Auðlindadeild Viðskipta- og raunvísindasviðs

LOK1126 og LOK1226

Optimum growth in turbot farming – protein substitution in feed for <500 g turbot

Lokaverkefni til B.S. gráðu í sjávarútvegsfræði

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Front picture: Turbot (*Scophthalmus maximus*), (Reference:www.fishbase.org)

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Undirskrift leiðbeinanda

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Rannveig Björnsdóttir, dósent Auðlindadeild

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Abstract

A dietary study was conducted to investigate the effect of partly substituted levels of fish meal in feed for <500 g turbot (*Scophthalmus maximus* Rafinesque 1810) on growth performance. The experimental diets were formulated to contain 53, 73 and 93 % fish meal protein and different levels of plant protein substitutes (wheat, corn gluten, soybean and canola meal) with three replicates for each diet. The fish (N = 252; initial mean weight ± SD, 140 ± 37 g) were tagged with PIT and randomly distributed into 9 rearing tanks (1.47 m^3) and handfed to satiation 6 days a week for a period of 138 days. The temperature and salinity level were kept at a constant level of (mean ± SD) 15.4 ± 0.6 °C and 21.4 ± 1.8 ‰, respectively.

Weight development was significantly affected by the dietary treatments. Fish fed lower fish meal diets displayed a slower weight development throughout the entire experiment compared to fish fed the high fish meal diet. However, no significant differences were shown between dietary treatment groups for specific growth rate, feed conversion ratio, daily feed intake and total feed consumption.

Results demonstrate that substitution of fish meal by plant protein raw materials down to 53 % fish meal does not affect growth of turbot < 500 g.

Keywords: turbot, growth, protein, substitution, feed conversion

Ágrip

Tilgangur tilraunarinnar var að kanna áhrif útskiptingar fiskimjöls fyrir plöntuhráefni á vöxt sandhverfu undir 500 g að þyngd. Þrjár gerðir tilraunafóðurs innihélt mismunandi hátt hlutfall fiskimjölis (93, 73 og 53 %) og mismunandi plöntu hráefni (hveiti, maís, soja og repju mjöl). Fóðurgerðir voru prófaðar í þrítekningu.

Sandhverfur (N=252, upphafsþyngd ± SD, 140 ± 37 g) voru örmerktar og þeim dreift tilviljunarkennt í níu 1.47 m^3 ker. Handfóðrað var til mettunar tvisvar á dag, 6 daga vikunnar yfir 138 daga tímabil. Vatnshiti (15.4 ± 0.6°C, meðaltal ± SD), selta (21.4 ± 1.8 ‰) og súrefni (101.7 ± 7.1 %) var mælt daglega og stillt eftir þörfum.

Hlutfall fiskimjöls í fóðrinu hafði marktæk áhrif á þyngdarvöxt fisksins sem fór vaxandi með auknu fiskimjölshlutfalli. Ekki reyndist þó vera marktækur munur í dagvexti né fóðurstuðli og étnu fóðurmagni fiska í tilraunahópnum.

Niðurstöður benda til þess að hægt sé að skipta út allt að 40% fiskimjöls fyrir plöntu hráefni í fóðri fyrir smærri sandhverfu án þess að það hafi áhrif á vöxt.. Þetta leiðir til verulegrar lækkunar framleiðslukostnaðar og stuðlar að aukinni sjálfbærni sandhverfueldis.

Lykilord: sandhverfa, prótein, útskipting, vöxtur, fóður

1 Introduction

1.1 Turbot (*Scophthalmus maximus*)

The turbot (*Scophthalmus maximus*) is a left-eyed flatfish of the family *Scophtalmidae*, a demersal predatory fish native to brackish and marine waters, mainly in the area from the Black and Mediterranean Sea to the North Atlantic and the Baltic Sea (Seafish Industry, 2002).

Wild turbot populations have been overfished for years (FAO, 2012) . Today, the majority of available turbot is cultivated in aquaculture (Figure 1-1). Limited global production, a firm flesh and a mild flavor have made the turbot a highly valuable "gourmet" fish.

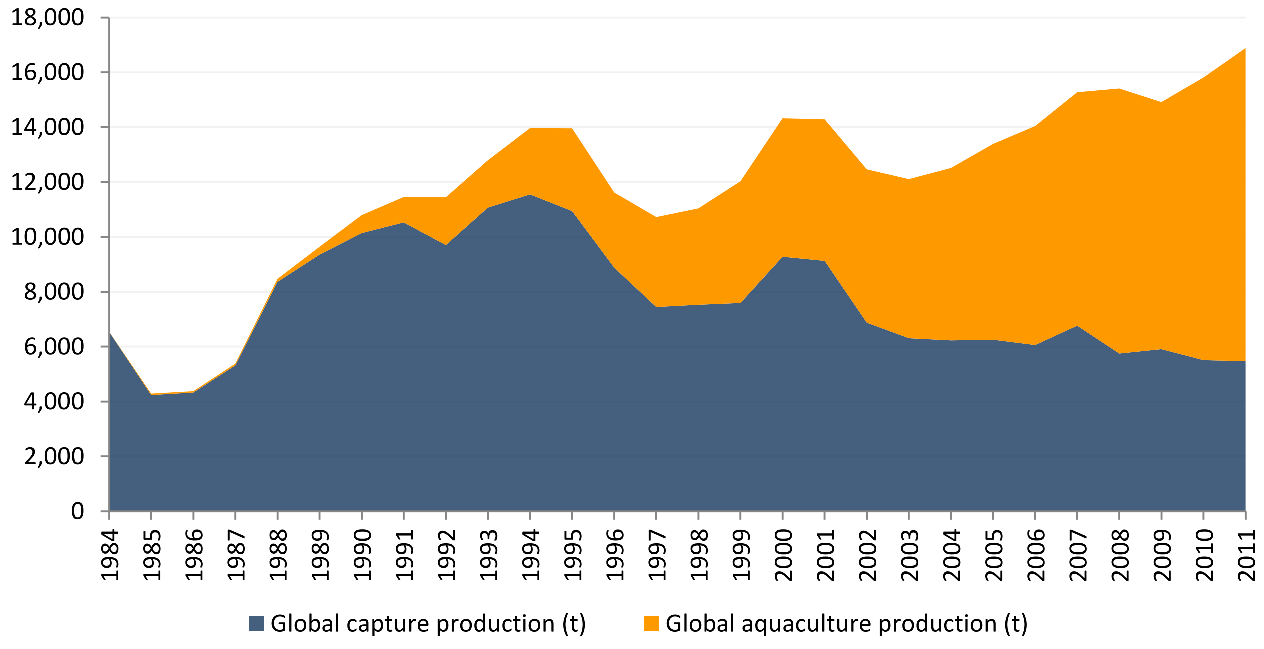

Figure 1-1: Global turbot production 1984 – 2011 (FAO FishStat)

History of turbot aquaculture

Turbot aquaculture was started in Scotland in the 1970s and subsequently established in Spain and France. In the beginning the production was restricted for some years by a limited juvenile supply but technological developments in the early 1990s led finally to an expansion (FAO, 2013). Today, Spain, France and Portugal are Europe's main producers with a well-established and large scaled on-growing industry (Figure 1-2). Although a number of other

European countries are involved, Spain, and here particularly the Galicia region, has become the main EU producer. China has been reporting an extensive turbot production since 2003, but these quantities appear to be difficult to confirm (Figure 1-3).

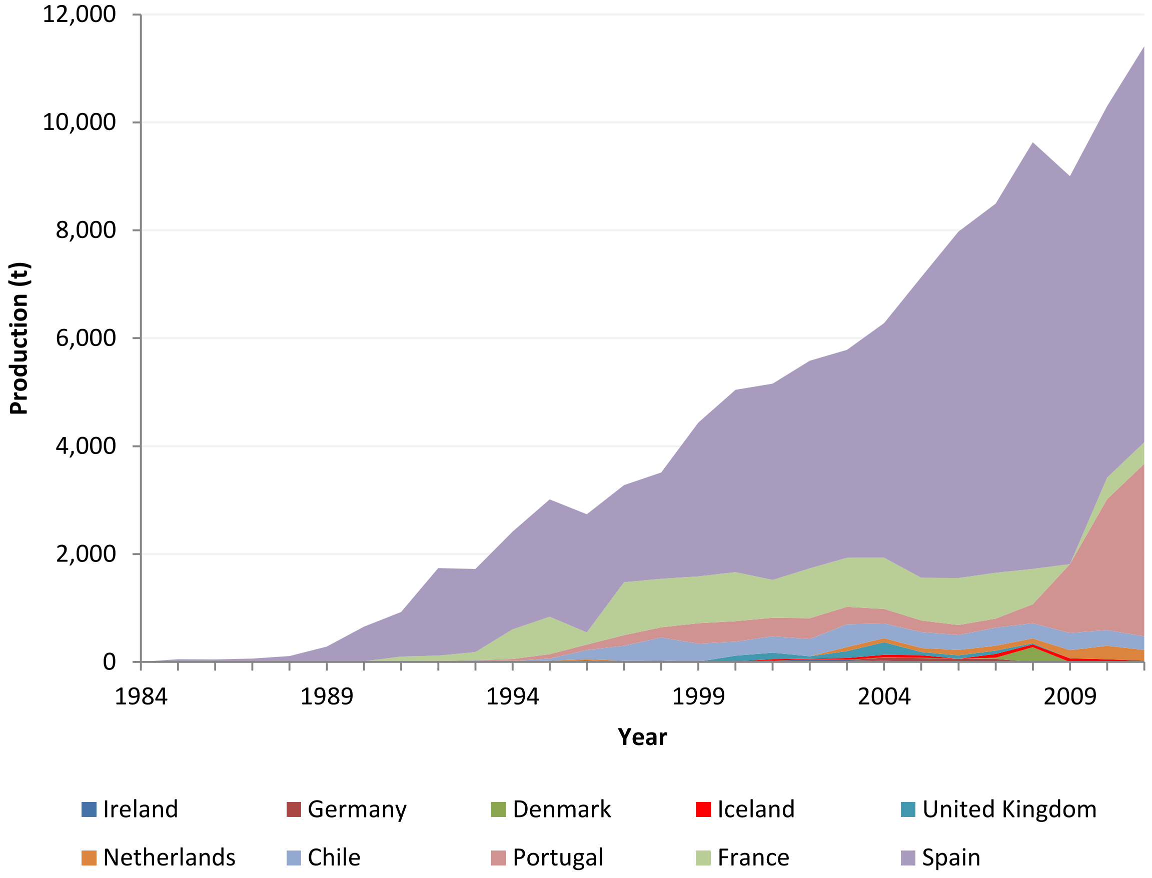

Figure 1-2: Main turbot producing countries (FAO FishStat)

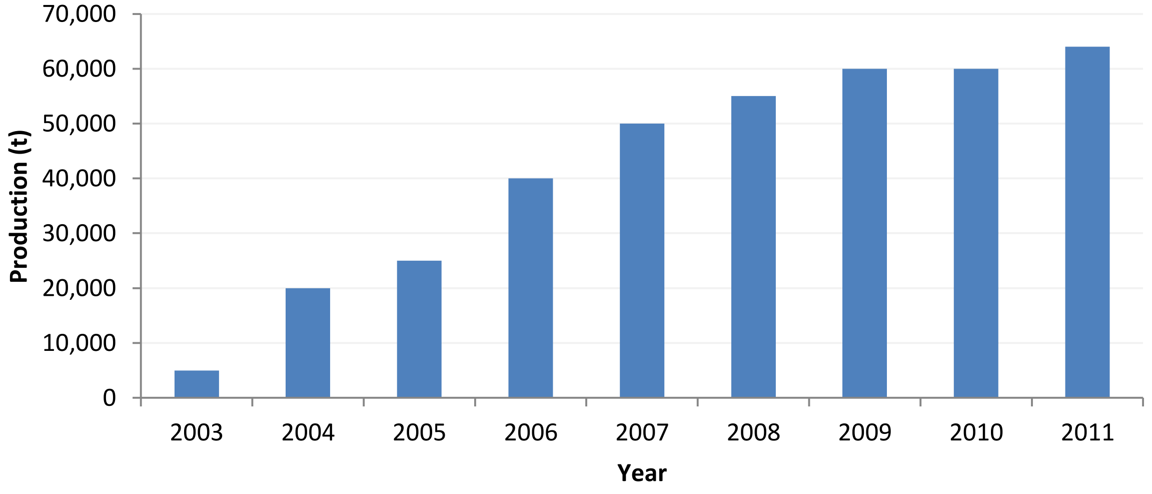

Figure 1-3: Reported Chinese aquaculture production of turbot – unconfirmed quantities (FAO FishStat)

1.2 Turbot farming

Production process

Reproduction

Reproduction of farmed turbot is carried out under strictly controlled conditions at technologically highly sophisticated hatcheries. Broodstock, often consisting of both farmed and captured individuals, are kept at low densities, under specific photoperiod and temperature conditions, which provides eggs all year round (EC, 2013).

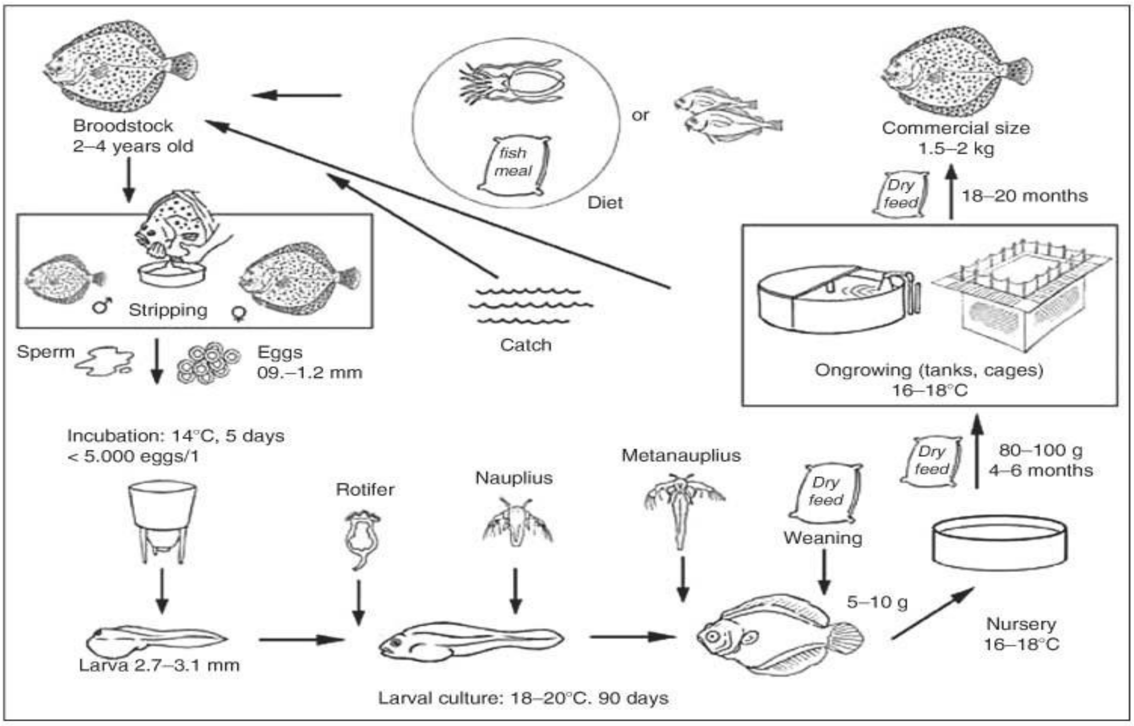

Figure 1-4: Production cycle of turbot (www.thefishsite.com)

Hatchery / nursery phase

The eggs are pelagic and are placed in incubation tanks until hatching. Larvae are reared at low densities, absorbing the yolk sac first. When they are able to open their mouths, they are fed zooplankton and subsequently *Artemia* nauplii. After four to five weeks they are adapted to commercial artificial diets and the juveniles fed on dry feed until they reach a weight of 5-10 g (Figure 1-4). Some hatcheries keep juveniles in a closed recirculation system, which allows a better control of the rearing environment.

On-growing and grow-out phase

At the end of the hatchery / nursery phase the juveniles are transferred to larger rearing units for a pre-fattening period of several months until they reach a weight of around 100 g. (EC, 2013). During this time stock density in the tanks that are normally 10 to 20 square meters in surface, increases from 10 to about 25 kg per square meter. Before transferred to larger on-growing tanks the fishes are usually graded to maintain size as homogeneous as possible in order to guarantee optimal feeding. This grading marks the end of the on-growing phase.

Grow-out of turbot usually takes place under intensive rearing conditions. Tanks get bigger with increasing fish size and are usually around 100 to 150 square meters in surface with a water depth of 50 to 90 cm. The average rearing density is between 20 and 40 kg per square meter (EC, 2013). Turbot takes about two to three years to grow to commercial size of 1.5 to 2 kg from fertilized egg (EC, 2013).

Feed and feeding routines

Turbot in the on-growing and grow-out phase is normally fed with dry, slowly sinking or floating pellets. Pellet size is adjusted individually to the size of the fish at each stage. Special diets were developed when turbot aquaculture expanded, containing a nutritional well balanced formulation to meet the requirements of turbot at different stages (see Nutritional requirements of turbot1.3).

Daily based manual feeding to apparent satiety is the most common procedure in turbot feeding in order to limit food deprivation and water pollution. Feeding behavior can be visually monitored from above the water and food supply is stopped as soon as uneaten pellets are observed. Visual feeding observation can be difficult in high stocking densities during the grow-out phase. Therefore floating feed has been developed which allows the observation of feeding behavior at the water surface and limits food deprivation.

Rearing conditions and methods

Previous research has shown the effect of temperature (e.g. Imsland, 1996, Imsland et al., 2000), and salinity (Imsland et al., 2001) on growth rate of turbot. Results indicate that the temperature to support optimal growth of turbot decreases rapidly with increasing size. Optimum temperature for growth of juvenile turbot in the size range up to 25 g is between 19 and 22 °C, for 25–75 g between 16 and 19 °C and between 13 and 16°C for 100 g turbot and larger. Growth and food conversion efficiency of turbot can verifiably be improved by rearing at intermediate salinities.

Farming of turbot during the on-growing and grow-out phase is normally taking place in outdoor, shore-based, square or circular tanks with open-circuit pumped sea water. The tanks are covered to protect the fish from sunburn. In some areas with seasonably varying sea water temperatures electricity is used to either heat the water to optimum temperature or cool it down. Still, only a small part of turbot is produced in recirculation aquaculture systems (RAS) although this would reduce the cost for heating and pumping. Trials have been carried out for turbot rearing in shallow raceways (e.g. Labatut & Olivares (2004)), which are already widely used for other species. Results indicate that RAS in combination with shallow raceways allow high fish densities and improve overall productivity.

1.3 Nutritional requirements of turbot

Most studies on nutritional requirements of turbot refer to the juvenile phase. In general, high protein requirements have been documented for turbot (Guillaume, 1991) although suggestions for protein levels vary in relation to fish size.

For the size range 0 – 10 g suggestions for optimum protein levels vary between 35% (Adron et al., 1976), 69.8% (Caceres-Martinez et al., 1984) and 50% (Danielssen & Hjertnes, 1993) crude protein (CP) in dry matter of feed. For larger turbot Cho et al.(2005) showed a minimum protein level of 55% CP for 47 g fish and Cho et al. (2003) reported that 49.4% CP in dry matter support optimum growth for turbot of 89 g. Other results indicate that a level of 69.4% CP was adequate to support optimum growth and protein sufficiency for 100 g turbot (Nijhof, 1993). A recent study on turbot >500 g (Leknes, et al., 2012) shows that CP level in feed for grow-out turbot can be reduced to 43.5% under optimal rearing conditions without affecting maximum growth and feed efficiency.

Although these results might be influenced by variations in protein quality and origin, protein/fat energy ratios, feeding routines and rearing conditions – a trend to decreasing protein demands following increasing fish size can be detected. Similar trends have been shown in studies on Atlantic halibut (Árnason, et al., 2009) and Atlantic cod (Árnason et.al. 2010). The fact that the minimum CP level in order to support optimum growth could be lower than the CP level in actually used commercial fish feed, as concluded for Atlantic halibut in (Árnason, et al., 2009), could affect feed production cost considerably.

Studies regarding lipid requirements in diets for < 500 g turbot suggest optimum levels of 16 to 17% (Nijhof, 1993, Sæther & Jobling, 2001, Cho et al.,2005). Studies on mineral and vitamin requirements of turbot could not be found.

1.4 Protein substitution in fish feed

A fast growing aquaculture industry with its increasing demand for fish meal and fish oil as the main protein and lipid sources in fish feed leads to limits in fish meal and fish oil production. High market prices for fish meal and – as a result of that - higher production costs force aquaculture industry to search for cheaper, but still effective and more sustainable alternatives.

Fournier et al. (2004) have indicated the possibility to substitute fish meal in diets for turbot for alternative protein raw materials, which would have an important implication on feed cost, protein economy and environmental sustainability. When substituting components in fish diets the balance of nutrients still has to satisfy the nutritional requirements of the fish which in fact seem to be different during the on-growing and growth-out phase (Árnason, et al., 2009). The fact that the protein level in most plant raw materials is lower than in marine fish meal allows a wide range of raw materials for diet formulation. Former trials on turbot have mainly focused on corn gluten meal, soy bean or lupine meal as an alternative protein source (Regost et. al., 1999, Fournier et. al., 2004).

Main plant protein raw materials

Wheat gluten is shown to be an excellent protein source, containing 70-80% protein which is reported to be highly digestible to rainbow trout other fish species (Sugiura et al.,1998). Studies show that up to 25% of fish meal can be replaced with wheat gluten without negative effects on growth or feed conversion ratios (Weede, 1997). However, a relatively high price level because of human consumption still limits the usage of wheat gluten in fish feed formulation.

Corn gluten is a low prized plant protein material and contains a minimum of 60% protein (Morales et al.,1994) which is for example 97% digestible to trout (Sugiura et al.,1998). Corn gluten can for example substitute up to 40% of fish meal without negative effects on growth or feed conversion ratios in trout (Morales et al. 1994), (Weede, 1997).

Soybean products are generally high in protein content, ranging from 45% in soybean meal to 70% and more in soy protein concentrate. Soybean meal is considered to be one of the most nutritious plant protein material because of its suitable protein content and amino acid profile and has been tested on different fish species, such as Japanese flounder (Kikuchi et al.,1994), (Kikuchi, 1999) and Atlantic halibut (Berge et al.,1999).

Studies on crude rapeseed and canola products as a partial replacement of fish meal in diets for turbot (Burel et al.,2000a,b) have shown lower nutritional qualities despite a well-balanced amino acid profile.

Rapeseed and other plant-derived protein sources contain antinutritional factors (ANF) which determine their quality for fish nutrition but certain processing techniques can reduce the level of ANF, increase protein level and improve the value of plant protein products (Naczk & Shahidi, 1990), (Chabanon et al.,2007) .

The substitution of fish meal for not only one but a composition of several more cost-efficient plant protein sources is a common approach in order to minimize the amino acid deficiencies in fish diets and meet the requirements of the specific fish species. The present study was set up in a 3 x 3 factorial design to investigate growth performance for three different dietary treatments with partly substituted levels of fish meal for various levels of wheat, soybean, corn gluten and canola meal in feed for <500 g turbot.

2 Materials and methods

2.1 Fish

The fish for this experiment was provided by The Marine Research Institute of Iceland (Hafrannsóknastofnun) which operates an in house mariculture laboratory in Grindavík at the Reykjanes peninsula in South West Iceland using filtered sea water (salinity 32 ‰) which is partly heated with 60°C hot geothermal water through a heat exchanger. The turbot broodstock consists of both captured and farmed individuals and by using different light and temperature conditions spawning periods are generated twice a year. Turbot eggs were hatched in January 2011 and fed with rotifers and *Artemia* nauplii during the first 30 days. During that period water temperature was increased from 13°C up to 22°C. Start feeding with dry food was initiated at day 25.

In October 2011, approximately three weeks ahead of experiment initiation, the juvenile turbot were transferred to the aquaculture research facilities at Verið ehf. in Sauðárkrókur (North Iceland) in order to acclimatize to new rearing conditions.

The experiment was based on 252 fish which were distributed randomly into 9 tanks and marked with Passive Integration Transponders (PIT tags) for calculations of growth rates. Initial weight was 140 ± 37 g (mean ± SD) and total average weight in each tank was 3.88 ± 0.17 kg (mean ± SD). No fish died during the experiment.

2.2 Rearing conditions

The circular tanks used in the experiment were made of black fiberglass with a diameter of 1.8 m^2 and a volume of 1.47 m^3. Average stocking density in the tanks at the initiation of the experiment (T_0) was 2.16 kg/m^3. The water outlet was centrically situated at the bottom of the tanks and waste water was led through a feed trap to monitor feed consumption. Tanks were set up in 2 rows. Rearing conditions (temperature, oxygen and salinity) could be adjusted separately for each row. The rearing parameters for this experiment were based on previous research on turbot. Water temperature was set to 15.4 ± 0.6 °C (mean ± SD) which is near optimum rearing temperature for turbot growth of this size (Imsland et al.,1996). Salinity level was kept at 21.4 ± 1.8 ‰ (mean ± SD) which is near optimal salinity level for turbot

(Imsland, et al., 2001). The fish were reared under hyperoxic conditions with oxygen saturation level kept slightly over 100% (see Table I in Appendix I).

An oxygen meter (YSI 550 A, Ohio, USA) was used to measure oxygen saturation levels; a digital pocket refractometer (Atago® Digital Pocket Refractometer, Tokyo, Japan) for salinity measurements and a glass thermometer for measuring water temperature. Oxygen level was measured daily near the water outlet in each tank. Water temperature and salinity were measured daily in the effluent water in each row and adjustments made if necessary.

The fish were reared under ambient light conditions (LD 16:8) throughout the whole experiment.

2.3 Fish feed and feeding routines

The feed used in this experiment was produced at the Laxá feed mill in Akureyri, Iceland. Three dry diets with different levels of fish meal and substituted plant protein (wheat meal, corn gluten meal, soy meal and canola meal) were fed. Raw material composition and the content of protein, lipids and gross energy are shown in Table 2-1.

The turbot were hand fed to satiation six days each week with 6 mm pellet with two feeding rounds each day, one in the morning and one in the afternoon. Excess feed was collected in feed traps and the number of uneaten pellets counted. The average pellet weight (N = 600, mean pellet weight = 0.281g) for each feed type was measured, and by multiplying the number of uneaten pellets with the mean weight of the pellet, the amount of uneaten feed could be calculated and subtracted from total feed supplied to the tank.

Table 2-1: Nutritional components and energy content of the feed used in the present experiment

Nutritional ingredients (%)								
Treatment	Wheat	Fish meal	Corn Gluten meal	Hipro soy	Canola meal	Fish oil	Vit./Min. Premix	**% Fish meal Protein (% FMP)**
85	21.4	58.4	0.0	0.0	0.0	19.2	1.0	**93**
86	9.8	45.5	1.9	15.0	6.7	20.2	1	**73**
87	8.0	33.0	17.0	17.0	2.9	21.1	1.0	**53**

Nutritional composition (%)

Treatment	Humidity	Dry matter	Crude protein	Crude lipid	Ash
85	9.8	90.2	43.3	22.1	10.8
86	8.6	91.4	41.3	24.5	9.8
87	8.4	91.6	42.2	23.0	10.7

Nutritional composition (% Dry matter)

Crude Protein	Crude lipid	Crude ash	GE MJ/kg
48.1	24.6	12.0	20.6
45.2	26.8	10.7	20.0
46.0	25.1	11.7	21.1

2.4 Experimental design and sampling

The experiment was initiated on 10 November, 2011 and terminated on 28 March, 2012. Each experimental feed was presented in triplicate tanks which were randomly distributed in separate rows in order to minimize possible tank effects.

Weight measurements were undertaken at day 0 (T_0, 10 November 2011), day 41 (T_1), day 71 (T_2), day 103 (T_3) and day 138 (T_4, 28 March 2012). Fish were starved one day ahead of sampling, and anesthetized with 3.33 ml l^{-1} of 2-phenoxyethanol, prior to weighing. A digital balance with ± 2g as margin of error was used for all weight measurements.

2.5 Calculations

Specific growth rate (SGR)

SGR was calculated according to the formula:

SGR (% day^{-1}): $100 \times (\ln BW_1 - \ln BW_0) \times (T)^{-1}$, where BW_0 and BW_1 are initial and final body weights, respectively and T is the count of days in the investigated period.

Feed conversion ratio (FCR) is based on the total biomass in each tank.

Calculations of FCR were made according to the following formula:

FCR: Period feed consumption x (period biomass growth) $^{-1}$

Total feed consumption (C_t) was calculated according to the following formula:

$C_t = F_s - F_c$, where F_s is supplied feed and F_c is collected feed in the feed traps. F_c was calculated by multiplying the total number of uneaten pellet with the average weight of one pellet. (Average pellet weight was measured separately for each of the dietary treatments).

Daily feed intake (DFI) is based on total feed consumption in each tank.

Measurements of DFI were based on the following formula:

DFI (% day^{-1}) = $100 * [C_t/((B_1 + B_2)/2) \times ((t_2 - t_1)^{-1})]$

(C_t) is the total feed consumption during the period between t_1 and t_2, and B_1 and B_2 are the total biomass at day t_1 and day t_2, respectively.

2.6 Statistics

The statistical software SPSS Statistics 21.0.0 (IBM, 2012) was used for all statistical analysis of the data in this experiment. A two way nested ANOVA (General linear model) was used to analyze differences in weight performance and SGR between groups and replicates (Zar, 1984) as well as for the overall group data from FCR, DFI and C_t. For the group data from FCR, DFI, and C_t a one-way ANOVA was used to test for possible group differences (Zar, 1984).

Statistical analysis of SGR, FCR and C_t were followed by a linear regression to reveal possible differences between dietary treatment groups (Zar, 1984) and a Leven's test to consider homogeneity of variance in data set.

If showing significant differences ANOVA analyses were followed by a Student-Newman-Keuls multiple comparison test to display differences between experimental groups (Zar, 1984).

A significance (α) level of 0.05 was used for all analysis.

3 Results

3.1 Weight performance and specific growth rate

Figure 3-1 displays the development of mean weights throughout the entire experimental period. Mean weight among dietary treatment groups at experiment initiation (day 0) varied between 139 g (73% FMP and 53% FMP) and 142 g (93% FMP) with no significant differences in weight between treatment groups observed (see Table VIII in Appendix II)

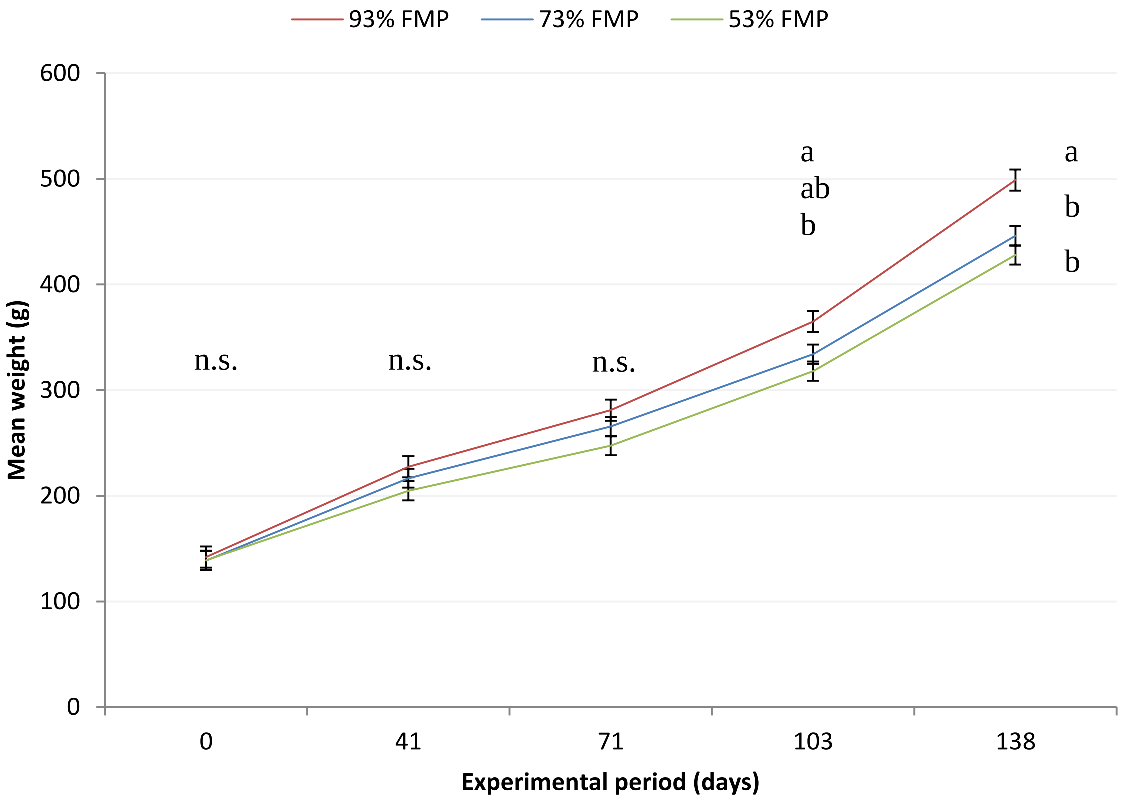

Figure 3-1: Mean weight of <500 g turbot, fed three different diets. Each line represents a different dietary treatment and shows mean weight ± SE at each sampling day throughout the entire experimental period (day 0 – day 138). (n.s./ a – no significant / significant differences between dietary treatment groups with "a" as the highest value).

At sampling day 41 mean weights ranged from 205 g (53% FMP) to 227 g (93% FMP). No significant differences between treatment groups were observed at day 41 (see Table IX in Appendix II), likewise at sampling day 71(Table X).

Significant differences were observed at sampling day 103 (Table XI) where the 93% FMP group had significantly higher mean weight compared to the 53% FMP group.

At experiments termination (day 138) mean weight for treatment 85 (93% FMP) was significantly higher (499 g) than for treatment 86 (446 g) and 87 (428 g) (Table XXXVI).

The development of specific growth rates (SGR) for each dietary treatment is displayed in Figure 3-2. SGR in the first experimental period from T_0 to T_1 is significantly lower for treatment group 87(0.95%) than for treatment 86 (1.08%) and 85 (1.15%) (see Table XXXVII in Appendix II).

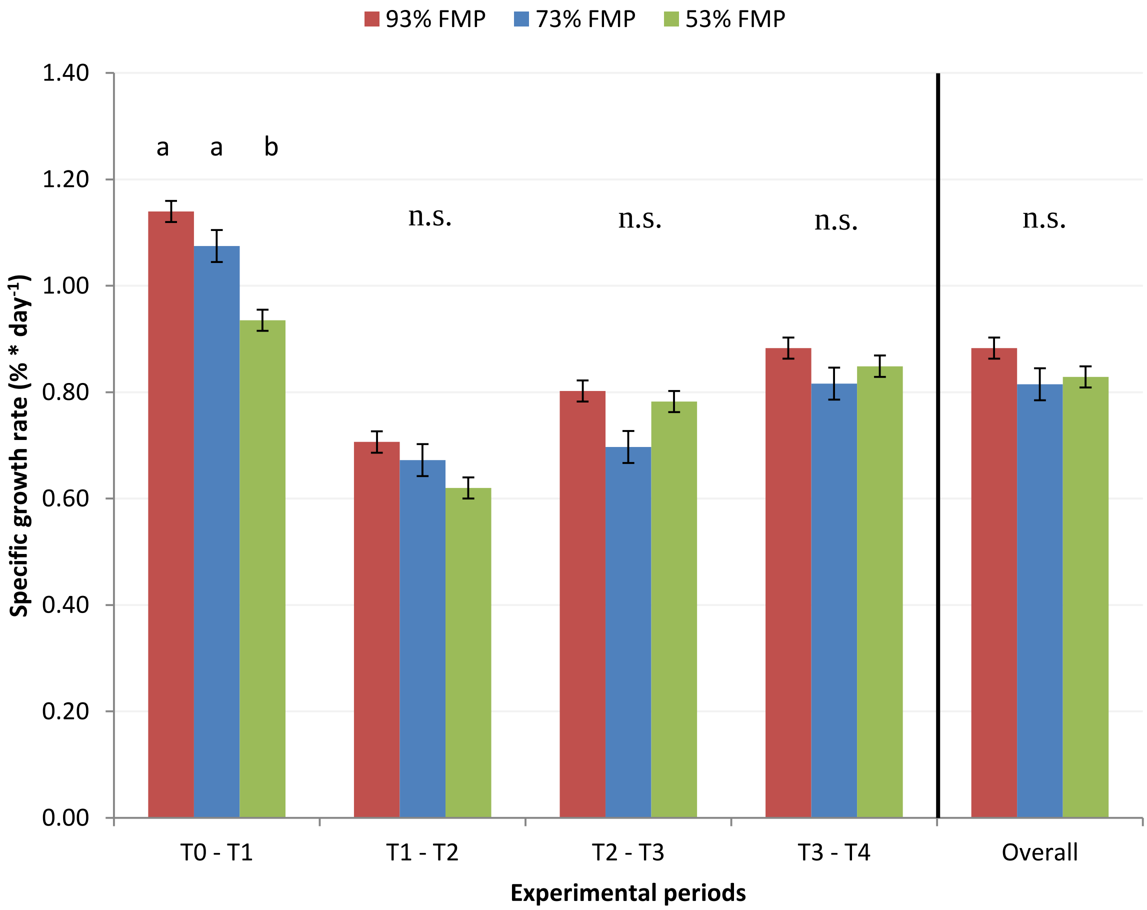

Figure 3-2: Specific growth rate (SGR) for < 500 g turbot fed three different diets. Each column represents a different dietary treatment and shows group means ± SE. (n.s./ a – no significant / significant differences between dietary treatment groups with "a" as the highest value).

For the period T1-T2 a significant drop in SGR (35 to 40%) for all treatment groups was observed which readjusted in the next period. However, no significant differences and deviations between treatment groups appeared in applied statistics for this period, as well as for T_2-T_3, T_3-T_4 and overall (see Table XXXVII in Appendix II).

3.2 Feed conversion ratio, feed consumption and daily feed intake

Figure 3-3 displays feed conversion ratio (FCR) between different dietary treatment groups with no significant differences observed by applied statistics for any experimental period and overall analysis (see Table XXIII and XXIV in Appendix II).

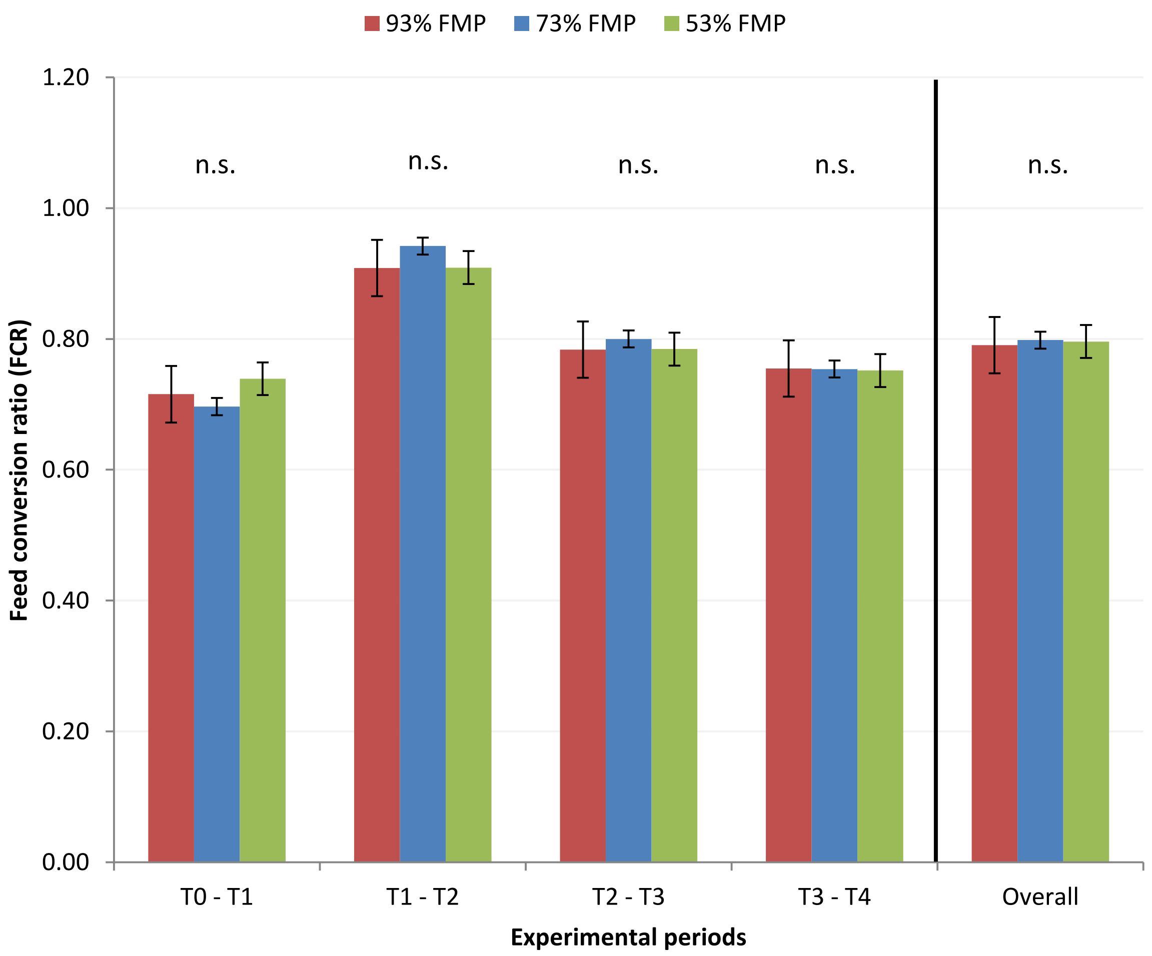

Figure 3-3: Feed conversion ratio (FCR) for < 500 g turbot fed three different diets. Each column represents a different dietary treatment and shows group means ± SE. (n.s. – no significant differences between dietary treatment groups)

FCR in the period T0 - T1 varies between 0.70 (73% FMP) and 0.74 (53% FMP), similar homogeneities between values for FCR are displayed for all experimental periods. However, FCR values take a rise in period $T_1 - T_2$ of 22 to 34% and readjust right afterwards, a deviant development also shown for specific growth rates and total feed consumption in the same experimental period.

Figure 3-4 shows total feed consumption (C_t) for the dietary treatment groups from all experimental periods and overall. Means for all periods ranged from 999 g (53% FMP) to 2565 g (93% FMP).

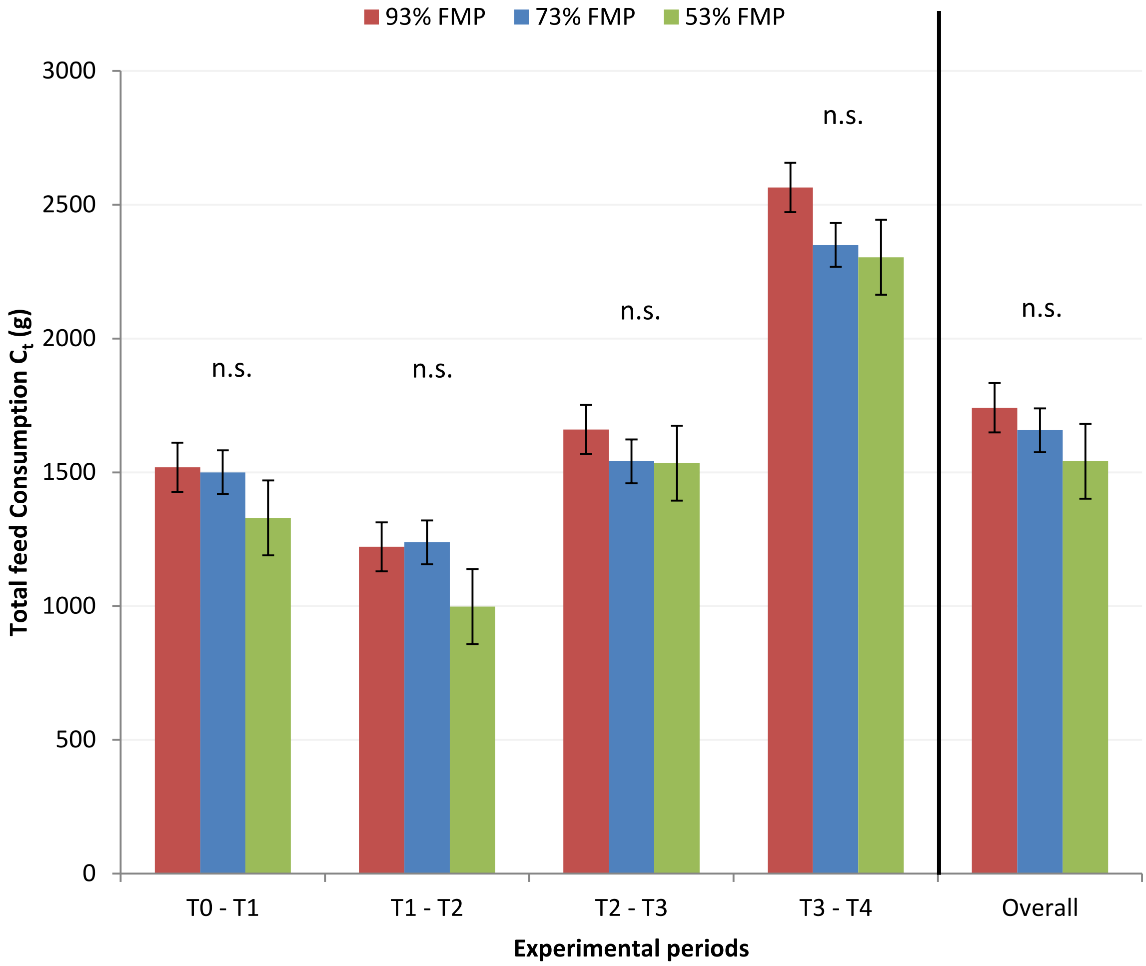

Figure 3-4: Total feed consumption (C_t) for < 500g turbot fed three different diets. Each column represents a different dietary treatment and shows group means ± SE. (n.s. – no significant differences between dietary treatment groups)

No significant differences in (C_t) between different dietary treatment groups were observed by applied statistics for any experimental period (see Tables XXIX and XXX in Appendix II) . For the periods $T_0 - T_1$ and $T_1 - T_2$ results displayed a distinguishably lower value for treatment 87 (53% FMP) which degenerated in the remaining periods. Total feed consumption increased for all treatments in period $T_1 - T_2$. However, a distinctive rise of up to 50% (93% FMP) was observed for all treatment groups in period $T_3 - T_4$.

Results for daily feed intake (DFI) among dietary treatment groups are displayed in Figure 3-5. Mean values for all experimental periods range from 0.59% (53% and 93% FMP) to 0.74% (FMP), no clear relation between DFI and levels of FMP could be observed as well as no significant differences in DFI between dietary treatment groups (see Table XXXV in Appendix II).

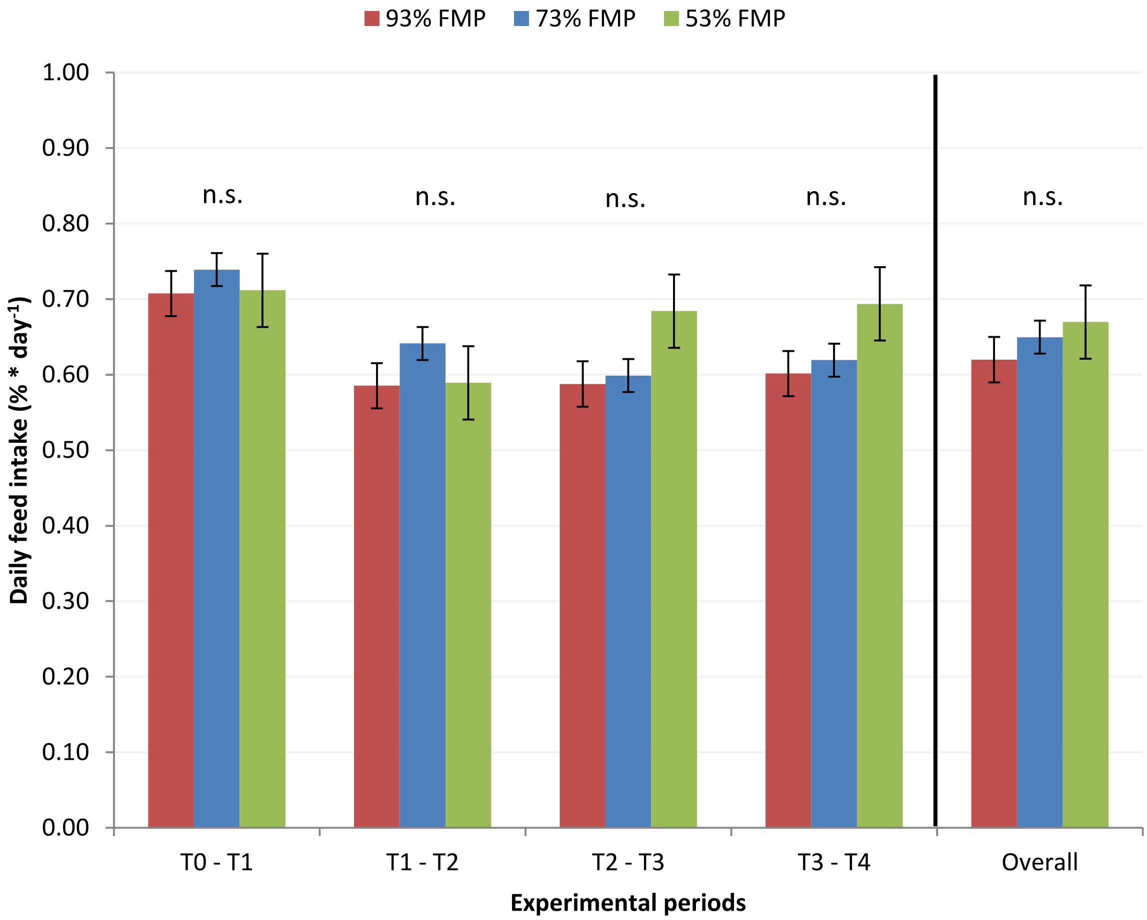

Figure 3-5: Daily feed intake (DFI) for < 500g turbot fed three different diets. Each column represents a different dietary treatments and shows group means ± SE. (n.s. – no significant differences between dietary treatment groups)

In periods $T_0 - T_1$ and $T_1 - T_2$ treatment group 86 (73% FMP) displayed the highest value for, while DFI was observed to be distinctively higher for treatment group 87 (53% FMP) in the periods $T_2 - T_3$ and $T_3 - T_4$. Treatment group 85 (93% FMP) displayed the lowest results for DFI in all experimental periods.

4 Discussion

Weight development results do not emerge significant differences between the different dietary treatment groups until the second part of the experiment. Higher mean weight was first observed in the last experimental period for the 93% FMP after more than 100 days (see Table XXXVII in Appendix II). However, treatment group 85 (93% fish meal protein) showed the highest mean weight throughout the entire experiment and a slight trend towards higher weight gain during for the periods T_2 – T_3 and T_3 – T_4 when compared to other treatment groups.

Specific growth rates (SGR) in this experiment (overall) ranged from 0.82 (73% FMP) to 0.88 % per day (93% FMP), average weight gain for all groups was 318 g. Significant differences in SGR between treatment groups were only observed in period T_0 – T_1 when SGR for treatment group 87 (53% FMP) was significantly lower (0.95%) than for the other treatment groups and is likely an expression of pre-term accustoming caused by the high level of plant protein. Accustoming for all treatment groups was observed in period T_1 – T_2 resulting in a drop in growth and a slowed weight gain.

However, a clear relation between the level of fish meal and specific growth rates as detected in weight development could not be observed. Treatment 85 (93% FMP) slowed down in growth performance after the first period, increasing growth rates for treatment 87 (53% FMP) were observed instead, passing results for treatment 86 (73% FMP) from periods T_2 – T_3 and T_3 – T_4.

To the authors knowledge, no similar feed trials with partly substituted levels of fish meal protein have been performed on turbot of this size range. However, trends in growth performance in this trial correspond to the pattern seen in trials carried out on juvenile and smaller turbot where fish meal protein was substituted by high concentrated rapeseed products (Slawski et al., 2011; Nagel et al., 2012)),. Results of these trials show no significant differences in SGR between treatment groups. However, weight performance was observed to lower according to lowered levels of fish meal protein in dietary treatments. Observations from this study correspond as well with results reported for 65 g turbot by Regost et al.(1999) which displayed similar growth rates for two experimental groups fed with 51% fish meal and 31% fish meal/20% corn gluten meal and results from Fournier et al.(2004) for a trial on 26 g turbot juveniles with treatment groups fed diets containing 40,30 and 20 % fish meal and varying levels of corn gluten meal, wheat gluten meal and lupin, which is a seed crop used widely as a substitute for fish meal.

Feed conversion ratio (FCR) in this experiment (overall) was equal among dietary treatment groups (0,79/0,80/0,80) and did only vary slightly between groups in different experimental periods. No significant difference was found in results for FCR between treatment groups. However, the treatment containing 93% FMP displayed the lowest overall FCR contrary to the results of Regost et al.(1999) but confirming the findings regarding FCR reported by Fournier et al.(2004).

Total feed consumption (C_t) and daily feed intake (DFI) did not display significant differences between dietary treatment groups. Treatment 87 displayed lower values for C_t for the periods T_0 – T_1 and T_1 – T_2 which are likely an expression of relatively longer accustoming caused by the high level of plant proteins. A clear relation between the level of FMP and total feed consumption was observed for the overall results, which goes along with the results of Fournier et al.(2004)..

In summary, the results of this study indicate that partial substitution of fish meal as the main protein supplier in fish feed leads to a slightly slowed weight development but, however, does not affect growth of smaller turbot (<500 g).

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Appendix I

Descriptive statistics

Table I: Experimental conditions showing O_2, temperature and salinity (mean T_0 – T_4 ± SD). Fish density is displayed in kg/ m^3 at initiation (T_0) and termination (T_4) of the experiment.

Tank	Feed	Tank Size m^2	Number of fish	O_2 - saturation % Mean±SD	Temperature °C Mean±SD	Salinity ‰ Mean±SD	Density kg/m^2 T_0 - T_4
2-1	86	1.8	28	102.4 ± 6.2	15.3 ± 0.6	21.4 ± 1.7	2.13 - 6.77
2-3	87	1.8	28	103.0 ± 6.0	15.3 ± 0.6	21.4 ± 1.7	2.08 - 5.88
2-4	85	1.8	28	101.8 ± 9.8	15.3 ± 0.6	21.4 ± 1.7	2.30 - 8.05
2-5	86	1.8	28	103.1 ± 5.8	15.3 ± 0.6	21.4 ± 1.7	2.07 - 6.67
3-1	87	1.8	28	100.5 ± 7.4	15.4 ± 0.6	21.4 ± 1.8	2.15 - 6.15
3-2	85	1.8	28	101.0 ± 7.4	15.4 ± 0.6	21.4 ± 1.8	2.13 - 7.53
3-3	86	1.8	28	100.8 ± 7.5	15.4 ± 0.6	21.4 ± 1.8	2.24 - 7.20
3-4	85	1.8	28	100.9 ± 6.8	15.4 ± 0.6	21.4 ± 1.8	2.25 - 7.76
3-5	87	1.8	28	101.3 ± 7.3	15.4 ± 0.6	21.4 ± 1.8	2.09 - 6.15

Table II: Weight measurements (g) of all fish from sampling T_0, T_1, T_2, T_3 and T_4 (day 0, 41, 71 103 and 138). (N) is the count of fish in each replicate.

Feed	Replicate	Day	Fish (N)	Mean weight (g)	Sum (g)	Min (g)	Max (g)	SD (g)	SE (g)
85	a	T_0	28	148	4148	76	286	42	8
85	a	T_1	28	234	6546	106	364	60	11
85	a	T_2	28	304	8518	150	466	78	15
85	a	T_3	28	386	10810	194	604	107	20
85	a	T_4	28	517	14488	226	820	153	29
85	b	T_0	27	133	3594	64	216	38	7
85	b	T_1	27	218	5890	104	352	61	12
85	b	T_2	27	264	7126	136	408	68	13
85	b	T_3	27	347	9378	158	530	97	19
85	b	T_4	27	480	12960	192	710	140	27
85	c	T_0	28	145	4054	76	286	45	8
85	c	T_1	28	230	6442	110	458	84	16
85	c	T_2	28	275	7694	120	564	101	19
85	c	T_3	28	361	10116	158	742	134	25
85	c	T_4	28	499	13964	214	996	183	35
86	a	T_0	28	137	3834	82	212	34	6
86	a	T_1	28	214	6000	118	316	58	11
86	a	T_2	28	265	7432	142	404	72	14
86	a	T_3	28	329	9202	172	532	96	18

Feed	Replicate	Day	Fish (N)	Mean weight (g)	Sum (g)	Min (g)	Max (g)	SD (g)	SE (g)
86	a	T_4	28	435	12182	234	718	134	25
86	b	T_0	28	133	3730	70	174	31	6
86	b	T_1	28	204	5700	92	286	55	10
86	b	T_2	28	258	7224	108	372	74	14
86	b	T_3	28	324	9078	118	478	97	18
86	b	T_4	28	429	12008	144	642	131	25
86	c	T_0	27	146	3954	74	248	38	7
86	c	T_1	27	233	6280	140	380	55	11
86	c	T_2	27	273	7382	140	462	66	13
86	c	T_3	27	350	9446	162	588	92	18
86	c	T_4	27	476	12846	234	806	131	25
87	a	T_0	24	135	3240	66	234	40	8
87	a	T_1	24	197	4738	86	368	63	13
87	a	T_2	24	248	5961	106	487	86	18
87	a	T_3	24	312	7496	156	636	112	23
87	a	T_4	24	413	9916	206	806	140	29
87	b	T_0	23	147	3378	68	216	32	7
87	b	T_1	23	217	5000	110	306	44	9
87	b	T_2	23	256	5890	138	376	53	11
87	b	T_3	23	336	7729	204	504	73	15
87	b	T_4	23	454	10442	262	692	109	23
87	c	T_0	26	136	3528	78	234	32	6
87	c	T_1	26	200	5202	112	332	51	10
87	c	T_2	26	238	6200	132	390	62	12
87	c	T_3	26	307	7988	170	500	80	16
87	c	T_4	26	418	10874	222	648	104	20

Table III: Mean weight (g) of all fish from day T_0, T_1, T_2, T_3 and T_4 (day 0, 41, 71, 103 and 138). Replicates are united under each dietary treatment. (N) is total count of fish.

Feed	Day	Fish (N)	Mean (g)	Min. (g)	Max. (g)	SD	SE
85	T_0	83	142	64	286	42	5
85	T_1	83	227	104	458	69	8
85	T_2	83	281	120	564	85	9
85	T_3	83	365	158	742	115	13
85	T_4	83	499	192	996	161	18
86	T_0	83	139	70	248	35	4
86	T_1	83	217	92	380	57	6
86	T_2	83	266	108	462	71	8
86	T_3	83	334	118	588	96	11
86	T_4	83	446	144	806	133	15
87	T_0	73	139	66	234	35	4
87	T_1	73	205	86	368	54	6
87	T_2	73	247	106	487	69	8
87	T_3	73	318	156	636	91	11
87	T_4	73	428	206	806	120	14

Table IV: Specific growth rate (SGR, % · day^{-1}) for all fish from period T_0 to T_4. Replicates are united under each dietary treatment.

Feed	Period	Fish (N)	Mean SGR %	Min. %	Max. %	SD	SE
85	T_0 - T_1	83	1.14	0.14	1.67	0.30	0.03
85	T_1 - T_2	83	0.71	0.27	1.16	0.21	0.02
85	T_2 - T_3	83	0.80	0.11	1.27	0.19	0.02
85	T_3 - T_4	83	0.88	0.44	1.30	0.16	0.02
85	Overall	83	0.88	0.11	1.67	0.27	0.03
86	T_0 - T_1	83	1.07	0.03	1.91	0.28	0.03
86	T_1 - T_2	83	0.67	0.00	1.57	0.26	0.03
86	T_2 - T_3	83	0.70	0.23	1.22	0.18	0.02
86	T_3 - T_4	83	0.82	0.34	1.10	0.14	0.02
86	Overall	83	0.82	0.00	1.91	0.28	0.03
87	T_0 - T_1	73	0.94	0.09	1.50	0.29	0.03
87	T_1 - T_2	73	0.62	0.18	1.09	0.20	0.02
87	T_2 - T_3	73	0.78	0.25	1.25	0.19	0.02
87	T_3 - T_4	73	0.85	0.40	1.16	0.14	0.02
87	Overall	73	0.83	0.09	1.50	0.24	0.03

Table V: Feed conversion ratio (FCR) for all fish from period T_0 to T_4. Replicates are united under each dietary treatment. (N) is the number of replicates for each dietary treatment.

Feed	Period	N	Mean FCR	Min.	Max.	SD	SE
85	T1	3	0.72	0.65	0.83	0.08	0.05
85	T2	3	0.91	0.78	1.08	0.12	0.07
85	T3	3	0.78	0.73	0.85	0.05	0.03
85	T4	3	0.76	0.70	0.84	0.06	0.03
85	Overall	12	0.79	0.65	1.08	0.11	0.03
86	T1	3	0.70	0.68	0.71	0.01	0.01
86	T2	3	0.94	0.91	0.97	0.02	0.01
86	T3	3	0.80	0.77	0.83	0.03	0.02
86	T4	3	0.75	0.75	0.76	0.00	0.00
86	Overall	12	0.80	0.68	0.97	0.09	0.03
87	T1	3	0.74	0.67	0.79	0.05	0.03
87	T2	3	0.91	0.85	1.00	0.06	0.04
87	T3	3	0.78	0.74	0.83	0.04	0.02
87	T4	3	0.75	0.72	0.78	0.02	0.01
87	Overall	12	0.80	0.67	1.00	0.08	0.02

Table VI: Total feed consumption (C_t) in (g) for all fish from period T_0 to T_4. Replicates are united under each dietary treatment. (N) is the number of replicates for each dietary treatment.

Feed	Period	N	Mean C_t (g)	Min. (g)	Max. (g)	SD	SE
85	T_0 - T_1	3	1518.6	1383.2	1586.8	95.8	55.3
85	T_1 - T_2	3	1221.3	1060.1	1539.9	225.3	130.1
85	T_2 - T_3	3	1659.8	1511.8	1776.2	110.2	63.6
85	T_3 - T_4	3	2564.9	2418.1	2662.4	105.7	61.0
85	Overall	12	1741.2	1060.1	2662.4	521.6	150.6
86	T_0 - T_1	3	1500.0	1391.7	1642.6	105.2	60.8
86	T_1 - T_2	3	1238.3	1059.4	1354.9	128.4	74.1
86	T_2 - T_3	3	1541.3	1477.1	1640.3	71.0	41.0
86	T_3 - T_4	3	2349.4	2195.6	2607.7	183.7	106.1
86	Overall	12	1657.3	1059.4	2607.7	435.7	125.8
87	T_0 - T_1	3	1329.6	1122.8	1608.1	204.5	118.1
87	T_1 - T_2	3	998.5	857.8	1068.8	99.4	57.4
87	T_2 - T_3	3	1534.7	1290.5	1826.4	221.4	127.8
87	T_3 - T_4	3	2303.3	1852.8	2847.9	411.6	237.7
87	Overall	12	1541.5	857.8	2847.9	545.5	157.5

Table VII: Daily feed intake (DFI, % · day^{-1}) for all fish from period T_0 to T_4. Replicates are united under each dietary treatment. (N) is the number of replicates for each dietary treatment.

Feed	Period	N	Mean DFI %	Min %	Max %	SD	SE
85	T1	3	0.71	0.63	0.77	0.06	0.03
85	T2	3	0.59	0.51	0.70	0.09	0.05
85	T3	3	0.59	0.54	0.63	0.04	0.02
85	T4	3	0.60	0.56	0.64	0.03	0.02
85	Overall	12	0.62	0.51	0.77	0.08	0.02
86	T1	3	0.74	0.72	0.77	0.02	0.01
86	T2	3	0.64	0.53	0.70	0.08	0.05
86	T3	3	0.60	0.57	0.62	0.02	0.01
86	T4	3	0.62	0.59	0.66	0.03	0.02
86	Overall	12	0.65	0.53	0.77	0.07	0.02
87	T1	3	0.71	0.62	0.85	0.10	0.06
87	T2	3	0.59	0.50	0.64	0.06	0.04
87	T3	3	0.68	0.59	0.81	0.09	0.05
87	T4	3	0.69	0.58	0.85	0.11	0.06
87	Overall	12	0.67	0.50	0.85	0.11	0.03

Appendix II

Applied statistics

Table VIII: Two - way nested ANOVA of weight data for all fish from T_0, day 0

Dependent Variable: MW0

		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	4663436,780	1	4663436,780	3475,523	,000
	Error	8125,121	6,055	1341,794[a]		
Feed	Hypothesis	491,013	2	245,506	,183	,837
	Error	8089,513	6,030	1341,600[b]		
Replicate(Feed)	Hypothesis	8048,237	6	1341,373	,932	,473
	Error	331202,195	230	1440,010[c]		

a. ,996 MS(Replicate(Feed)) + ,004 MS(Error)

b. ,998 MS(Replicate(Feed)) + ,002 MS(Error)   c. MS(Error)

Table IX: Two - way nested ANOVA of weight data for all fish from T_1, day 41

Dependent Variable: MW41

		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	11135721,408	1	11135721,408	3181,565	,000
	Error	21193,849	6,055	3500,076[a]		
Feed	Hypothesis	19412,915	2	9706,457	2,774	,140
	Error	21101,354	6,030	3499,590[b]		
Replicate(Feed)	Hypothesis	20994,140	6	3499,023	,934	,471
	Error	861448,227	230	3745,427[c]		

a. ,996 MS(Replicate(Feed)) + ,004 MS(Error)

b. ,998 MS(Replicate(Feed)) + ,002 MS(Error)   c. MS(Error)

Table X: Two - way nested ANOVA of weight data for all fish from T_2, day 71

Dependent Variable: MW71

		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	16671252,548	1	16671252,548	3210,817	,000
	Error	31454,215	6,058	5192,215[a]		
Feed	Hypothesis	43094,691	2	21547,346	4,151	,074
	Error	31307,267	6,031	5190,959[b]		
Replicate(Feed)	Hypothesis	31136,964	6	5189,494	,891	,502
	Error	1340014,569	230	5826,150[c]		

a. ,996 MS(Replicate(Feed)) + ,004 MS(Error)

b. ,998 MS(Replicate(Feed)) + ,002 MS(Error)   c. MS(Error)

Table XI: Two-way nested ANOVA of weight data for all fish from T_3, day 103

Dependent Variable: MW103

		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	27370985,724	1	27370985,724	3830,357	,000
	Error	43419,713	6,076	7145,805[a]		
Feed	Hypothesis	87731,504	2	43865,752	6,144	,035
	Error	43126,806	6,041	7139,110[b]		
Replicate(Feed)	Hypothesis	42787,791	6	7131,298	,678	,668
	Error	2420911,027	230	10525,700[c]		

a. ,996 MS(Replicate(Feed)) + ,004 MS(Error)

b. ,998 MS(Replicate(Feed)) + ,002 MS(Error)   c. MS(Error)

Table XII: Two-way nested ANOVA of weight data for all fish from T_4, day 138

Dependent Variable: MW138

		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	49876048,196	1	49876048,196	3824,143	,000
	Error	79288,064	6,079	13042,411[a]		
Feed	Hypothesis	211571,954	2	105785,977	8,119	,019
	Error	78726,347	6,043	13028,711[b]		
Replicate(Feed)	Hypothesis	78076,350	6	13012,725	,652	,689
	Error	4590580,473	230	19959,046[c]		

a. ,996 MS(Replicate(Feed)) + ,004 MS(Error)

b. ,998 MS(Replicate(Feed)) + ,002 MS(Error)   c. MS(Error)

Table XIII: One - way ANOVA of SGR for all fish from period T_0 - T_1, day 0 - 41

Dependent Variable: SGR1

	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	10,091	1	10,091	6054,727	,000
Feed	,063	2	,031	18,807	,003
Error	,010	6	,002		

Table XIV: One-way ANOVA of SGR for all fish from period $T_1 – T_2$, day 41 – 71

Dependent Variable: SGR2

	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	4,067	1	4,067	222,914	,000
Feed	,006	2	,003	,172	,846
Error	,109	6	,018		

Table XV: One-way ANOVA of SGR for all fish from period $T_2 – T_3$, day 71 - 103

Dependent Variable: SGR3

	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	5,398	1	5,398	1400,026	,000
Feed	,017	2	,008	2,164	,196
Error	,023	6	,004		

Table XVI: One-way ANOVA of SGR for all fish from period $T_3 – T_4$, day 103 - 138

Dependent Variable: SGR4

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	6,588	1	6,588	3293,889	,000
Feed	,007	2	,004	1,756	,251
Error	,012	6	,002		

Table XVII: Two-way nested ANOVA of SGR (overall) for all fish from the entire period $T_0 – T_4$, day 0 – 138

Dependent Variable: SGR

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	25,418	1	25,418	73205,000	,000
	Error	,002	6	,000[a]		
Feed	Hypothesis	,048	2	,024	68,456	,000
	Error	,002	6	,000[a]		

Replicate(Feed)	Hypothesis	,002	6	,000	,010	1,000
	Error	,923	27	,034[b]		

a. MS(Replicate(Feed))

b. MS(Error)

Table XVIII: Linear regression of SGR (overall) for all fish from the entire period T_0 – T_4, day 0 – 138

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	,213[a]	,045	,017	,16525

a. Predictors: (Constant), Feed

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	,044	1	,044	1,619	,212[b]
	Residual	,928	34	,027		
	Total	,973	35			

a. Dependent Variable: SGR

b. Predictors: (Constant), Feed

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	4,531	2,901		1,562	,128
	Feed	-,043	,034	-,213	-1,272	,212

a. Dependent Variable: SGR

Table XIX: One way ANOVA of feed conversion ratio (FCR) for all fish from period T_0 - T_1, day 0 - 41. Replicates are united under each feed treatment.

Dependent Variable: FCR1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.

Intercept	4,637	1	4,637	963,778	.000
Feed	,002	2	,001	,259	,780
Error	,029	6	,005		

Table XX: One way ANOVA of feed conversion ratio (FCR) for all fish from period T_1 – T_2, day 41 - 71. Replicates are united under each dietary treatment.

Dependent Variable: FCR2

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	7,654	1	7,654	748,804	,000
Feed	,002	2	,001	,099	,907
Error	,061	6	,010		

Table XXI: One way ANOVA of feed conversion ratio (FCR) for all fish from period T_2 – T_3, day 71 - 103. Replicates are united under each dietary treatment.

Dependent Variable: FCR3

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	5,601	1	5,601	2507,960	,000
Feed	,000	2	,000	,065	,938
Error	,013	6	,002		

Table XXII: One way ANOVA of feed conversion ratio (FCR) for all fish from period T_3 – T_4, day 103 to 138. Replicates are united under each dietary treatment.

Dependent Variable: FCR4

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	5,108	1	5,108	2286,985	,000
Feed	,000	2	,000	,000	1,000
Error	,013	6	,002		

Table XXIII: Two way nested ANOVA of feed conversion ratio (FCR) for all fish from the entire period T_0 – T_4, day 0 - 138. Replicates are united under each dietary treatment.

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	22,785	1	22,785	1475,800	,000
	Error	,093	6	,015[a]		
Feed	Hypothesis	,001	2	,000	,018	,982
	Error	,093	6	,015[a]		
Replicate(Feed)	Hypothesis	,093	6	,015	1,709	,157
	Error	,244	27	,009[b]		

a. MS(Replicate(Feed))

b. MS(Error)

Table XXIV: Linear regression of feed conversion ratio (FCR) for all fish from the entire period $T_0 - T_4$, day 0 - 138. Replicates are united under each dietary treatment.

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	,035[a]	,001	-,028	,099509

a. Predictors: (Constant), Feed

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	,000	1	,000	,042	,839[b]
	Residual	,337	34	,010		
	Total	,337	35			

a. Dependent Variable: FCR

b. Predictors: (Constant), Feed

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	,437	1,747		,250	,804
	Feed	,004	,020	,035	,205	,839

a. Dependent Variable: FCR

Table XXV: One-way ANOVA of total feed consumption (C_t) from period T_0 - T_1, day 0 - 41. Replicates are united under each dietary treatment.

Dependent Variable: Ct 1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	18906959,192	1	18906959,192	609,312	.000
Feed	65052,339	2	32526,169	1,048	,407
Error	186179,930	6	31029,988		

Table XXVI: One-way ANOVA of total feed consumption (C_t) from period $T_1 - T_2$, day 41 - 71. Replicates are united under each dietary treatment.

Dependent Variable: Ct 2

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	11958202,017	1	11958202,017	310,094	.000

Feed	107469,226	2	53734,613	1,393	,318
Error	231379,054	6	38563,176		

Table XXVII: One-way ANOVA of total feed consumption (C_t) from period T_2 – T_3, day 71 - 103. Replicates are united under each dietary treatment.

Dependent Variable: Ct 3

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	22428496,229	1	22428496,229	677,684	.000
Feed	29730,202	2	14865,101	,449	,658
Error	198574,892	6	33095,815		

Table XXVIII: One-way ANOVA of total feed consumption (C_t) from period T_3 – T_4, day 103 - 138. Replicates are united under each dietary treatment.

Dependent Variable: Ct 4

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	52095241,408	1	52095241,408	485,985	.000
Feed	117006,589	2	58503,294	,546	,606
Error	643171,622	6	107195,270		

Table XXIX: Two way nested ANOVA for total feed consumption C_t for the entire period T_0-T_4. Replicates are united under each dietary treatment.

Dependent Variable: Ct

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	97612950,939	1	97612950,939	756,536	.000
	Error	774157,354	6	129026,226[a]		
Feed	Hypothesis	241108,838	2	120554,419	,934	,443
	Error	774157,354	6	129026,226[a]		
Replicate(Feed)	Hypothesis	774157,354	6	129026,226	,418	,861
	Error	8339245,570	27	308860,947[b]		

a. MS(Replicate(Feed))

b. MS(Error)

Table XXX: Linear regression for total feed consumption C_t for the entire period T_0-T_4. Replicates are united under each dietary treatment.

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	,160[a]	,026	-,003	517,785

a. Predictors: (Constant), Feed

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	239084,874	1	239084,874	,892	,352[b]
	Residual	9115426,889	34	268100,791		
	Total	9354511,763	35			

a. Dependent Variable: Ct

b. Predictors: (Constant), Feed

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	10230,243	9089,949		1,125	,268
	Feed	-99,809	105,692	-,160	-,944	,352

a. Dependent Variable: Ct

Table XXXI: One way ANOVA of daily feed intake (DFI) for all fish from period T_0 - T_1, day 0 - 41. Replicates are united under each dietary treatment.

Dependent Variable: DFI 1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	4,658	1	4,658	668,719	.000
Feed	,002	2	,001	,128	,882
Error	,042	6	,007		

Table XXXII: One way ANOVA of daily feed intake (DFI %) for all fish from period T_1 – T_2, day 41 - 71. Replicates are united under each dietary treatment.

Dependent Variable: DFI 2

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	3,288	1	3,288	368,340	.000
Feed	,006	2	,003	,345	,721
Error	,054	6	,009		

Table XXXIII: One way ANOVA of daily feed intake (DFI %) for all fish from period T_2- T_3, day 71 - 103. Replicates are united under each dietary treatment.

Dependent Variable: DFI 3

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	3,498	1	3,498	678,629	.000
Feed	,017	2	,008	1,623	,273
Error	,031	6	,005		

Table XXXIV: One way ANOVA of daily feed intake (DFI %) for all fish from period T_3- T_4, day 103 - 138. Replicates are united under each dietary treatment.

Dependent Variable: DFI 4

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	3,665	1	3,665	512,739	.000
Feed	,014	2	,007	1,005	,420
Error	,043	6	,007		

Table XXXV: Two way nested ANOVA of daily feed intake (DFI %) for all fish from the entire period $T_0 - T_4$, day 0 - 138. Replicates are united under each dietary treatment.

Dependent Variable: DFI

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	15,040	1	15,040	925,724	.000
	Error	,097	6	,016[a]		
Feed	Hypothesis	,015	2	,008	,464	,649
	Error	,097	6	,016[a]		
Replicate(Feed)	Hypothesis	,097	6	,016	2,662	,037
	Error	,165	27	,006[b]		

a. MS(Replicate(Feed))

b. MS(Error)

Table XXXVI: Results from SNK test showing differences / homogeneities in mean weight between experimental groups

Day 0 (T_0)			Day 41 (T_1)			Day 71 (T_2)			
Feed	N	Subset 1	Feed	N	Subset 1	Feed	N	Subsets 1	Subsets 2
86	83	138,77	87	73	204,66	87	73	247,27	
87	73	138,99	86	83	216,63	86	83	265,52	265,52
85	83	142,12	85	83	227,45	85	83		281,18
Sig.		**,843**	**Sig.**		**,052**	**Sig.**		**,133**	**,197**

Day 103 (T_3)				Day 138 (T_4)			
Feed	N	Subsets 1	Subsets 2	Feed	N	Subsets 1	Subsets 2
87	73	317,99		87	73	427,84	
86	83	334,05	334,05	86	83	446,22	
85	83		365,11	85	83		498,94
Sig.		**,325**	**,058**	**Sig.**		**,413**	**1,000**

Table XXXVII: Results from SNK test showing differences / homogeneities in specific growth rate between experimental groups

Period T_0 - T_1				Period T_1 – T_2			Period T_2 – T_3		
Feed	N	Subsets 1	Subsets 2	Feed	N	Subset 1	Feed	N	Subset 1
87	3	,9467		87	3	,6367	86	3	,7167
86	3		1,0833	86	3	,6800	87	3	,7867
85	3		1,1467	85	3	,7000	85	3	,8200
Sig.		**1,000**	**,106**	**Sig.**		**,838**	**Sig.**		**,184**

Period T_3 – T_4			Overall		
Feed	N	Subset 1	Feed	N	Subset 1
86	3	,8267	87	12	,8042
87	3	,8467	86	12	,8267
85	3	,8933	85	12	,8900
Sig.		**,240**	**Sig.**		**,500**

Table XXXVIII: Results from Levene's test for homogeneity of variance

Variables	F	df1	df2	Sig.
Weight 0, day T_0	,607	8	230	,771
Weight 1, day T_1	1,498	8	230	,159
Weight 2,day T_2	1,541	8	230	,144
Weight 3, day T_3	1,497	8	230	,159
Weight, overall	1,425	8	230	,187
SGR 1, $T_0 – T_1$	1,754	2	6	,251
SGR 2, $T_1 – T_2$	,310	2	6	,744
SGR 3, $T_2 – T_3$	,291	2	6	,757
SGR 4, $T_3 – T_4$	,078	2	6	,925
SGR, overall	,394	8	27	,914
FCR, overall	,487	8	27	,855
C_t, overall	,233	8	27	,981
DFI, overall	,720	8	27	,672

(*) Test applied only to overall group data, insufficient number of observations (N) from single periods

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