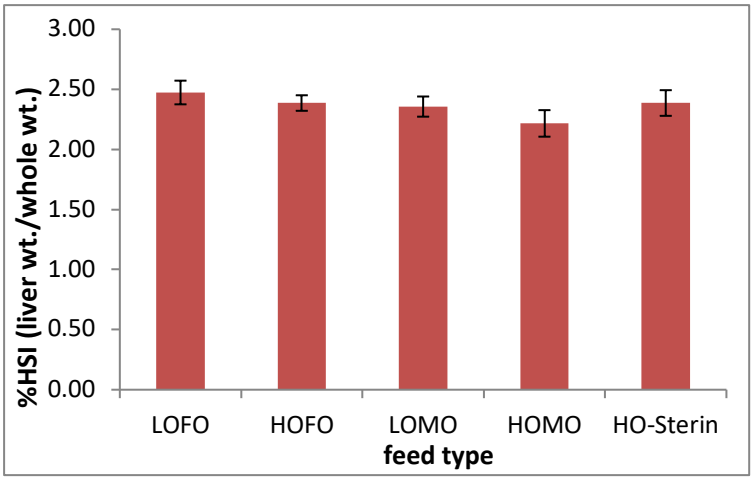

Figure 9: *Effect of lipid source and lipid amount in diet on liver index (HSI) at the final weight of the fish (average +/- SEM, n=12).*

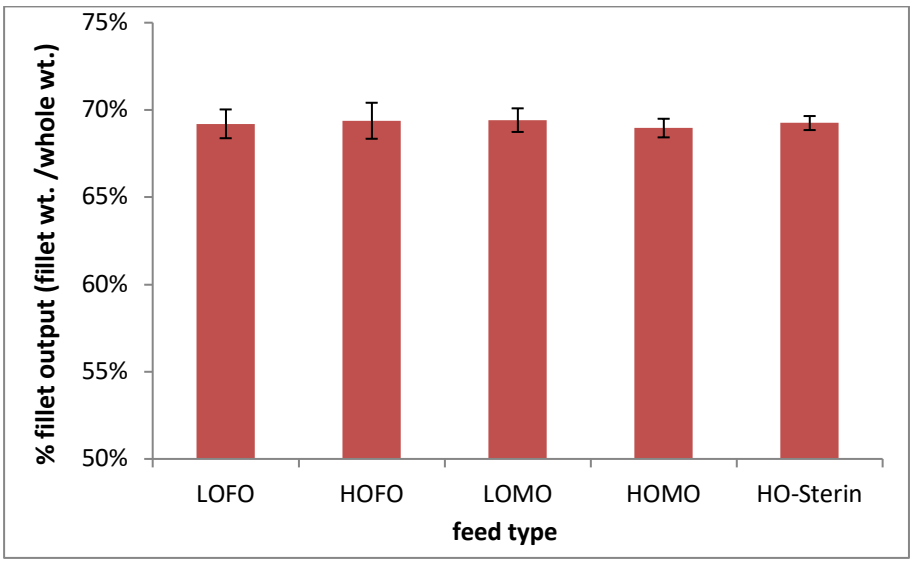

Figure 10: *Effect of lipid source and lipid amount in diet on fillet yield (% output of un-trimmed fillets from whole bled fish) at final sampling day (average +/- SEM, n=12).*

Fillet composition

There are certain variations in the nutrient content in the fillets at the final sampling, primarily on the lipid content. The composition of the smaller fish (period 1, average weight = 620g) is shown in table 6. The lipid content in the fillets is considered as moderately high for a salmonid species, in the range of 12,5-14,7%. However it is not possible to find consistency of lipid level or lipid source in the diets and lipid content in fillets. The protein content in fillets is quite similar in all groups, in the range of 17,0-17,7%. The dry-matter content of fillets is not affected by diet composition.

The composition of the bigger fish (final weight, average weight = 1470g) is shown in table 7. The protein content of the fillets is similar in all groups, except in the group getting the HO/Sterin diet (possibly an analytical error). The protein content is similar to the composition of the smaller fish, in

the range of 17,4-17,9%. The lipid content of the bigger fillets is higher than in the smaller fillets but there are more variations between treatment groups. The lipid content is in the range of 16,7-20,9%. The lowest fillet lipid content is in the group getting low fat level and mixed oil source. Remarkably the lipid in fillets is higher in the group getting low fish oil level than in the group getting higher level of fish oil in the diet.

Table 6: *Nutrient composition of the fillets from fish fed the experimental diets for 132 days, (average life weight of fish samples 620g, n= 12 in each treatment group).*

Type (lipid level/origin)	LO/FO	HO/FO	LO/MIX O	HO/MIX O	HO/Sterin
Feed nr.	71	72	73	74	75
Moisture (%)	67,4	67,4	66,8	66,4	67,6
DM (%)	32,7	32,6	33,2	33,6	32,4
Protein (% CP)	17,7	17,2	17,5	17,3	17,0
Lipid (% CL)	13,6	12,5	14,7	13,9	14,5
Ash (%)	1,1	1,1	1,1	1,1	1,1
In DM					
Protein (%)	54,2	52,8	52,6	51,5	52,5
Lipid (%)	41,7	38,3	44,1	41,4	44,6
Ash (%)	3,4	3,4	3,3	3,3	3,4

Table 7: *Nutrient composition of the fillets from fish fed the experimental diets, at the end of the experiment, after 281 days. (average life weight of fish sample was 1470g, n=12 in each treatment group).*

Type	LO/FO	HO/FO	LO/MIX	HO/MIX	HO/ Sterin
Feed Nr	71	72	73	74	75
As is:					
Humidity %	62,0	61,6	62,7	61,0	60,6
Dry matter %	38,0	38,4	37,3	39,0	39,4
Protein %	17,8	17,7	17,9	17,4	15,9
Lipid (Soxhlet) %	19,1	17,0	16,7	20,9	19,5
Ash %	1,0	1,0	1,0	1,0	1
In DM:					
Protein %	46,8	46,1	48,0	44,6	40,4
Lipid (Soxhlet) %	50,3	44,3	44,8	53,6	49,5
Ash %	2,6	2,6	2,7	2,6	2,5

Fatty acid composition

Feed (second period)

The fatty acid composition in the feed was only measured in the latter part of the experiment (period 2) when the fish was fed 6mm pellets.

As expected there are considerable differences in fatty acid composition, in the different diets, (table 8 and figures 11-15). The diets containing palm oil and rapeseed oil had higher content of saturated fatty acids and higher n-6/ n-3 ratio since the content of n- 6 fatty acids is much higher in plant oils than in fish oil. The content of n-3 fatty acids, in particular EPA and DHA, is also lower in the feeds with plant oil. The diet with 13,8% sterin inclusion is intermediate in fatty acid composition compared to all fish oil- and mix oil diets regarding most of the fatty acids, except for the content of saturates which is in the same range as in the fish oil diets.

Table 8: *Fatty acid profile in the experimental diets (period 2, 6mm pellets) as percentage of the lipid composition.*

Feed type	LO/FO	HO/FO	LO/MIX	HO/MIX	HO/Sterin
Feed nr	71	72	73	74	75
C6:0	0,0	0,0	0,1	0,1	0,0
C8:0	0,0	0,0	1,6	1,5	0,0
C10:0	0,0	0,0	1,4	1,4	0,0
C12:0	0,0	0,0	13,6	14,4	0,0
C14:0	6,3	6,5	7,6	8,1	3,7
C14:1	0,1	0,1	0,0	0,0	0,0
C15:0	0,3	0,3	0,1	0,1	0,3
C16:0	11,5	11,5	10,0	10,1	13,6
C16:1n7	0,2	0,2	0,1	0,1	0,2
C16:1n9	4,1	4,4	1,5	1,6	3,0
C16:2n4	0,3	0,3	0,1	0,1	0,2
C17	0,3	0,3	0,1	0,1	0,3
C18:0	1,2	1,1	2,2	2,2	2,6
C18:1n5+7+9	17,5	16,5	27,6	26,7	39,7
tC18:2n6	0,4	0,0	0,1	0,1	0,3
C18:2n6	6,1	4,9	13,7	12,2	12,5
C18:3n6	0,3	0,3	0,4	0,4	0,5
C18:3n3	2,0	1,7	4,6	4,5	4,7
C20:1n11	12,2	13,0	4,2	4,5	3,4
C18:4n3	2,0	2,0	0,6	0,6	0,8
C20:2n6	0,1	0,1	0,1	0,0	0,1
C20:3n6	0,0	0,1	0,2	0,2	0,2
C22:1n11+9+7	20,5	21,2	5,5	5,9	4,4
C20:5n3 EPA	5,8	5,9	2,0	2,2	3,9
C22:5n3	0,6	0,6	0,2	0,2	0,4
C22:6n3 DHA	6,0	5,9	2,3	2,5	3,7
Sum FA	97,6	96,9	99,5	99,6	98,4
Unidentified FA	2,4	3,1	0,5	0,4	1,6
Sum saturates	19,6	19,7	36,6	38,0	20,5
Sum n6	6,9	5,4	14,3	12,8	13,6
Sum n3	16,3	16,0	9,7	9,9	13,5
n6/n3	0,4	0,3	1,5	1,3	1,0
Sum EPA + DHA	11,7	11,8	4,3	4,6	7,6

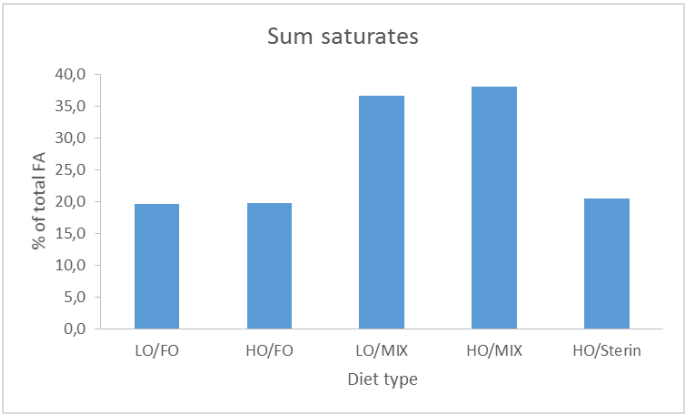

Figure 11: *Effect of lipid source and lipid proportion on the ratio of saturated fatty acids (%) in the diet lipids used in growth period 2.*

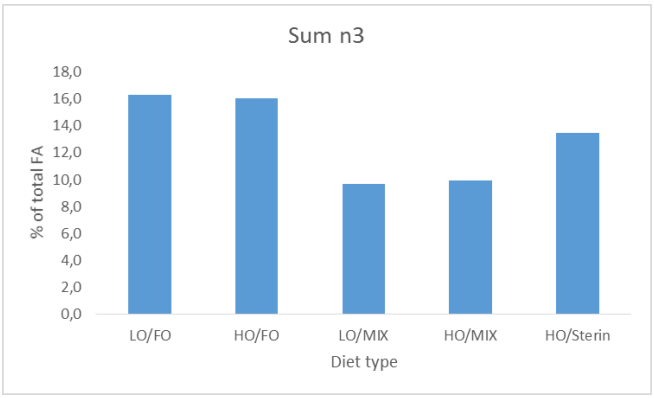

Figure 12: *Effect of lipid source and lipid proportion on the ratio of n-3 fatty acids (%) in the diet lipids used in growth period 2.*

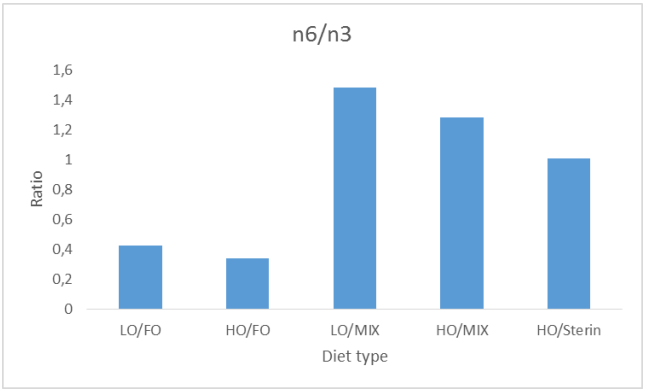

Figure 13: *Effect of lipid source and lipid proportion on the relationship between n-6/ n-3 fatty acids ratio in the diet lipids used in growth period 2*

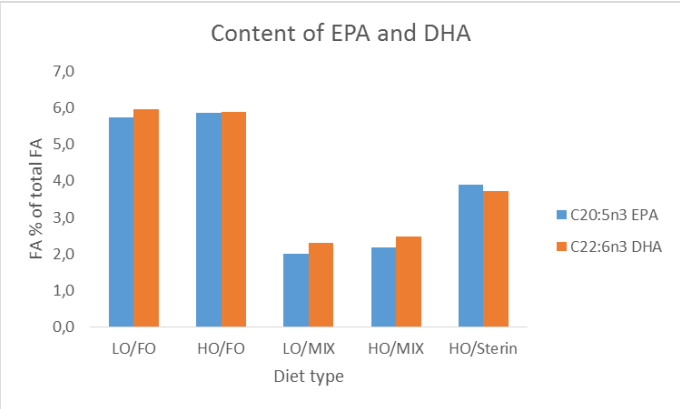

Figure 14: *Effect of lipid source and lipid proportion on the EPA and DHA fatty acids content as percentage of the diet lipids used in growth period 2.*

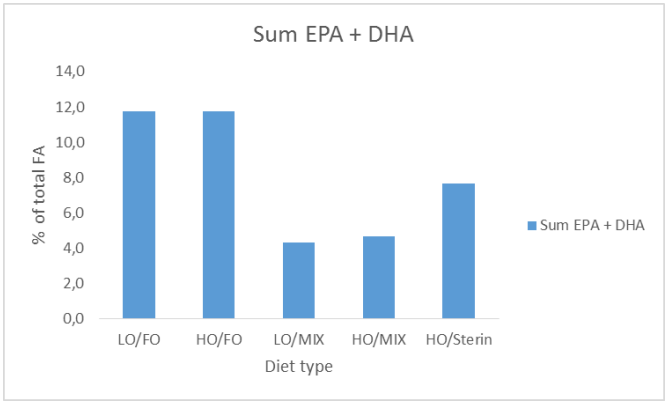

Figure 15: *Effect of lipid source and lipid proportion on the sum of EPA and DHA fatty acid contents percentage of the diet lipids used in growth period 2.*

Fillets

The content of fatty acids in fillets is shown in table 9 and figures 16 -20. As expected the fatty acid composition in fillets is reflecting FA composition in the experimental diets. The only exception seen is the ratio between EPA and DHA which in the diets is approximately 1:1 (figure 14) but considerable higher content of DHA than EPA found in the fillets (figure 19).

Table 9: *Fatty acid profile in fish fillets (period 2) as percentage of the lipid composition.*

Feed type	LO/FO	HO/FO	LO/MIX	HO/MIX	HO/Sterin
Feed nr	71	72	73	74	75
C6:0	0,0	0,0	0,0	0,0	0,0
C8:0	0,0	0,0	0,0	0,0	0,0
C10:0	0,0	0,0	0,3	0,3	0,0
C12:0	0,0	0,0	7,1	8,0	0,0
C14:0	4,7	4,9	5,8	6,0	3,4
C14:1	0,1	0,1	0,1	0,1	0,1
C15:0	0,2	0,3	0,1	0,1	0,2
C16:0	12,8	12,2	12,4	11,5	11,9
C16:1n7	0,2	0,2	0,3	0,0	0,3
C16:1n9	6,4	6,6	4,6	0,3	5,2
C16:2n4	0,3	0,3	0,1	4,0	0,2
C17	0,3	0,3	0,1	0,1	0,2
C18:0	1,6	1,5	2,4	0,1	1,9
C18:1n5+7+9	24,9	24,6	31,8	34,6	36,7
tC18:2n6	0,2	0,0	0,0	0,0	0,0
C18:2n6	6,3	6,0	12,5	12,4	9,9
C18:3n6	0,3	0,0	0,5	0,5	0,4
C18:3n3	1,6	1,7	3,2	3,4	3,1
C20:1n11	10,6	11,0	5,4	5,4	6,2
C18:4n3	1,6	1,8	0,8	0,8	1,2
C20:2n6	0,3	0,2	0,7	0,6	0,4
C20:3n6	0,2	0,2	0,5	0,5	0,3
C22:1n11+9+7	11,9	11,5	4,7	4,5	5,7
C20:5n3 EPA	4,2	4,6	1,4	1,5	3,4
C22:5n3	1,0	1,0	0,4	0,4	0,9
C22:6n3 DHA	7,6	7,8	4,0	4,0	6,3
Sum FA	97,2	96,8	99,3	99,1	97,6
Unidentified	2,8	3,2	0,7	0,9	2,4
Sum saturates	19,4	18,9	28,0	26,0	17,3
Sum n6	7,2	6,5	14,2	14,0	10,9
Sum n3	15,9	16,9	9,9	10,1	14,8
n6/n3	0,5	0,4	1,4	1,4	0,7
Sum EPA + DHA	11,7	12,4	5,4	5,5	9,7

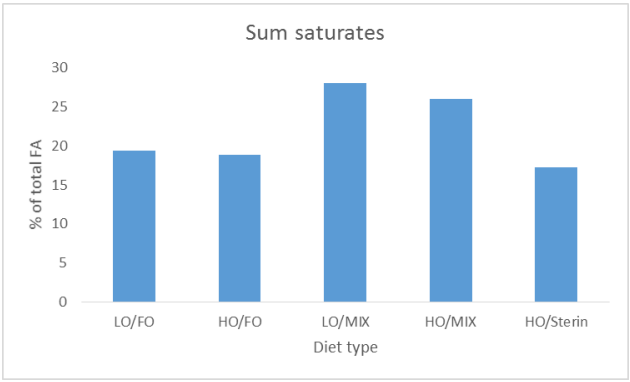

Figure 16: *Effect of lipid source and proportion in diet on sum of saturated fatty acids as percentage of the fillet lipids.*

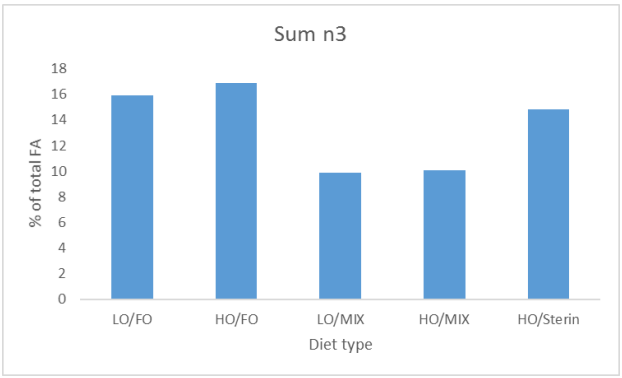

Figure 17: *Effect of lipid source and proportion in diet on sum of n-3 fatty acids as percentage of the fillet lipids.*

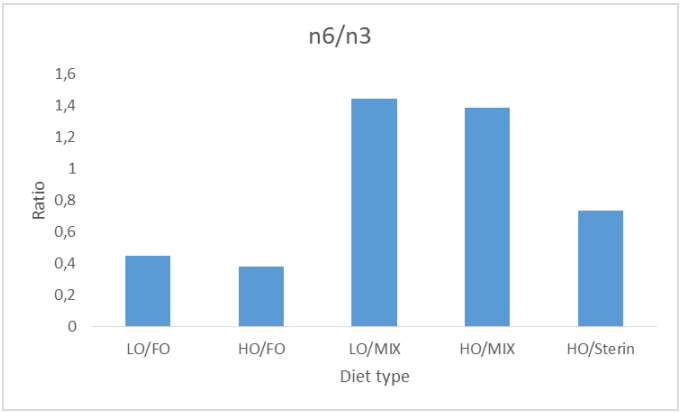

Figure 18: *Effect of lipid source and lipid proportion in diet on n-6 / n-3 fatty acid ratio in the fish fillet lipids.*

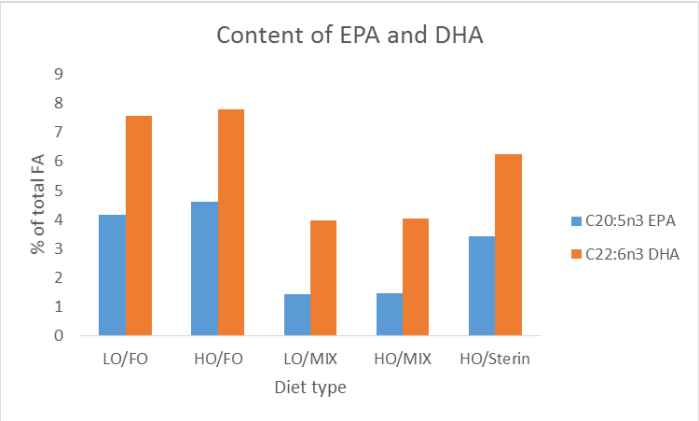

Figure 19: *Effect of lipid source and proportion in diet on EPA and DHA content in fillet lipids.*

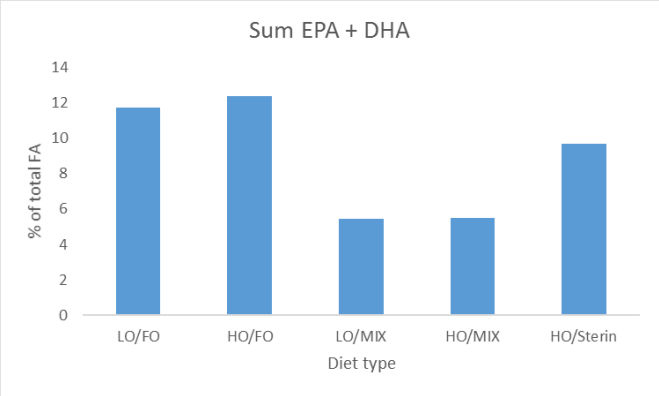

Figure 20: *Effect of lipid source and proportion in diet on sum of EPA and DHA in fillet lipids.*

Correlations

Figures 21-24 shows the relationship between fatty acid content proportionally of total lipids in the feeds and the fillets as is. The sum of saturated fatty acids and EPA content in fillet lipids is lower than in the lipids of the feed. That is indicating higher metabolism (catabolism) or transformation of these fatty acids than conversion or retention. The opposite is found for DHA indicating retention of consumed DHA and/or elongation of shorter n-3 fatty acids (18:3 n-3) taking place.

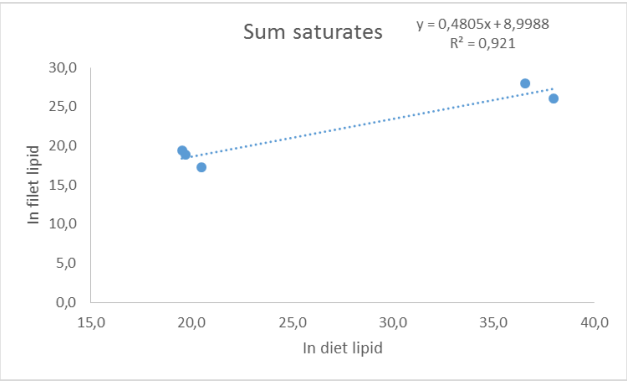

Figure 21: *Relation between sum of saturated fatty acids in diet lipids and in fillet lipids.*

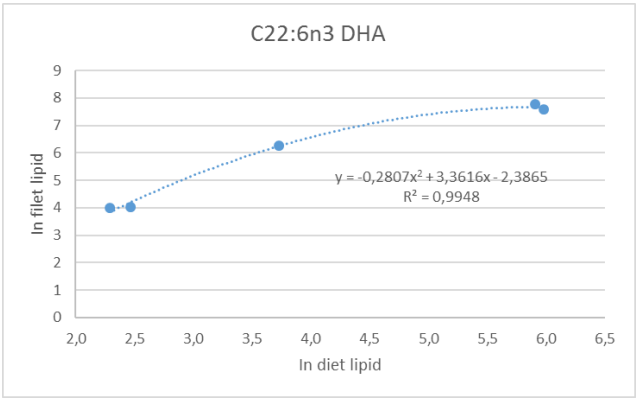

Figure 22: *Relation between DHA in diet lipids and in fillet lipids.*

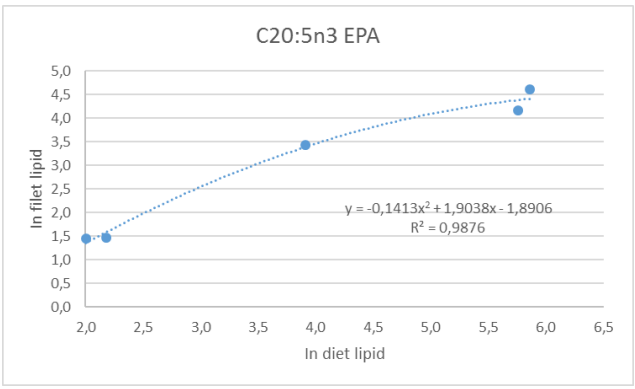

Figure 23: *Relation between EPA in diet lipids and in fillet lipids.*

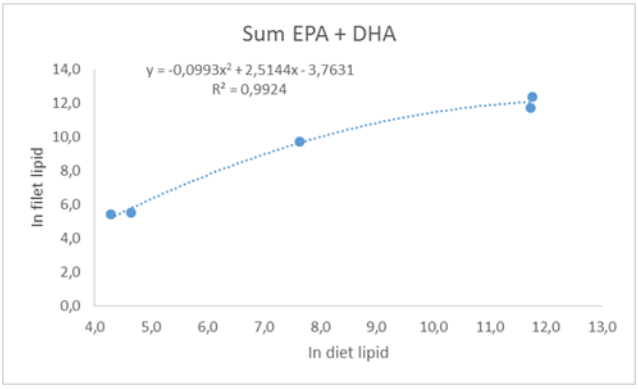

Figure 24: *Relation between EPA+ DHA in diet lipids and in fillet lipids.*

Color

Colorants in feed

Even though all diets were added equal amounts of colorant according to the formulation, chemical analyses showed different content in the produced diets (figure 25).

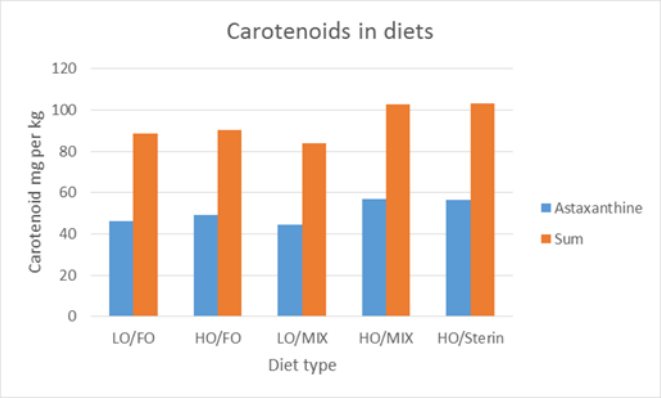

Figure 25: *Content of Astaxanthin and sum of carotenoids in the experimental diets.*

Fillet color

Measurements of color in the fillets at final sampling as well as the relation between the different measurement methods are shown in figures 26-30. The measurements show a certain variation in color between the treatments.

Visual color

The visual color is estimated with SalmoFan™ score under constant white light. The results are shown in figure 26.

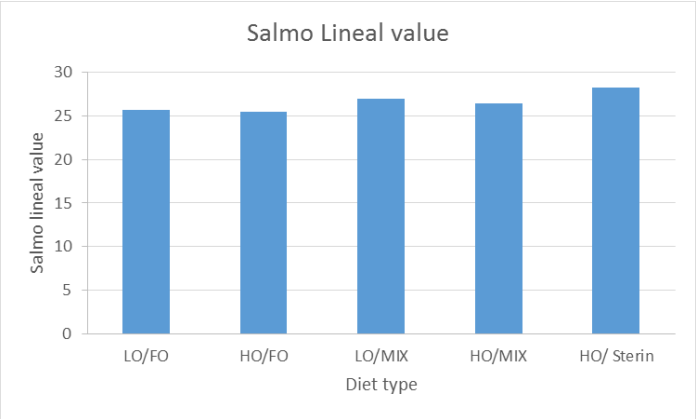

Figure 26: *Effect of lipid source and lipid proportion in diet on visual fillet color measured by SalmoFan™*

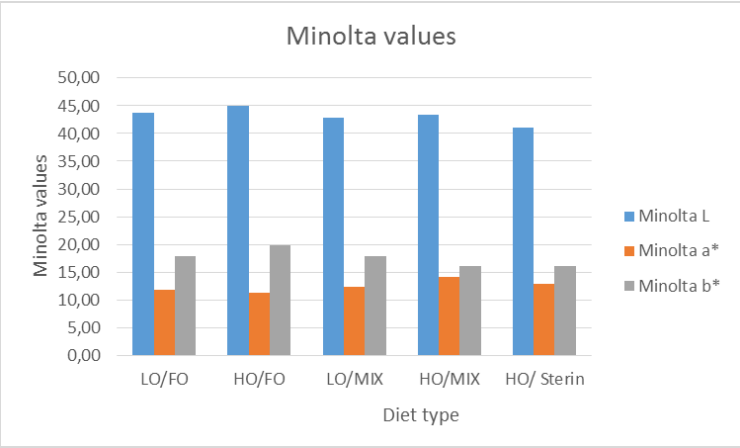

Figure 27: *Effect of lipid source and lipid proportion in diet on fillet color measured by Minolta chromo meter.*

Chemically analysed colorants

The results of carotenoid chemical analysis are shown in fig. 28. The carotenoid content is a blend of different types of carotenoids. In all diets Astaxanthin is the most pronounced and is commonly around 2 mg/kg. The total carotenoid content in the fillets is lowest in the group fed high lipid and fish oil (HO/FO), 4mg/kg. The highest carotenoid content is analysed in the group fed diet high in lipid content but a mixture of lipid sources (HO/mix), 5.6 mg/kg.

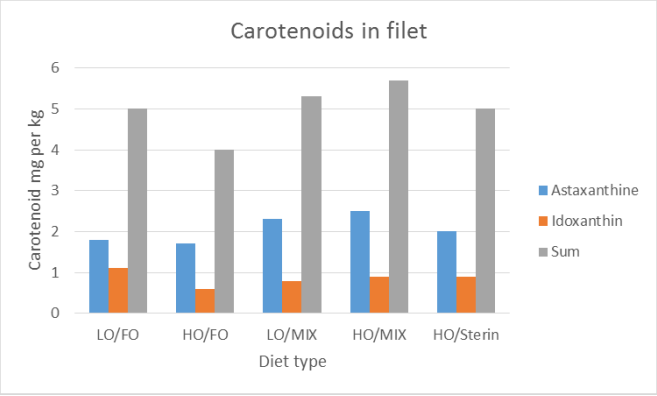

Figure 28: *Content of carotenoids in fillets (mg/kg) in the experimental groups at final sampling.*

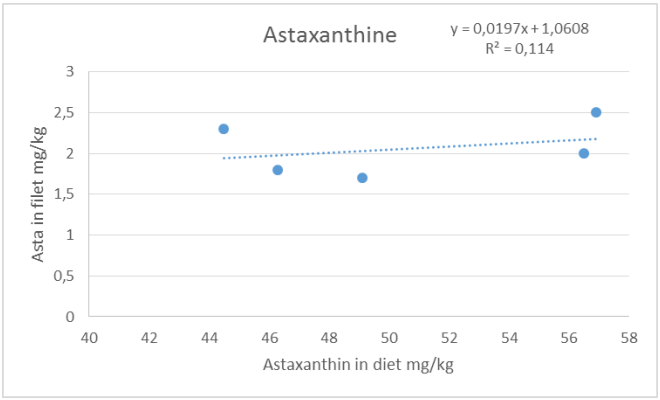

Figure 29: *Relation of chemically analysed astaxanthin content (mg/kg) in diets and fillets.*

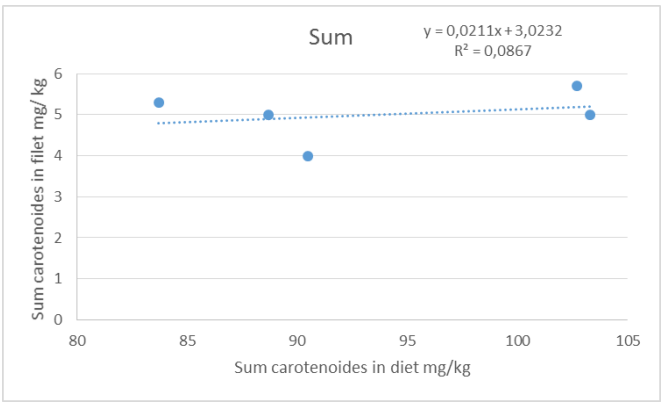

Figure 30: *Relation of chemically analysed sum (mg/kg) of carotenoids in diets and fillets.*

Part II. Effect of colorants and coloring regime

The aim of the second part of the study was to compare the effect of the organic colorants Panaferd and Aquasta on flesh color of Arctic charr. In addition, the effect of four different regimes for Aquasta content were tested.

Material and methods

Fish and management

The experiment was conducted in partial recirculation systems at Verið experimental station in Sauðarkrokur Iceland. The fish of initial body mass: 113,1g ±1.4 (mean ± se) was distributed among 15 tanks (0.8 m3) in 5 separated lines with identical systems, 45 fish in each tank. Each treatment was in triplicate. The fish were allowed to adapt to the tanks and the experimental diets for two weeks before the experiment commenced. The first measurement was performed after two-week period followed by 5 growth measures throughout the experimental period. The weight (g) and length (cm) was measured on all individual fishes each measuring days. After 210 days in 0,8 m3 tanks the fish was moved to 1,6m3 tanks, were they were grown till the end of the trial, after 330 days. When measured, the fish were anaesthetized with 2-phenoxy ethanol (0,3ml/l).

The temperature, salinity and oxygen levels were recorded daily and adjusted if necessary. The profile of temperature (°C), salinity (PPT) and oxygen saturation (%) during the trial period is shown in figure 31, 32 and 33.

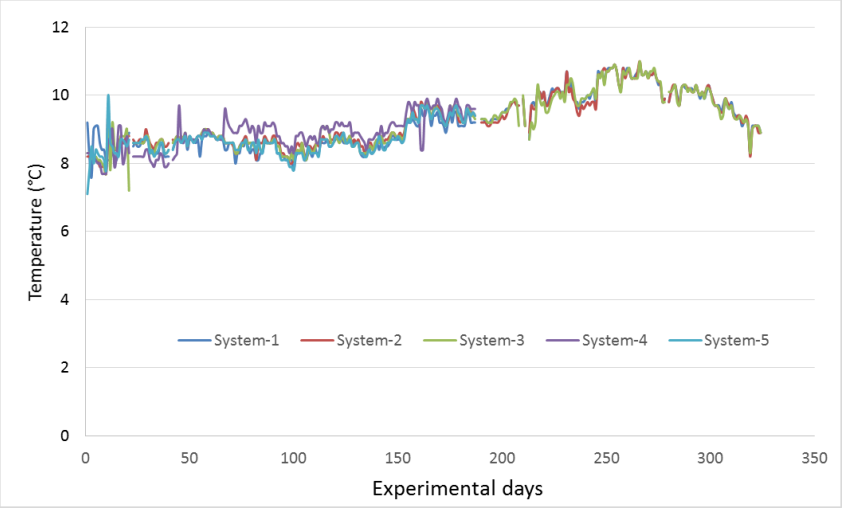

Figure 31: *Temperature (°C) in the experimental systems during the experimental period.*

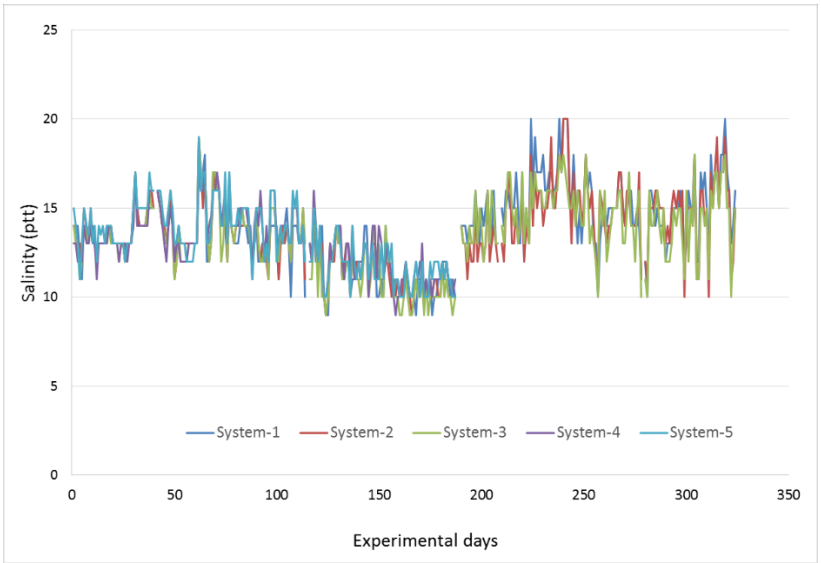

Figure 32: *Salinity (ppt) in the experimental systems during the experimental period.*

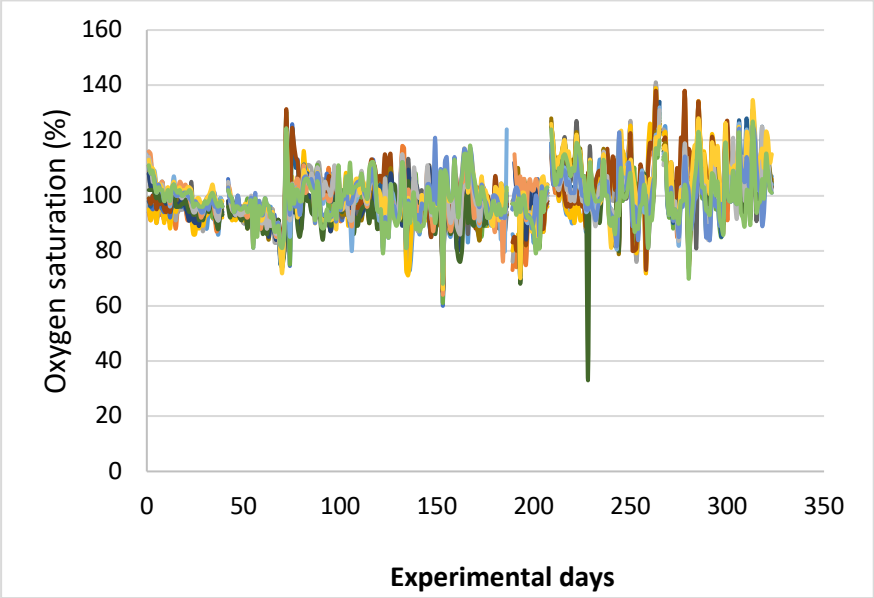

Figure 33: *Oxygen saturation (%) in effluent water in all individual experimental tanks during the experimental period.*

Feed and feeding

The diets used in the experiment were commercial diets, Eco 3.0, Eco 4.0 and Eco 6.0 from Laxa Feedmill, Akureyri, Iceland. The basis formulation of the feeds used in the experiment is shown in table 10. The planned addition of colorants in the diets is shown in table 11 and figure 34. Differences in recovery of colorants during the production process of the experimental diets led to different inclusion of colorants from initial plan. The actual analysed content of colorants in the experimental diets is shown in table 12 and figure 35.

Table 10: *Basic formulation of experimental diets within different pellet size (% of ingredients).*

	Eco 3.0	ECO 4.0	Eco 6.0
Fish meal	43,4	31,0	28,0
Corngluten meal	15,0	20,1	20,1
Rapeseed meal	0,0	10,4	15,1
Wheatgluten meal	10,3	5,5	0,0
Hypro soya	1,8	0,0	1,3
Wheat	10,0	10,0	14,3
Fish oil	13,6	16,1	14,1
Rapeseed oil	4,3	4,8	5,5
Premix	1,0	1,0	1,0
MonoCa-Phosphate	0,6	1,1	0,7

Table 11: *Colorant type and planned colorant composition in the experimental diets.*

	Fish size (g) / pellet diameter	**100–200g 3mm**	**200–500g 4mm**	**600g+ 6mm**
Feed nr.	**Colorant**	**Colorant in feed (mg/kg)**		
f-0	Panaferd	30	60	50
f-1	Aquasta	30	60	50
f-2	Aquasta	50	50	50
f-3	Aquasta	70	50	30
f-4	Aquasta	5	70	30

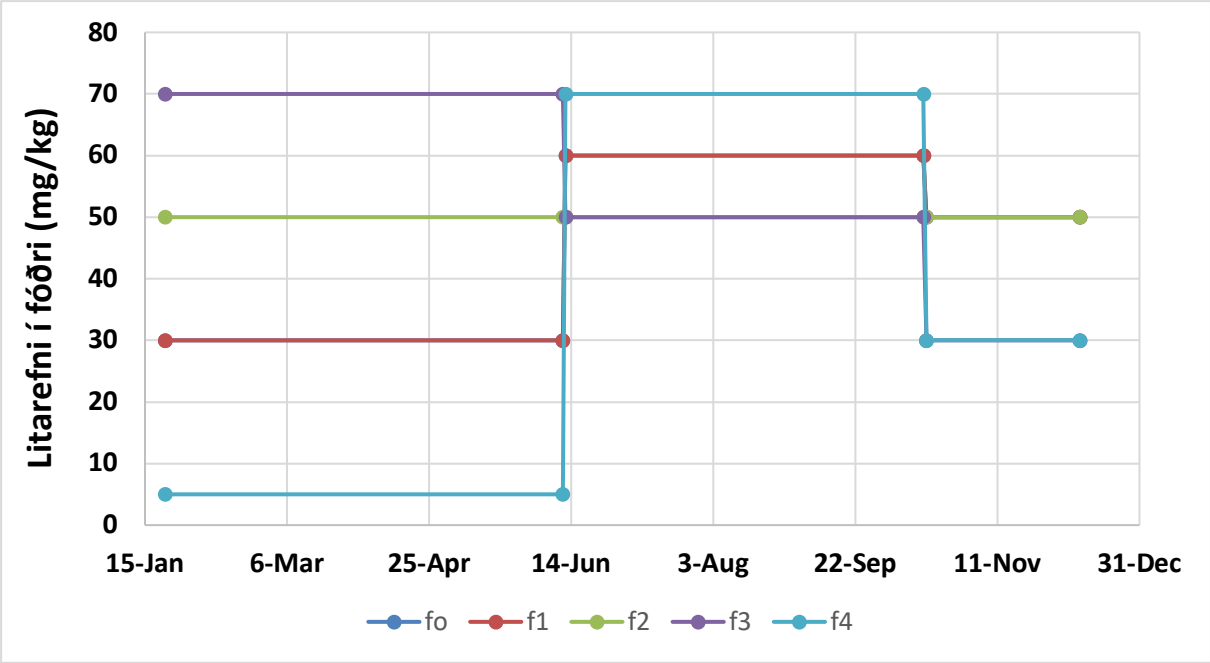

Figure 34: *Colorant type and planned content in experimental diets and changes in concentration during the experimental period.*

Table 12: *Colorant type and actual colorant composition in the experimental diets*

	Fish size (g)/pellet diameter	**100-200g 3mm**	**200-500g 4mm**	**600g+ 6mm**
Feed nr.	**Colorant**	**Colorant in feed (mg/kg)**		
f0	Panaferd	27	48	66
f1	Aqasta	16	48	72
f2	Aqasta	24	37	72
f3	Aqasta	28	38	45
f4	Aqasta	6	51	45

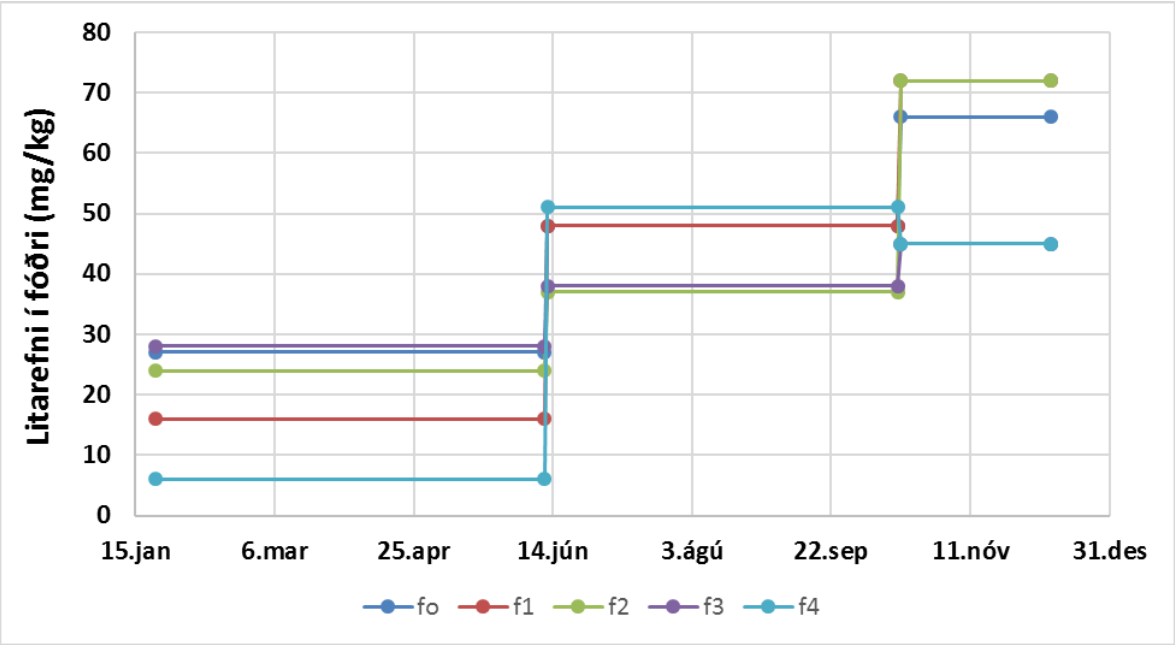

Figure 35: *Colorant type and actual content (mg/kg) in experimental diets and changes in concentration during the experimental period.*

The fish were fed continuously with automatic belt feeders, slightly overfed (10-15%), according to biomass and appetite, 6 days a week. Feed traps were fitted to the outflow of all tanks to catch uneaten pellets. The number of uneaten pellets was counted 1-2 times per day and the weight of the uneaten feed estimated from the number of pellets and the mean weight of dry pellets.

Calculations and measures

Specific growth rate (SGR) was calculated by following formula: SGR%= 100*(Ln(final weight) – Ln (start weight))/number of feeding days.

TGC: Thermal growth coefficient: TGC = 1000*(Wf1/3 – Wi1/3)/ day degrees, where Wf is the final weight and Wi is the initial weight.

Feed conversion ratio (FCR) was calculated according to following:

FCR = kg feed eaten/ kg growth

Condition factor (K): 100x (weight (g) / length^3 (cm))

Hepato somatic index (HSI): 100 x (liver weight/ Body weight)

% Gut loss: 100* (gutted weight (g) / whole weight (g))

% Fillet yield: 100*(fillet weight (g) / whole weight (g))

Analysis

At the end of the trial, samples, 4 fish pr. tank, i.e. 12 fishes for each treatment group where collected for analysing harvesting output and flesh color. The average weight of whole fish in these samples was 1883 g.

The moisture content of the diets was determined in a 5 g sample dried at 110°C overnight and then allowed to cool in a desiccator before the sample was reweighed (AOAC, 2000). Crude protein was calculated from total nitrogen content (0.5 g sample) determined in a Kjeldahl system following acid digestion and titration of sample distillate according to the ISO standard (ISO 5983, 2005). Crude lipid was determined gravimetrically following ethyl-ether extraction from a dried sample according to Ba 3-38 (AOCS, 1998) in a Soxhlet extractor. Ash content was determined as total inorganic matter by incineration of a 10 g sample at 550°C overnight followed by cooling in a desiccator before reweighing according to ISO standard (ISO 5984, 2002).

Fillet color was measured using Minolta chromo meter where L value (100= white and 0= black), a* value (a+ is red and a- is green) and b* value (b+ is yellow and b- is blue) were analysed. Measurements were taken at three spots on the fillet, in the neck region, in the centre of the fillet and at the tail end of the fillet.

Visual fillet color was also estimated by giving score according to SalmoFan™ color card in the centre of the fillet.

Chemical determination of carotenoids in feed and fillet were performed according to the following procedures:

Determination of carotenoids in diets containing Panaferd was performed according to “Analytical methods for Panaferd”, JX Nippon Oil and Energy Corporation, version 2.1, 2015. The method separates and quantifies the eight carotenoids that are present in Panaferd. The carotenoids are extracted from the sample matrix with a mixture of THF, hexane and methanol. The separation is performed by normal phase chromatography on an HPLC system by using two Si columns, connected in series. The carotenoids are detected at 470 nm.

Determination of astaxanthin in diets containing Aquasta was performed according to “HPLC method for determination of astaxanthin in formulated fish feed containing red yeast, *Xanthophyllomyces dendrorhous (Phaffia rhodozyma)*. Nofima Ver. 1.2 2. mai 2013”. Intracellular astaxanthin is made available for extraction by combined homogenization, sonication, and digestion of yeast cells with lysing enzyme. The digested aqueous sample is further homogenized with methanol and a portion of resulting preparation is extracted with chloroform to remove the carotenoids. A portion of chloroform extract is evaporated to dryness and reconstituted in mobile phase for subsequent assay by HPLC. The assay is normal phase on a silica column with visible wavelength detection at 470 nm.

Chemical analyses of carotenoids in fillet were performed in the following manner: Pooled homogenised muscle samples were thawed, and carotenoids were extracted from samples of accurately weighed minced muscle using a 1:1:3 mixture of distilled water, methanol (containing 500 mg l− 1 BHT), and chloroform according to Bjerkeng et al. (1997). The solvent was removed from an aliquot under reduced pressure, and re-dissolved in mobile phase (acetone/n-hexane/methanol 20:80:0.1), filtered through a 0.45 µm filter (Minisart SRP 15, Göttingen, Germany) and analysed isocratically by HPLC system II (See Bjerkeng et al. 1997).

Statistical analysis

Data was analysed using general lineal model and one-way ANOVA to determine existing significant difference of the measured variable to the different experimental diets. The significance level was set at 5% ($p < 0,05$).

Results

Colorant recovery

As seen from table 11 and 12 the actual content of colorants fed, in the three periods of the experiment turned out to be different from planned due to different recovery of added colorants during the feed production. This is in particular the case in the feeds getting Aquasta as colorant (see table 13).

Table 13: *Colorant composition of the experimental diets. Added colorants, analysed colorant composition and recovery rate of colorant in diet.*

Pellet diameter (mm)		**3mm**			**4mm**			**6mm**		
Feed nr.	Colorant	added mg/kg	analysed mg/kg	recovery %	added mg/kg	analysed mg/kg	recovery %	added mg/kg	analysed mg/kg	recovery %
f-0	Panaferd	30	27	90	60	48	80	50	66	131
f-1	Aquasta	30	16	53	60	48	80	50	72	144
f-2	Aquasta	50	24	48	50	37	74	50	72	144
f-3	Aquasta	70	28	40	50	38	76	30	45	150
f-4	Aquasta	5	6	120	70	51	73	30	45	150

Weight development and Growth

The development of growth and growth rate SGR are shown in figures 36, 37, 38 and TGC in figure 39. Overall the growth of the fish in this experiment was good except for the fish getting treatment f2 due to poor outcome in one tank of the triplicates. The reason for this is not clear and might have happened by chance or been some unexplained technical failure in one of the triplicate tanks of this treatment. No deviations were observed in the regular water quality measurements. This tank effect is affecting the mean value in the group f2. The results are expressed this way instead of skipping one of the replicates from the particular group. A significant difference in SGR was found for the different treatments in the first two intermediate periods even though the fish was fed with the same basal diet (figure 37). The increase in growth between day 210 and day 268 is most probably due to transportation of the fish in bigger tanks, from 0,8 to 1,6m3 in volume. These change gives shift to lower density of fish and probably more favourable growth conditions. The average temperature also rises by 2°C which is likely also stimulating the growth. No difference was found in SGR in the last three periods or in the overall SGR between the experimental groups (figure 38).

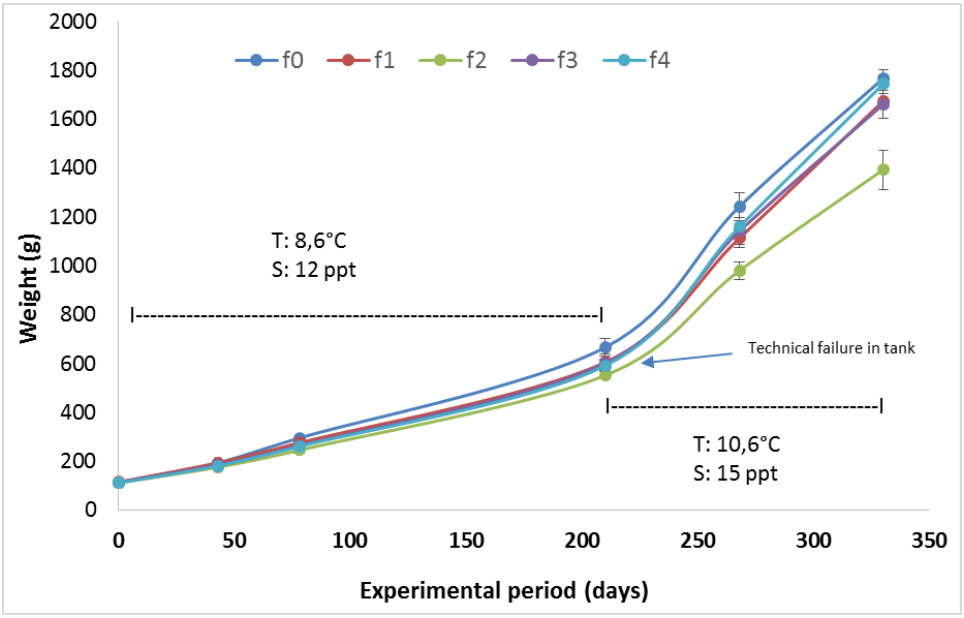

Figure 36: *Growth of the experimental groups during the experimental period. The trials were run in two phases in different culture systems. Average temperature and salinity shown (mean and error bars is SEM. n=3).*

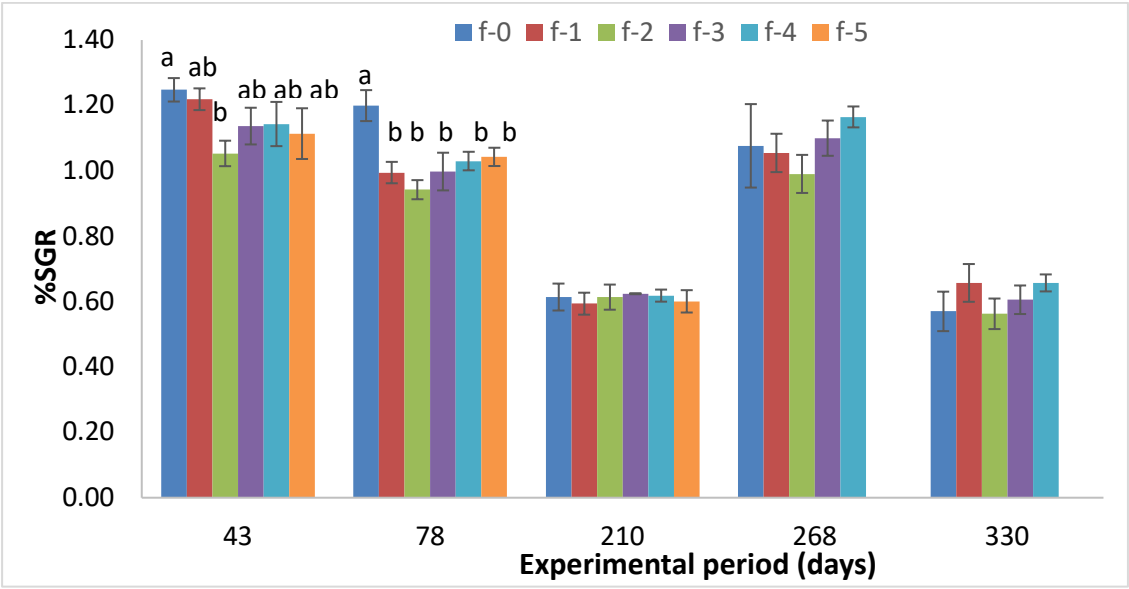

Figure 37: *Specific growth rate of experimental groups during the experimental period (days). SGR calculated in time periods from day-0-43, 43-78, 78-210, 210-268 and 268-330 respectively.*

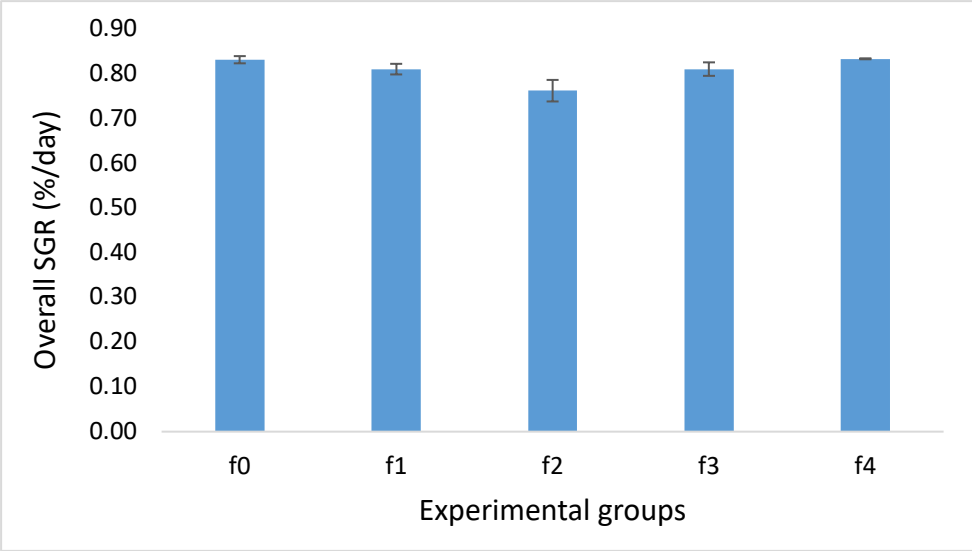

Figure 38: *The average specific growth rate (%SGR ±SEM) of experimental groups during the entire experimental period (330 days).*

The Thermal Growth Coefficient (TGC) shown in figure 39 shows a similar trend as the SGR and the actual values indicate that the fish is thriving well, particularly in a period after the shift from the 0,8m3 tanks to the 1,5m3 tanks. The sum of day-degrees was 2989 and the average TGC was from 2,12 (f-2) to 2,42 (f-0 and F-4).

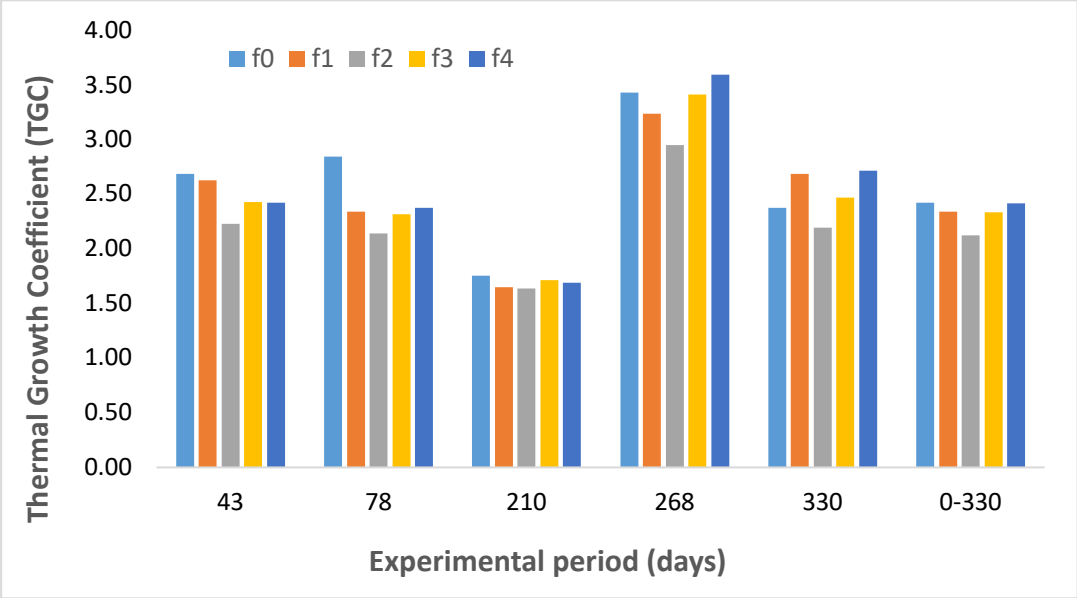

Figure 39: *The average thermal growth coefficient (TGC) in the experimental groups, between measuring days and during the entire experimental period (n=3).*

Feed intake and utilisation

There was no difference in feed intake between the different treatments in the experiment (results not shown).

The average feed conversion ratio FCR was 1.2 (figure 40). There were no significant differences found in FCR between groups.

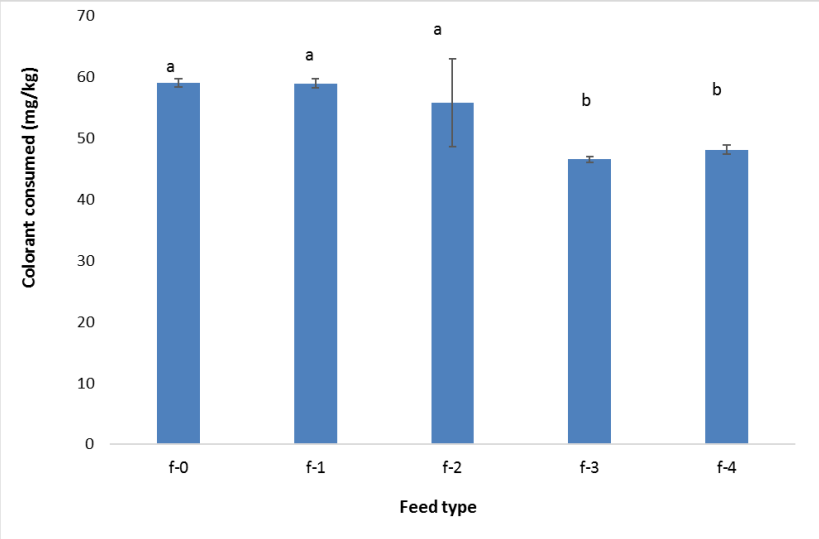

Figure 40: *Net feed conversion ratio (FCR) in the diet groups during the experimental period (mean and SEM, n=3).*

Sum of colorants consumed

Related to the coloring regime and feed intake of the fish the overall consumption of astaxanthin varied among the feeds given. The results are shown in figure 41. The f-3 and f-4 group fishes were consuming significantly less colorants in their diets compared to f-0, f-1 and f-2 groups. This is in consistency to the analysed astaxanthin content in the fillets (table 16) but is not reflected in the visual estimate done with Salmofan value (fig. 42).

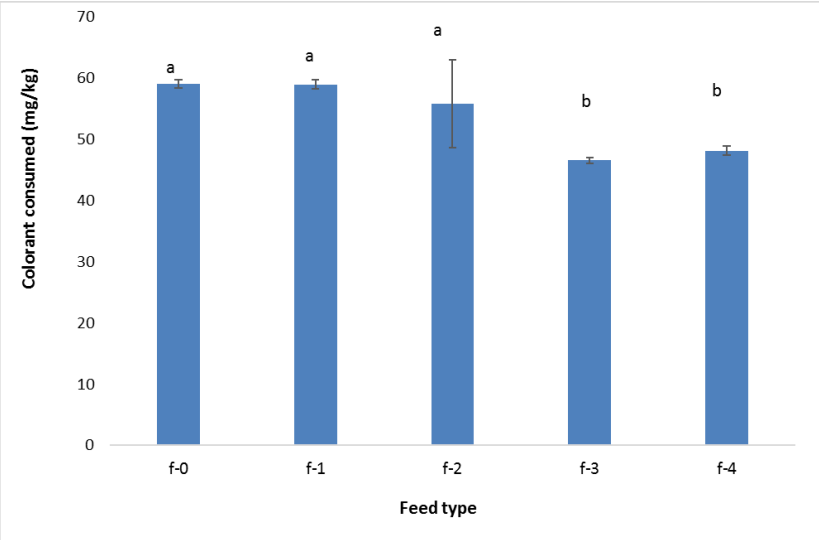

Figure 41: *Consumed Astaxanthin per kg fish produced, in the experimental groups during the whole growth period. (average ± SEM), n=3.*

Harvesting and yield- results

There are limited variations between treatment groups on conditions and yield, as expected (table 14). Maturity ratio is based of visual estimate of external signs of maturity at the final measuring day. The maturation is in the range of 10,8-24,5%, which can be expected in fish of this size and age (2+). The maturation ratio is highest in the treatment group (f-2) with the lowest average weight. Earlier mentioned, fish in one tank of the triplicate in this treatment group had problems, lowest growth rate, highest mortality rate and early maturation ratio, of unexplained reasons.

The condition factor for the fish is high, in the range of 1,71-1,82 and is reflected in the excellent fillet yield. Fillet yield of un-trimmed fillets, made by hand-filleting is close to 70% from whole bled fish. Gut loss was in the range of 9,7-11,7 and the hepato-somatic index was in the range of 2,5-3,4 with high variation in one group (f03). No differences were detected in the yielding factors between groups.

Table 14: *Samples taken for measuring harvesting output and condition of fish in the experimental groups. Five fish from each tank pooled for each treatment group (n=15).*

	f-0		f-1		f-2		f-3		f-4	
	average	SEM	average	SEM	average	SEM	average	SEM	average	SEM
Length (mm)	482,2	4,1	479,6	5,3	467,5	4,9	477,7	5,9	470,9	4,0
Weight (g)*	1932,4	62,2	1993,2	51,8	1756,1	61,7	1895,7	96,3	1841,1	71,5
K	1,72	0,05	1,82	0,06	1,71	0,04	1,72	0,04	1,8	0,0
Gutted weight (g)	1745,7	57,6	1758,9	55,7	1575,3	58,1	1706,9	88,2	1652,5	66,1
% gut-loss	9,7	0,3	11,7	1,6	10,4	0,4	10,0	0,3	10,0	0,2
HSI (%)	2,5	0,1	2,5	0,1	2,5	0,1	3,4	1,1	2,5	0,1
Fillet yield (%)**	70,4	0,5	69,3	1,3	69,6	0,6	70,3	0,5	70,3	0,7
Maturity at end (%)	10,8	2,5	15,6	3,4	24,5	1,1	18,8	0,9	13,6	0,9

*bled whole fish, ** Ratio from whole fish, filleted by hand, untrimmed fillet.

Chemical composition of fillets

The chemical composition of the fillets from fish in the treatment groups at harvesting is shown in table 15. The moisture content was in the range of 62,7-64,8%, the Lipid content in the range of 14,3-17,9, the protein content in the range of 18,6-19,3 and the ash content in the range of 1,1-1,4.

Table 15: *Chemical composition of fillets from fish in the treatment groups at the end of the experiment.*

Experimental feed	Moisture (%)	DM%	Lipid (%)	Protein (%)	Ash (%)
f-0	62,7	37,3	16,0	18,6	1,2
f-1	62,8	37,2	17,9	18,7	1,1
f-2	63,6	36,4	16,4	19,0	1,2
f-3	64,8	35,2	14,3	19,0	1,4
f-4	64,6	35,4	15,0	19,3	1,2

Fillet coloring and relations

The concentration of astaxanthin in the fish flesh (initial fish weight = 113,1g) at the beginning of the feeding trials was 0,61mg/kg for astaxanthin and 1,04mg/kg for astaxanthin and idoxanthine combined. There is a considerable increase in both astaxanthin and idoxanthin in the fish flesh during the experimental period as one can expect by feeding more colorants (table 16). The Panaferd colorant source may lead to some difference in astaxanthin vs idoxanthin ratio and content in fillets compared to fish who got and consumed similar amount of Aquasta (group f-0 vs f-1 and f-2). Final values of analysed Astaxanthin are low compared to A. salmon but in addition the fillets contain significant amounts of Iodoxanthin.

Table 16: *Analysed content of astaxanthin and idoxanthin (mg/kg) in fish fillets, from fish of initial size at start (a.w. 113g) and from fish at the end of the experiment (a.w. 1883g).*

		Astaxanthin (mg/kg)	Astaxanthin (mg/kg)	Idoxanthin (mg/kg)	Idoxanthin (mg/kg)	∑ Asta. + Ido. (mg/kg)	∑ Asta. + Ido. (mg/kg)
Color in initial fish		0,61		0,43		1,04	
Colorant in diet	feed no.		Increase ratio		Increase ratio		Increase ratio
Panaferd	f-0	1,33	2,18	1,70	3,95	3,03	2,91
Aquasta	f-1	1,23	2,02	2,30	5,35	3,53	3,39
Aquasta	f-2	1,13	1,85	2,13	4,95	3,27	3,14
Aquasta	f-3	0,99	1,62	1,80	4,19	2,79	2,68
Aquasta	f-4	0,86	1,41	1,57	3,65	2,43	2,34

The average color score using SalmoFan™ is shown in fig. 42, the test done on the twelve fillets per treatment. No statistical differences were found between treatment groups and the overall average value was 25,9 on the SalmoFan™ score card.

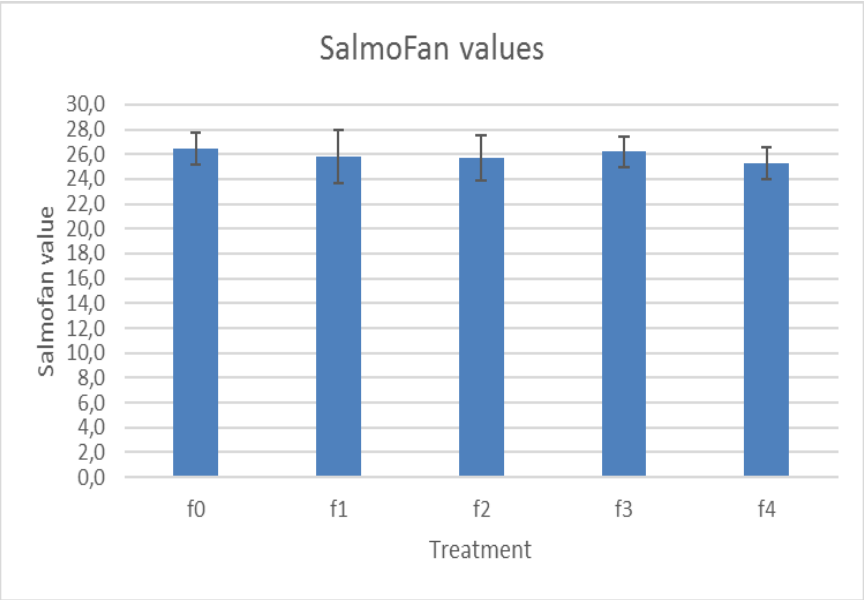

Figure 42: *Effect of dietary treatment on visual fillet color measured by SalmoFan,™(n=12)*

The average results of measurements using Minolta chromo meter, at three points in twelve fillets per treatment, are shown in fig. 43-45. Twelve fillet samples were taken from each treatment group, four from each replicate.

There appears to be limited variability in the L* and b* values but a tendency to a dietary effect on the a* value.

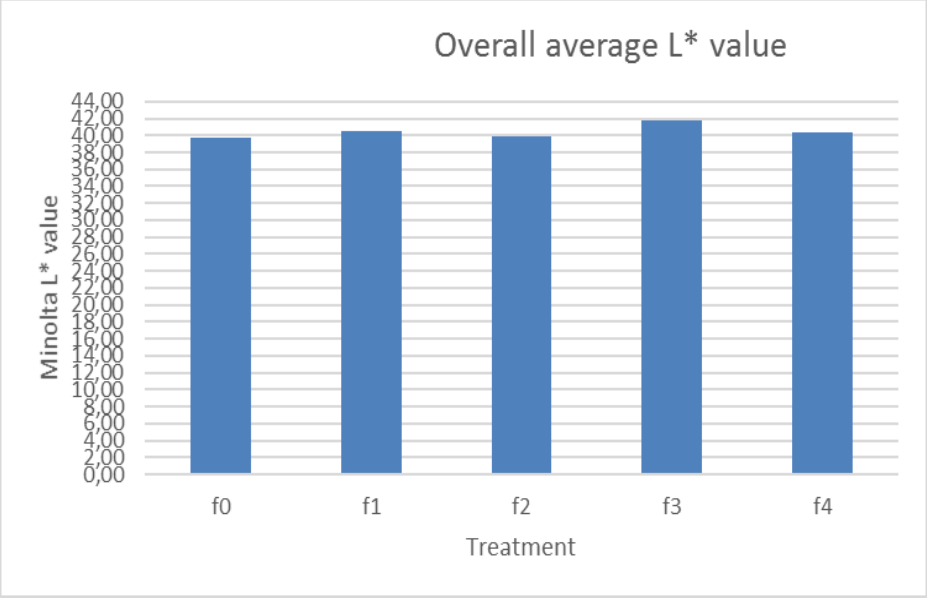

Figure 43: *Effect of dietary colorant origin and content on fillet color measured as L* value by Minolta chromo meter.*

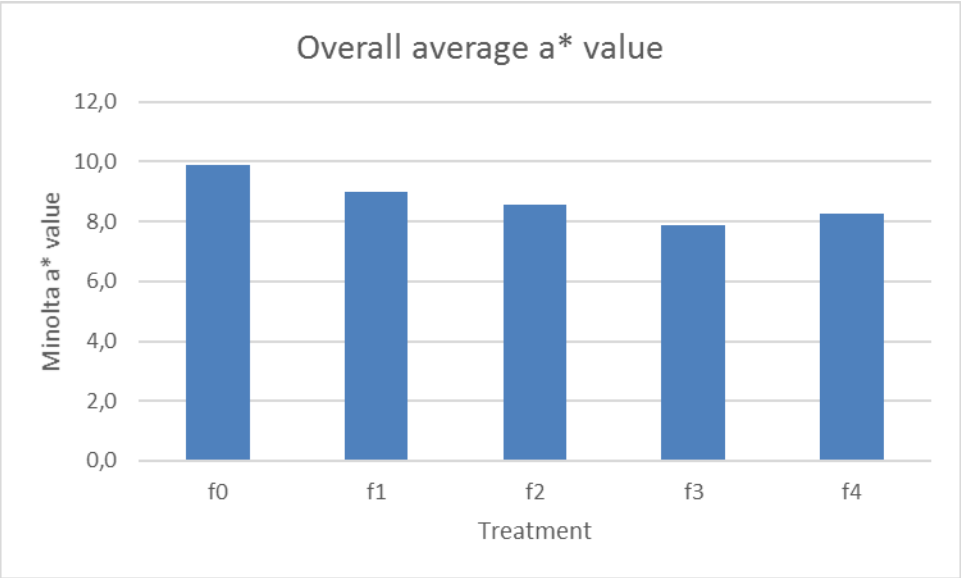

Figure 44: *Effect of dietary colorant origin and content on fillet color measured as a* value by Minolta chromo meter.*

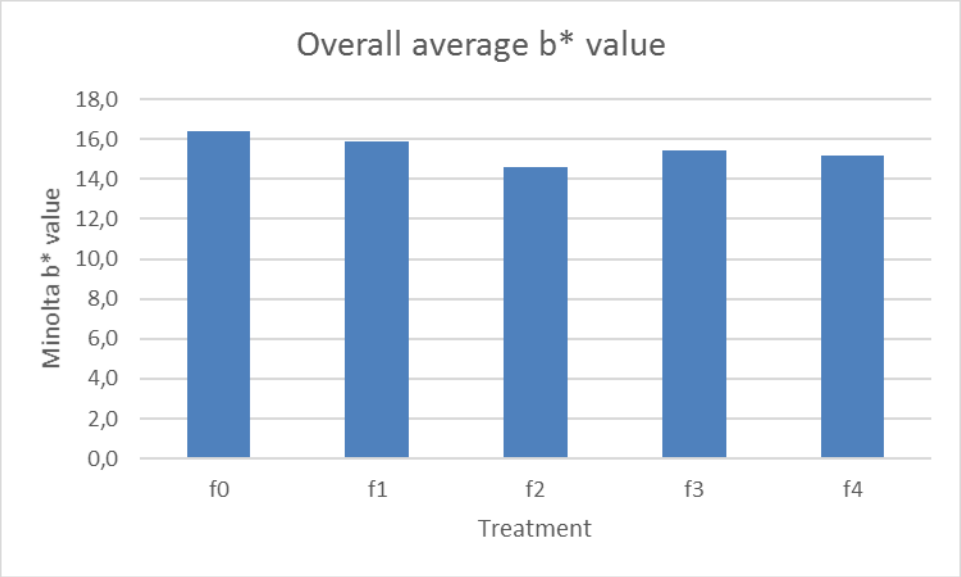

Figure 45: *Effect of dietary colorant origin and content on fillet color measured as b* value on Minolta chromo meter.*

The correlation between intake of Astaxanthin (figure 41) and analysed content of Astaxanthin and sum of Astaxanthin and Idoxanthin in fillets shows an effect of consumed colorant in feed on Astaxantin and Idoxanthin in the fillets (figures 46 and 47). More colorant consumed is reflected in the fillet color chemical composition.

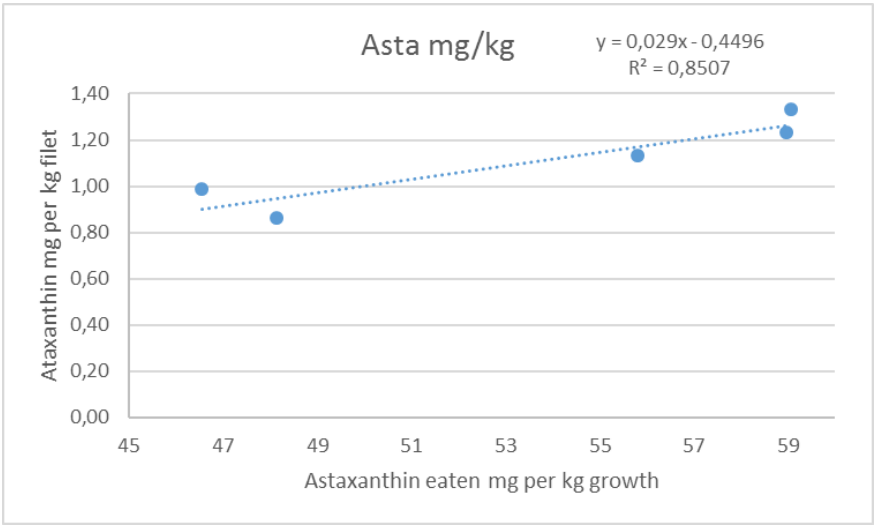

Figure 46: *The relationship between consumed Astaxanthin and analysed Astaxanthin in fillet at final sampling.*

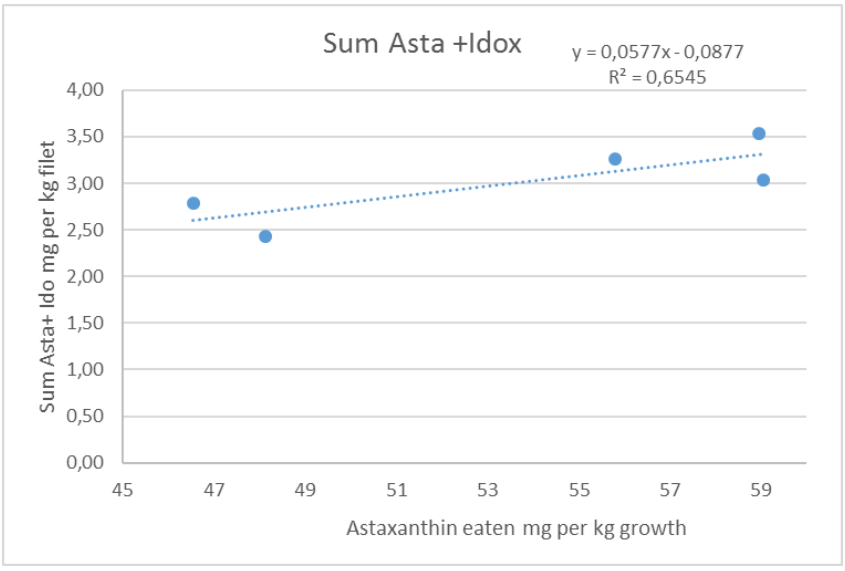

Figure 47: *The relationship between consumed Astaxanthin and sum of analysed Astaxanthin and Idoxanthin in fillet at final sampling.*

Discussion

Part I

In this part of the experiment, the fish increased its weight almost 10 times, from 160g to nearly 1460 g in average, during the experimental period of 281 days. This is in line with or better growth than in previous experiments with Arctic charr at similar temperature (average 7.2°C) and salinity (13,9 ‰). This can also be estimated as acceptable growth in these experimental culturing condition, considering fish size and temperature, when compared to earlier studies (Gunnarsson et al 2011; Gunnarsson et al. 2012). There was no significant difference in the growth response between treatment groups, i.e. no effect of replacing three quarters of the fish oil by a 50/50 mixture of rapeseed oil and palm oil or replacing 25% of the fish oil by Stearin figure 4 and table 4 & 5). No effects were detected of the Gross energy reduction in the diets by 1,1 Mega joule per kg. Neither was there any differences found in percent daily weight gain between groups, measured as Specific Growth Rate (SGR- figure 5). The feed intake, measured as feed eaten as percentage of average biomass per day, was not statistically different between treatments (figur 6 & 7). That indicate no palatability effect of replacing fish oil by either plant oils or Stearin. In the present experiment, no effects of different lipid ratio or gross energy content in the diets were detected on feed conversion ratio. i. e. feed eaten per kg growth. This finding are in contrast to other studies on Atlantic salmon (Hillestad et al 1998., Hemre & Sandnes 1999) but correspond to the results of Bendiksen et al. (2003).

No differences were found in harvesting output, measured as gutting loss and fillet yield, between the different treatments which could be related to lipid content or type of lipid in the diets. The fillet output of 70 percent as untrimmed fillet from whole un-gutted fish is remarkably high, indicating the high condition factor of well-fed Arctic charr of this size. The liver size (HSI) was also unaffected of different diet composition. The nutrient composition of fillets was measured after132 days from experimental start and at the end, after 281 days. Some variations were detected during the growth period, particularly in the lipid content which attended to increase as the fishes grew bigger. No clear effect of lipid ratio was detected on nutrient content in fillets. This is in line with normally moderate effect of dietary lipid (energy) on fillet fat in salmonids. A clear effect was found of dietary fat origin on the fatty acid composition in the lipid fraction in the fillets. The fatty acid composition in the fillet was reflecting the composition in the diets, with few exceptions: The sum of DHA and EPA was similar in the fillet lipid as in the dietary lipid fraction. However, looking at DHA and EPA separately DHA was in higher ratio in the fillet fat than in the dietary fat. The opposite was found for the EPA. This indicates that DHA has higher retention and is preserved rather than metabolized, perhaps in favour of EPA. Other explanation might be that elongation of consumed n-3 fatty acids is directed towards synthesis of DHA in preference of EPA. This is in line with the findings of Heissenberger et al. (2009).

The analyses of colorants in the experimental diets showed some variation in the content of both Astaxanthin and sum of analysed Carotenoids. Highest content (and equal) was detected in the diets containing HO/MIX and HO/Sterin lipids. The effect of this dietary difference can be seen in all of the three measurements or estimates of colorants in fillets. There is a good consistency between the fillet color score measured by SalmoFan™ (26,5) and L*value, a* value and b* value measured by Minolta chromo meter. The chemical analyses showed Axtaxanthin concentration in fillets close to 2 mg per kg, which is low compared to values normally found in Atlantic salmon (Bendiksen et al. 2003; Bjerkeng et al. 2000). Idoxanthin was in range of 0,5-0,9mg per kg and sum of all analysed Carotenoids up to a range of 4-5,8mg per kg fillet. It can be questioned if the correlation between visual colour and chemically analysed Carotenoids is the same in Arctic charr and other Salmonids. The analysed content

of Idoxanthin and the ratio of idoxantin to Astaxanthin is indicating minimal effect of maturation on flesh colour he at the end of the experiment. There were no external signs of maturation of fishes sampled for flesh color and fillet output.

Part II

During the feed extrusion process in the diet preparation, mix of dry raw materials is subject to high pressure and temperature. Analysis of colorant concentration indicate a considerable loss of added colorants as an effect of the extrusion. However, the losses can vary considerably. Limited knowledge is existing on the processing loss of the organic colorants Panaferd and Aquasta, which were tested in this experiment. Laxa Feedmill has been producing feed using these colorants for some time, assuming a process loss of around 30 percent. This assumption was used when formulating the diets in this experiment. Unfortunately, the chemical analyses of the actual content of carotenoids was delayed, resulting that actual content of Astaxanthin in the experimental diets became quite different from the original plan. Even the different ratio in planned inclusions was deviated due to variable Astaxanthin recovery of added colorant in the diets (table 13). Attempt was made to adjust the inclusion plan in the last phase of the experiment (6 mm pellets) by overdosing the colorants in the formulation, according to the recovery in the previous phases. Chemical analysis shows considerable over-shoot of astaxanthin content in this formulation with recovery ratio above 100% (table 13). These results of fluctuating colorant recovery in the feed emphasize the importance of correct information on colorant stability in the feed production process. It is urgently needed for the quality assumption of the feed.

The extrusion process is in general considered to be beneficial for the quality of the feed. The process involves water, heat, pressure, and mechanical stress in the organic compounds of the ingredients. Carotenoids like astaxanthin are potentially sensitive to such treatments and stability is also affected by storage methods and conditions. Anderson and Sunderland (2002) studied effect of extruder moisture and dryer processing temperature on astaxanthin stability. In their study, using synthesised astaxanthin, (CAROPHYLLR Pink® 8%) in common salmon feed formulation, the retention was in average of 86%, through extrusion. They concluded that astaxanthin stability in the final product of fish feed was most dependant on extruder discharge moisture and dryer processing temperature. Haaland et al. (1993) reported retention values for astaxanthin in extruded feed in the range of 86-94%. In this experiment a synthetic astaxanthin was not used but comparison made of more organic astaxanthin sources, Panaferd and Aquasta. Panaferd is a product of dried sterilised cells of a red carotenoid-rich bacterium (Paracoccus carotinifaciens). AQUASTA is a feed additive consisting of dried, inactivated, astaxanthin-rich yeast, containing nominally 1% w/w astaxanthin. Stability studies with Panaferd performed during feed processing have shown astaxanthin losses due to extrusion as high as 32 to 36 %. However, astaxanthin losses ranging from 8 to 11 % and canthaxanthin losses from 8 to 22 % were found in another study. A weekly loss of 1% during feed storage for both pigments have also been reported (NN-The EFSA Journal (2007) 546, 1-30). The stability of AQUASTA has also been examined during preparation of feeding stuffs. No significant degradation of astaxanthin or change in relative proportions of isomers occurred during manufacture of the mash has been reported. Samples of the pellets were used in a stability test, packed in bags typically used for fish feed (20 kg capacity), and stored at 25ºC and 60% relative humidity for one month. A loss of 20-30% astaxanthin was found after one month in these storage conditions. The data confirms that astaxanthin is sensitive to

oxidative damage and losses of this magnitude are not unexpected (NN-The EFSA Journal (2005) 320, 1-19). The potential instability of both Panaferd and Aquasta during feed processing and storage might explain the difference of added and analysed astaxanthin in the 3mm and 4 mm diets. The overshot in the 6 mm diet is left to be explained. This fluctuation and instability of colorants during processing and storage of feed needs more detailed research. In this part of the experiment, the fish increased its weight nearly 18-fold during the 331 days experimental period. An overall average SGR was around 0,8 percent per day with no major difference detected between groups. The thermal growth coefficient (TGC) though the period is about 2,35-2,42 except for the f-2 group where it was a collapse in one tank and TGC slightly lower (2,12; fig. 39). At the temperature range applied, this is considered as a very good growth of Arctic charr. The growth development in the different periods of the experiment showed an effect of moving the fish from 0,8m3 tanks to the 1,6m3 tanks. This is indicating the density or the tank space may have limited the growth rate of fish of that size, particularly at the final stage in both systems. Even though the nutrient composition of all diets was the same, except for the inclusion of colorants, a significant difference was found between different treatments in the two first periods (78 days) where fish in one replicate of treatment for diet f2 showed considerable drawback in growth. Later on, the growth in this group turned to normal but the average weight in this group remained smaller until the end of the trial. Anyway, it was decided to keep the outlier tank in the calculations although it might be expressing differences which are not related to treatments applied.

As expected no significant differences were found in average feed intake or in average feed conversion ratio. The variation within the replicate of diet f2 was higher than in other groups, probably due to drop in feed intake in one tank, affecting the average value of that treatment group. The harvesting output and nutrient composition of the fillets was also similar between treatment groups.

The actual coloring regime for the different treatments in the experiment (table 12 and figure 35) led to differences of overall intake of Astaxanthin between treatments groups. Treatments f3 and f4 consumed less Astaxanthin than the tree other treatments, measured as mg per kg fish produced. Chemical Analyses of the two main carotenoids, Astaxanthin and Idoxanthin and the sum of the two, in fillets showed corresponding differences to the intake of Astaxanthin. The content of Astaxanthin and the sum of Astaxanthin and Idoxanthin were moderate compared to values found in Atlantic salmon of similar development stage. No differences were detected in the measurements of visual color, either estimated by SalmoFan™ or measured by Minolta chromo meter. The a* value in the chromo meter analyses corresponded to the intake of Astaxanthin. Although the fish in group f-3 and f-4 consumed in total about 15mg/kg less carotenoids, according to calculations and analysed colorants in diets (fig. 41 and table 12) there was no visual color difference in the fillets measured by SalmoFan™ score or the L* and b* value in Minolta measures, although the a* value was lowest in these groups. This is indicating no detectible gain of increasing the amount of colourant above 50 mg/kg in the diets.

Closer look at the analyses of carotenoids in the fish fillets showed the ratio between Idoxanthin and Astaxanthin as 0,7 at the beginning of the experiment. At the end the ratio was on average 1,7. According to several authors the transformation of astaxanthin to idoxanthin, in Arctic charr, seems to diminish with increasing weight/age until the onset of sexual maturity (Aas et al.,1997; Hatlen et al., 1997; Bjerkeng et al., 2000). This is not confirmed in the present experiment, as relationship between signs on maturation on the fish at slaughter and analysed content of Idoxanthin was quite moderate.

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