www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 16: 267-273 (2016) DOI: 10.4194/1303-2712-v16_2_06

RESEARCH PAPER

Effect of Fish Protein Replacement in Diets for Juvenile Turbot *Scophthalmus Maximus*

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Received 11 November 2015
Accepted 21 March 2016

Abstract

A dietary study was conducted to investigate the effect of partly substituted levels of fish meal in feed for juvenile (140-500 g) turbot (*Scophthalmus maximus* Rafinesque) 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 meal, corn gluten meal, soybean meal and canola meal) with three replicates for each diet. Fish fed 53% fish meal diets had lower final mean weights compared to fish fed the 93% fish meal diet, whereas the 73% fish meal group displayed intermediate values. However, only minor differences were found in specific growth rate, feed conversion ratio, daily feed intake and total feed consumption between dietary treatment groups. Overall the findings demonstrate that substitution of fish meal by plant protein raw materials down to 53 % fish meal protein (44% replacement) does not affect overall specific growth rate, feed conversion ratio or feed intake in juvenile turbot (<500 g).

Keywords: Diets, protein replacement, growth, feed conversion, Turbot.

Introduction

A fast growing aquaculture industry with its increasing demand for fish meal (FM) and fish oil as the main protein and lipid sources in fish feed leads to pressure on FM and fish oil production. High market prices for FM and higher production costs force the aquaculture industry to search for cheaper, effective and more sustainable alternatives. Fournier *et al.* (2004), Bonaldo *et al. (*2011), Slawski *et al.* (2011) and Nagel *et al.* (2012) have investigated the possibility to substitute FM in diets for turbot, *Scophthalmus maximus* (Rafinesque), but those previous trials have been conducted with small (<100 g) juveniles and data for larger juveniles (100-500 g) are currently lacking. When substituting components in fish diets the balance of nutrients still has to satisfy the nutritional requirements of the fish. The fact that the protein level in most plant raw materials is lower than in marine FM reduces their use to substitute FM. Recent work of Leknes *et al.* (2012) has shown that the protein need of turbot is lower than in today's commercial diets, thus allowing a wide range of plant protein (PP) raw materials to be used in diet formulation. Leknes *et al.* (2012) suggested a minimum crude protein (CP) in diet for turbot between 500 and 1000 g was around 43% whereas commercial grow-out feed for turbot (>500 g) range from 52 to 54% (E. Waatevik, Skretting Ltd., Stavanger, Norway, pers. comm.). Furthermore, nutrient profile and anti-nutritional factors (AFNs) content will differ between plant row material and marine FM limiting the use of plant substitute in fish feed. E.g. the vegetable ingredients currently used to substitute FM and fish oil (FO) in fish diets lack n−3 HUFA. This decrease in dietary essential fatty acids is reflected in muscle fatty acid profile (Regost *et al.*, 2003). To be nutritionally attractive for commercial use, candidate alternative feedstuffs for marine fish feeds must have relatively high protein content (48%–80%, ideally higher than 60%) with a reasonable balance of essential amino acids, and have low levels of carbohydrates (e.g. fibre and starch) and anti-nutritional factors (Gatlin *et al.*, 2007).

Former trials on turbot have mainly focused on corn gluten meal, soy bean meal or lupine meal as an alternative protein source (Regost *et al.*, 1999; Fournier *et al.*, 2004). Wheat gluten has been shown to be an excellent protein source, containing 70-80% protein which is reported to be highly digestible. Wheat gluten has been included in diet for turbot at increasing levels, showing a good potential in substituting FM (Fournier *et al.*, 2004; Bonaldo *et al.*, 2011, 2015). Earlier studies on turbot show that up to

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in cooperation with Japan International Cooperation Agency (JICA), Japan

15% of FM can be replaced with wheat gluten without negative effects on growth or feed conversion ratio (FCR) (Bonaldo *et al.*, 2011). For European sea bass, *Dicentrarchus labrax*, a 50% and 70% fish meal (protein basis) replacement with wheat gluten did not have negative effect on growth performance (Messina *et al.*, 2013) and no difference in protein digestibility has been found in sea bass feed 30% wheat gluten diets (Robaina *et al.*, 1999). However, a relatively high price still limits the usage of wheat gluten in fish feed formulation. Corn gluten is a low priced PP material and contains a minimum of 60% protein (Morales *et al.*, 1994) which is 96% digestible for turbot (Fournier *et al.*, 2004). Corn gluten can substitute up to 30% of FM without negative effects on growth or FCR in turbot (Sevgili *et al.*, 2015). 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 PP materials because of its protein content and suitable amino acid profile and has been tested on different fish species, such as Japanese flounder, *Paralichthys olivaceu*s (Kikuchi, 1999) and Atlantic halibut (Berge *et al.*, 1999). Studies on crude rapeseed and canola products as a partial replacement of FM in diets for turbot (Burel e*t al.*, 2000a, b) have shown lower nutritional values despite a well- balanced amino acid profile.

The objective of the present study was to determine if different levels of PP substitutes (wheat meal, corn gluten meal, soy bean meal and canola meal) could be used in feed for 150-500 g turbot without affecting growth and feed conversion negatively.

Materials and Methods

Pre-Experimental Protocol

The fish in this experiment was provided by The Marine Research Institute of Iceland. 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 of Hólar University Collage (Sauðárkrókur, Iceland) in order to acclimatize to new rearing conditions. During the acclimation period the juveniles were feed commercial fed from Laxá feedmill (Akureyri, Iceland) containing 47% protein, 22% fat and 17% carbohydrates.

The experiment was based on 252 fish, which were distributed randomly into 9 tanks (3 replicate tanks per diet) and marked with Passive Integration Transponders (PIT tags) for calculations of growth rates. Initial weight was 140 ± 37 g (mean ± SD) and total average biomass in each tank was 3.88 ± 0.17 kg (mean ± SD). No fish died during the experiment.

Rearing Conditions

The 9 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 (T0) was 2.2 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. 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 the optimum rearing temperature for turbot growth of this size (15.4 °C, Imsland *et al.*, 1996). Salinity level was kept at 21.4 ± 1.8 ‰ (mean ± SD) which is near optimal salinity level for turbot (19 ‰, Imsland *et al.*, 2001). The oxygen saturation level kept slightly over 100% (101-105%). The fish were reared at 16 hours light: 8 hours darkness regime throughout the whole experiment.

Experimental Set-up

The experiment was initiated on 10 November, 2011 and terminated on 28 March, 2012. Three fish meal protein (FMP) substitution levels were used in the present trail: 93% FMP (no substitution), 73% FMP and 53% FMP. Each experimental feed was presented in triplicate tanks which were randomly distributed in separate rows in order to minimize possible tank effects. Hence the experimental set-up the present trial is 3 x 3 nested design (replicates nested in diet) design.

Weight measurements were undertaken at day 0 (T0, 10 November 2011), day 41 (T1), day 71 (T2), day 103 (T3) and day 138 (T4, 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 ± 2 g error margin was used for all weight measurements.

Fish Feed and Feeding Routines

The extruded diets used in this experiment were practical type diets produced at the Laxá feedmill in Akureyri, Iceland. Three dry diets with different levels of FM and substituted PP (wheat meal, corn gluten meal, soy meal and canola meal) were fed i.e. 93% FMP, 73% FMP and 53% FMP (Table 1). All diets were formulated to contain 42.5% crude protein and 25% crude lipid taking care of sufficient coverage of the first limiting amino acids Lysine and Methionine. Raw material composition and the content of protein, lipids and calculated gross energy (GE, MJ kg^{-1}) are shown in Table 1. Gross energy was measured in a bomb calorimeter (C 200; IKA, Staufen, Germany).

Table 1. Raw material composition, nutritional composition and energy content of the diets used in the present experiment

Raw material composition of diets (%)	FMP 93	FMP 73	FMP 53
Wheat	21.4	9.8	8.0
Fish meal[1]	58.4	45.5	33.0
Corn Gluten meal[2]	0.0	1.9	17.0
Hipro soy[3]	0.0	15.0	17.0
Canola meal[4]	0.0	6.7	2.9
Fish oil (capelin)	19.2	20.2	21.1
Vit./Min. Premix[5]	1.0	1.0	1.0
Nutritional composition (% dry matter) and energy content			
Crude protein	48.1	45.2	46.0
Crude lipid	24.6	26.8	25.1
Crude ash	12.0	10.7	11.7
GE (MJ kg^{-1})	20.6	20.0	21.1

1) Mix of Capelin and herring bone meal (68.1% CP) SVN, Neskaupsstaður, Iceland

2) Heins &Co. AG, Zurich, Switzerland, containing 60% CP (minimum).

3) Agrotrace SA, Geneva, Switzerland, containing 46% protein (minimum), 1.8% fat (minimum) and 3.25% crude fiber (minimum).

4) Emmelev A/S, Otterup, Denmark, containing 31% CP (minimum)

5) Premix designed for Laxa Feedmill. Content per kg premix: Vit.A 250.000 IU, Vit. D_3 150.000 IU, Vit. E 9.999 IU, Vit K_3 600 mg, Vit. B_1 700 mg, Vit. B_2 1500 mg, Pantothenic acid 4000 mg, Nicotinic acid 3000 mg, Biotin 30.000 mcg, Vit. B_{12} 2.000 mcg, Folic Acid 300 mg, Vit. B_6 1.499, Vit. C 10.000 mg, Iron 3.000 mg, Zinc 20.000 mg, Manganese 1.000 mg, Cobalt 10 mg, Iodine 300 mg, Selenium 3 mg

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 one hour after each feeding round and the number of uneaten pellets counted. The average pellet weight (*N* = 600, mean pellet weight = 0.281 g) 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. Test feeding of a tank with only seawater in it confirmed 100% recollection of fed pellets after automatic feeding for 22 hours.

Growth and Feed Conversion Efficiency

Specific growth rate (SGR) was calculated according to the formula:

$$SGR = 100 \, [\, (\ln(W_2)-\ln(W_1)) \, / \, (t_2-t_1) \,]$$

where W_2 and W_1 are weights on days t_2 and t_1, respectively.

Total feed consumption (C_T) was calculated as total feed supplied – total remaining feed in feed traps. C_T was calculated on a daily basis and then summarized for each tank.

Daily feeding rate (DFI) was calculated as

$$\text{DFI} \ (\% \ day^{-1}) = 100 \, [C \, / \, ((B_1 + B_2)/2)] \, (t_2 - t_1)^{-1}$$

where, C is feed consumption (g) in the period and B_1 and B_2 are fish biomass (g) on days t_1 (start) and t_2 (final) respectively.

Feed conversion ratio (FCR) was calculated feed consumed per unit biomass gain:

$$FCR = C \, / \, (B_2 - B_1)$$

Statistics

All statistical analyses were conducted using SPSS Statistics 21.0.0 and Statistica 12.0. To assess normality of distributions a Kolmogorov-Smirnov test (Zar, 1984) was used and homogeneity of variances was tested using Levene's F test (Brown and Forsythe, 1974). Two-way Model III nested ANOVAs (Searle *et al.*, 1992), where replicate tanks (random effect) are nested within diets (fixed effects), were used to test data on mean weights and SGR at different sampling dates, as well as the overall data for FCR, DFI and Ct and were followed by and a Student-Newman-Keuls (SNK) post-hoc test in the case of significant ANOVA's. The equation of the full nested model had the form:

$$X_{ijk} = \mu + \alpha_i + \beta_{j(i)} + \varepsilon_{ijk}$$

where: μ is the general level;
α_i is the treatment effect for $diet_i$;
$\beta_{j(i)}$ is the replicate factor (here: $tank_j$) nested within diet α_i;
ε_{ijk} is the model error term.

For the group data (FCR, DFI, and Ct) in each experimental period a one-way ANOVA was used to test for possible group differences (Zar, 1984). A significance level (α) of 0.05 was used if not stated otherwise.

Results

Growth

Significant differences in mean weight were observed at sampling day 103 where the 93% FMP group had significantly higher mean weight compared to the 53% FMP group (SNK test, $P<0.05$). At experiments termination (day 138) mean weight for the 93% FMP group was significantly higher (499 g) compared to the 53% FMP group (428 g) (SNK test, $P<0.05$, Figure 1), with the 73% FMP group (458 g) between.

Specific growth rate (SGR) in the first experimental period from T_0 to T_1 was significantly lower for 53% FMP group (0.95%) compared to the 73% FMP (1.07%) and the 93% FMP (1.14%) groups (SNK test, $P<0.05$, Table 2). But From the T1 period and onwards no differences were found in SGR, and the overall mean SGR did not differ between the groups (two way ANOVA, $P>0.15$, Table 2).

Feed Conversion Ratio, Feed Consumption and Daily Feed Intake

No significant differences were observed in FCR between different dietary treatment groups (two way nested ANOVA, $P>0.75$, Table 2) throughout the study. No significant differences in total feed consumption ($C_{t,}$ two way nested ANOVA, $P>0.30$, Table 2) or daily feed intake (DFI, two way nested ANOVA, $P>0.25$, Table 2) between different dietary treatment groups were observed during the study period.

Discussion

In the present study 44% replacement of FM by PPs did not affect SGR, FCR, C_t or DFI negatively for turbot in the size interval 140-500 g. However, the final mean weight was higher in the 93% FMP group compared to the 53% FMP group, with the 73% FMP group between the other two groups. This was probably due to lower growth in the 53% FMP in the first rearing period and could be attributed to an acclimation to the replacement feed as seen in previous replacement studies with turbot (Leknes *et al.*, 2012). To the authors' best knowledge, no similar feed trials with partly substituted levels of FMP have been performed with turbot of this size range. However, trends in growth performance in this trial correspond to the pattern seen in trials carried out on smaller turbot where FMP was substituted by high concentrated rapeseed (RPC) products (73-148 g, Slawski *et al.*, 2011; 32-98 g Nagel *et al.*, 2012). Results of these trials shown no significant differences in SGR between treatment groups up to 33% replacement, but lower growth and increased FCR after that. Dietary incorporation of other PPs revealed similar turbot performance at the same FM substitution level; e.g. Regost *et al.* (1999) observed unaffected feed efficiencies and growth performance of turbot fed diets containing a 33% replacement of FM by corn gluten meal. More recently, Bonaldo *et al.* (2011) found similar result with a wheat gluten/soy bean meal substitution of FM in experimental diets for turbot. Higher FM replacement levels negatively affected fish performance and nutrient utilization as 66% replacement level of FM by a RPC (Slawski *et al.*, 2011; Nagel *et al.*, 2012) or 52% FM replacement by PPs (Bonaldo *et al.*, 2011) showed significantly reduced feed intake, feed efficiency and growth performance. The data of the present experiment indicate that that up to 44% FM replacement by PP

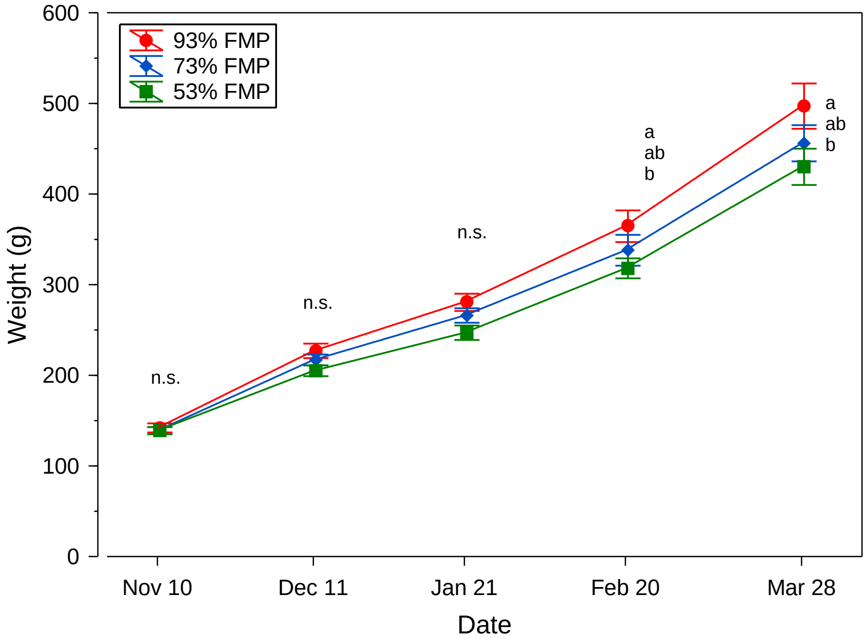

Figure 1. Mean weight of juvenile turbot, fed three different diets. Vertical lines indicate SE ($n = 3$ for each symbol). Different letters indicate significant difference between the experimental groups (Student-Newman-Keuls, $P<0.05$), n.s., not significant.

Table 2. Mean (SE) specific growth rate (SGR), feed conversion ratio (FCR), total feed consumption (CT) and daily feed intake (DFI) for juvenile turbot fed three different diets. Results are shown for each experimental period (*N*=3 for each treatment group) and the overall mean value for all periods combined. Different letters indicate significant difference between the experimental groups (Student-Newman-Keuls, P<0.05)

Treatment group				
Period		53% FMP	73% FMP	93% FMP
10 Nov. – 21 Dec.	SGR	0.94 (0.03)^{b}	1.07 (0.03)^{a}	1.14 (0.03)^{a}
	FCR	0.74 (0.03)	0.70 (0.01)	0.72 (0.05)
	C_t	1329 (118)	1500 (61)	1518 (55)
	DFI	0.71 (0.06)	0.74 (0.01)	0.71 (0.03)
22 Dec. – 20 Jan.	SGR	0.62 (0.02)	0.67 (0.03)	0.71 (0.02)
	FCR	0.91 (0.04)	0.94 (0.01)	0.91 (0.07)
	C_t	998 (57)	1238 (74)	1221 (130)
	DFI	0.59 (0.04)	0.64 (0.05)	0.59 (0.05)
21 Jan. – 20 Feb.	SGR	0.78 (0.02)	0.70 (0.02)	0.80 (0.02)
	FCR	0.78 (0.02)	0.80 (0.02)	0.78 (0.03)
	C_t	1534 (127)	1541 (41)	1559 (64)
	DFI	0.68 (0.05)	0.60 (0.01)	0.59 (0.02)
21 Feb. – 28 Mar.	SGR	0.85 (0.02)	0.82 (0.02)	0.88 (0.02)
	FCR	0.75 (0.01)	0.75 (0.01)	0.76 (0.03)
	C_t	2303 (237)	2349 (106)	2564 (61)
	DFI	0.69 (0.06)	0.62 (0.02)	0.60 (0.02)
Overall	SGR	0.83 (0.02)	0.82 (0.03)	0.88 (0.03)
	FCR	0.80 (0.02)	0.80 (0.01)	0.79 (0.03)
	C_t	1541 (157)	1657 (125)	1741 (150)
	DFI	0.67 (0.03)	0.65 (0.02)	0.62 (0.02)

can be achieved in turbot from 140 to 500 g. In fact growth (SGR) and feed efficiency (FCR) were not affected, neither it was voluntary feed intake as inferred from CT and DFI. This is higher replacement than found for smaller sized fish, where maximum FM substitution is reported not to exceed 33% (Slawski *et al.,* 2011; Nagel *et al.,* 2012). This could be a size related effect as growth was 16% lower in turbot in the size range 31-98 g feed 33% replacement diet (Nagel *et al.,* 2013), whereas the same replacement level resulted in 4% lower growth for turbot between 73-148 g (Slawski et al., 2011). An effect of fish meal replacement by soybean products on fish growth has been indicated (Sales, 2009). Another possibility to explain this apparent size effect could be due to lower protein demand for larger turbot. A closer look on data from Caceres-Martinez *et al.* (1984), Cho *et al.* (2005), Lee *et al.* (2003) and Leknes *et al.* (2012) indicates an exponential curve and a trend towards a decreased protein amount with increasing fish size. Decreasing protein demand with increasing fish size has been reported in recent feed experiments by Árnason et al. (2009, 2010) on Atlantic halibut and Atlantic cod.

No differences were seen in FCR, C_T or DFI between the three experimental diet groups in the present study. This is similar to results presented by Regost *et al.* (1999) and Fournier *et al.* (2004). In contrast Bonaldo *et al.* (2011) found higher FCR in juvenile turbot fed 52% PP compared to fish fed 25 and 39% PP (no control group available). In the study of Bonaldo *et al.* (2011) the increase of FCR in juvenile turbot (size range 24-126 g) fed diets with 52 % PP coincided with reduced protein utilization (PER) in line with earlier studies evaluating PP inclusion in turbot diets (Regost *et al.,* 1999; Day and González, 2000). The finding that PER decreased significantly for juvenile turbot fed diets PP52 and PP66 (Bonaldo *et al.,* 2011) while the protein apparent digestibility coefficient remained almost constant, suggests that the proportion of dietary protein used for catabolic processes (energy production), instead of anabolic ones (protein synthesis), increases with the level of FM replacement, as already stated by Day and González (2000). As no differences were seen in SGR or FCR in the present study this may suggest that anabolic processes were similar in all three experimental groups.

The dietary cost was influenced by the content of FM protein in the diet (Table 3). Hence the relative raw material price in the diet was 92.6 and 88.3 for the %FM protein 73, 53% respectively compared to the control (93% FM protein). Taking into account the effect of the diets on FCR, the raw material cost (and thereby feed cost) per kg growth was reduced by 6% and 11% by replacing the fishmeal protein by 20 or 40% respectively. Feed costs represent the largest cost share for most farmed species (Asche and Bjørndal, 2011). Given that the feed cost is 55% of production costs in turbot farming, reducing the FM protein in the diet by 20 - 40%, compared to an all fishmeal diet, could reduce the production cost of turbot by 3.3 – 5% given similar growth as found in this study.

In conclusion, the results of this study indicate that partial substitution of FM as the main protein

Table 3. Effect of different inclusion of fishmeal protein in diet on raw material cost and cost per kg of growth in turbot

% Fish meal protein	RM price € per kg feed	Rel. price	FCR	RM cost € per kg growth	Relative cost
93	0.90	100.0	0.79	0.71	100.0
73	0.83	92.6	0.80	0.66	93.8
53	0.79	88.3	0.80	0.63	89.4

supplier in fish feed does not affect overall specific growth rate, feed conversion ratio or feed intake in juvenile turbot up to 44% FM replacement for turbot from 140 to 500 g.

Acknowledgements

The authors would like to thank technical staff at the Verið aquaculture research station a Sauðárkrókur for valuable assistance prior to and during the experimental period. Financial support was given by the AVS fund (R 11-001) and the European Commission (MAXIMUS, 286200).

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