Feeding turbot based on ingredients from the Baltic Sea region: Comparative study with conventional feed ingredients

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Description

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Abstract

The European aquaculture industry still depends on imported raw materials like fish meal and soy bean meal from overseas for fish feeds. To evaluate the potential of regional feed ingredients for nutrition of the carnivorous turbot (*Psetta maxima*) a long-term study was conducted to compare conventional and regionally sourced feed ingredients with regards to growth performance, health and filet quality of fish.

One conventional diet (FM30) containing 30% fish meal and 20% soy bean meal and two diets, in which both ingredients were partly (FM15) or totally (FM0) substituted by regionally originated mussel meal, rapeseed protein concentrate and yeast were produced. In total 1476 juvenile turbot (21.6 g) were allocated in triplicates in a recirculating aquaculture system and fed daily for 292 days until apparent satiation.

Results showed that comparable growth rates could be achieved in fish fed completely regionally sourced diets (FM0) in comparison with conventional fish and soy based diets (FM30). However, best growth performance was observed in fish fed a mix of all test ingredients (FM15), where a similar feed conversion ratio (FCR) but an increased daily feed intake (DFI) in comparison with FM30 was observed. The FCR decreased in group FM0 and the DFI increased in both diets FM0 and FM15 in comparison with FM30. Furthermore hepatosomatic index (HSI) was highest and protein efficiency ratio (PER) lowest in treatment FM0. No significant differences were observed in-between dietary treatments with regards to other investigated physiological parameters and health status of fish such as splenic index, haematocrit and mortality. Furthermore in the sensory panel no significant differences in filet quality were observed between group FM30, FM15 and FM0.

It was concluded that turbot can be grown entirely based on regional ingredients without adverse effects on growth performance, health and product quality of fish. However, in future slightly reduced performance of fish fed regionally originated feed source could be overcome by optimized processings and thereby improved nutritive characteristics.

Keywords
Turbot nutrition; Mussel meal; Yeast; Rapeseed protein; Feed

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Contents

1. Introduction

In aquaculture there is still a strong need for new feed sources in order to overcome limited fish meal supply and rising fish meal prices (FAO 2012; Tacon & Metian 2008). This development might even potentially limit the growth of the aquaculture industry (Naylor et al. 2009).

Turbot (*Psetta maxima*) is a promising candidate for an aquaculture developement in the Baltic Sea region. Turbot are carnivorous and depending on high dietary inclusion levels of fish meal. Contrary, turbot has got several advantages comparably to many other fish species, because it can be farmed at high densities (Person-Le Ruyet 2002), utilizes feed very efficiently (Person-Le Ruyet 2002; Tacon & Metian 2008) and gains relatively high market price (FAO 2010). Consequently, various studies were conducted recently to identify new protein sources in turbot nutrition. It was shown that fish meal can be partly substituted by various alternatives like soy bean meal, wheat gluten, corn gluten or rapeseed protein, but higher dietary inclusion of plant feed sources always led to adverse effects on growth performance (Bonaldo et al. 2011; Fournier et al. 2004; Nagel et al. 2012; Nagel et al. 2013).

However, the use of soy bean meal, which is the most widely used fish meal alternative in fish nutrition (FAO 2012), is questionable with regards to its sustainability and limited supply (Bindraban et al. 2009).

In addition, using high inclusion rates of both soy and fish meals from overseas for fish nutrition in the European Union, European countries became net nutrient importers (FAO 2011). This means that the European aquaculture industry is mainly based on raw materials and nutrients from overseas, which both depleted tropical regions and provided European environment with excessive nutrient supply.

A certain amount of these nutrients is introduced in common water bodies and thus could have a potential environmental impact. Actually there are environmental problems in many water bodies in the European Union because of excessive release of nutrients from human activities such as fish farming. Effects of this development can be observed in the Baltic Sea where algae blooms became regular and even “dead” anoxic areas developed, which resulted in changes in zoobenthos and fish communities (Bonsdorff et al. 1997).

Even if fish farms are just responsible for a minor part of nutrient excesses to common water bodies, legislations of several countries are very strict with regard to licensing and permits for fish farming. So there is hardly no way for expanding fish farms and even harder to get permissions for new farms. As a result, the development of the aquaculture industry in the Baltic Sea region stagnated over remaining years (Paavola et al. 2013).

The government of Åland is actually discussing a new incentive-based aquaculture regulation. Briefly it is based on water improvement tools, like the use of regional grown mussels as feed ingredient, which overcompensate the nutrient surplus of feed based fish production. Thus new permissions for farmers will become available and do not limit the growth of the aquaculture industry in principle (Granholm 2014).

This procedure could be an ideal model for aquaculture development in the Baltic Sea where nutrients continuously circulate within the region. Therefore regionally avaible feed ingredients are necessary. As potential substitutes for imported feed ingredients rapeseed protein concentrate, mussel meal and yeast were already identified. Mussel meal can be produced from residuals (e.g. small ones/ with broken shell) which cannot be used for human nutrition or can be grown especially for feed pro-

duction in smart farms throughout the Baltic Sea (Åland Aquaculture Week 2012). Furthermore mussels extract nutrients from the surrounding water, so that they can be used to absorb a part of the surplus of phosphorous and nitrogen out of the eutrophic Baltic Sea. Additionally they have a high nutritional value for fish nutrition (Nagel et al. 2013). In addition, rapeseed protein concentrate can be prepared from rapeseed expeller, which is a residual from rapeseed oil or biofuel production. Yeasts can be grown on waste streams (Kiessling et al. 2014). So the advantage of these ingredients is not only the regional availability, but also that they are not directly competiting with human food sector. Additionally, by using residuals and by-products from industries it is possible to put nutrients back into the food chain and make them available for human consumption via high quality fish products.

Consequently, the aim of this study was to clarify if regional ingredients from the Baltic-Sea region could partly or totally substitute conventional imported feed ingredients. For this purpose, a long-term study with juvenile turbot fed to market size was conducted and growth performance and sensoric product quality were assessed.

2. Material and methods

2.1. Diet preparation

Three isonitrogenous and isocaloric diets were designed according to the nutritional requirements of turbot (Table 2). The first diet (FM30) contained fish meal and soy protein concentrate at amounts of 30% and 20%, respectively. This diet served as control diet as it was designed closest to a commercial diet for turbot nutrition according to Tacon & Metian (2008) and Person-Le Ruyet (2002). Fish meal and soy protein concentrate were partly (FM15) or totally (FM0) substituted by the regionally available feed ingredients mussel meal, rapeseed protein concentrate and yeast (Table 1). Used mussel meal was obtained and processed as described in Lindahl (2013).
Pellets of 4, 6 and 10 mm in diameter were extruded followed by oil addition in a vacuum chamber.

Table 1. Experimental diets used in the long-term study with turbot (g kg^{-1})

	FM30	FM15	FM0
Ingredient			
Fish meal	300	150	0
Soy protein concentrate	200	100	0
Rapeseed protein concentrate	0	75	150
Yeast	0	75	150
Mussel meal	0	100	200
Corn gluten	110	110	110
Wheat gluten	173.0	191.5	210.0
Wheat starch	111.9	81.6	51.4
Fish oil	83.1	94.0	104.9
Vitamins/Minerals	5/5	5/5	5/5
$CaHPO_4$	10	10	10
Lysine HCl	2.00	2.87	3.74

Table 2. Proximate, amino acid composition and antinutritional factors in experimental diets

	FM30	FM15	FM0
Proximate composition (g kg^{-1})			
Moisture	62	56	61
in dry matter:			
Crude protein	578	559	552
Crude lipid	147	155	165
Crude ash	61	55	50
Crude fibre	10	7	6
NFE	204	224	227
Gross energy (MJ kg^{-1})	23.32	23.45	23.73
Minerals (g kg^{-1})			
Calcium	8.4	6.8	4.9
Phosphorus	10.5	10.8	10.5
Total P – Phytate-bound P	8.3	8.2	7.7
Ca: P-ratio	0.80	0.63	0.47
Amino acids (g $16gN^{-1}$)			
Lysine	5.24	5.27	5.04
Methionine	2.13	2.09	1.94
Cysteine	1.47	1.70	1.83
Threonine	3.62	3.79	3.75
Valine	4.48	4.56	4.39
Isoleucine	4.24	4.31	4.13
Leucine	8.55	8.62	8.30
Phenylalanine	4.83	4.90	4.71
Tyrosine	3.41	3.52	3.44
Histidine	2.28	2.31	2.25
Arginine	5.12	5.26	5.13
Tryptophan	1.00	1.05	1.07
Antinutritional factors			
Glucosinolates (µmol mg^{-1})[1]	0	7.5	15
Phytic acid (g kg^{-1})	7.83	9.04	10.06
Phytate-bound-P (g kg^{-1})[2]	2.21	2.55	2.84

[1]calculated as: content of glucosinolates in rapeseed protein concentrate × % rapeseed protein concentrate in diet; [2]calculated assuming a P content of 282 mg g^{-1} in phytic acid molecule

2.2. Fish husbandry

The study was conducted in a recirculating aquaculture system (RAS) at the Gesellschaft für Marine Aquakultur mbH (GMA Büsum, Germany) (Image1). Fish were obtained from Maximus A/S (Gudnaes-strandvej 17, 7755 Bedsted Thy, Denmark) and transferred to the RAS. Fish were fed with a commer-cial diet (Aller Metabolica 4.5 mm, Aller Aqua A/S, Christiansfeld, Denmark (CP 52%, CL 15)) prior to the long-term study for three weeks at an intensity of 0.5% of body weight per day. Prior to the trial fish were starved for 2 days. Fish were individually weighed to determine condition factor. Experimental

fish were divided into nine equal groups of 164 fish each. Test diets were randomly allocated to the tanks to achieve a triplicate design in the feeding trial.

During the growth trial (292 feeding days) fish were fed manually until apparent satiation every day. Feeding regime was adapted to the behavior of fish, starting with two feedings a day (8:00 and 16:00) but switched to one feeding after six experimental months.

The RAS consisted of 10 circular tanks with 2.5 m^2 surface area for fish husbandry and 2.5 m^3 water volumes each. Waste water treatment took place via drum filter (60µm mesh size) followed by side streams/ bypasses to both a protein skimmer with ozone addition and a moving bed filter for biological water treatment.

Figure 1. Recirculating aquaculture system which was used for the long-term study with turbot

Oxygen level was controlled online and maintained via an oxygen cone to 9.3 ± 0.6 mg O_2 l^{-1}. Water temperature was maintained via a heat exchanging device and stepwise adapted for optimal growth conditions in accordance with Imsland et al. (2008) at first 18°C (17.8 ± 0.8) and once fish weight exceeded 100 g to 16.5°C (16.9 ± 0.7). Mean pH of the rearing water was 7.5 ± 0.2 and was stabilised with sodium bicarbonate ($NaHCO_3$). Photoperiod was set to 14 hours light and 10 hours darkness. Daily make up water was below 5% of total water volume per day. Ammonia and nitrite were measured daily with Microquant test kits for NH_4^+ and NO_2^- (Merck KGaA, Darmstadt, Germany). Mean TAN- and NO_2-N-levels during the trial were 0.2 ± 0.2 mg l^{-1} and 0.2 ± 0.1 mg l^{-1}, respectively. Nitrate (NO_3^-) level was determined spectrophotometrically from time to time at 400nm wave length using HACH LANGE DR 2800 spectrophotometer (Hach-Lange GmbH, Berlin, Germany) and powder pillow detection kit based on the cadmium reduction method. Nitrate level never exceeded 57 mg l^{-1} NO_3-N, which was the highest value at the end of the trial. The mean salinity was 26 ± 3 g kg^{-1} (HI 96822 Seawater Refractometer, Hanna Instruments Inc., Woonsocket-RI-USA).

2.3. Sampling

At the end of the trial fish were starved for 18 hours prior to sampling. Five fish of each tank were randomly selected for determination of hepatosomatic index, splenic index, final condition factor and haematocrit.

For determination of the haematocrit blood was gained with heparinised syringes (Injekt 5 ml, Sterican 21G × 1.5", Braun, Melsungen, Germany) at the caudal vein. Haematocrit was determined instantly with micro-hematocrit-tubes (Na-heparinised, 75 ×1mm, Brand GmbH + Co KG, Wertheim, Germany) and a haematocrit tube card reader (210 centrifuge, Hettich, Tuttlingen, Germany).

2.4. Sensory panel

Following the growth trial, eight fish of each tank were randomly selected and transferred from the RAS into clean seawater system. Additionally fish were starved for 14 days to prevent off-flavour problems.

After that period fish were killed and filleted immediately. Filets were stored for 48 hours at 4°C prior to sensory panel test. Upper filets of turbot were used to determine potential differences and preferences in-between dietary treatments with regards to odour, colour, firmness and flavour of fish filets. Fish fed diet FM 30 were set as reference group in each pair.

Prior to the sensory panel test, filets were cut into equal pieces of 5 cm and adapted to room temperature for one hour. Then filets were heated in an air circulation oven for 9 minutes at 180°C and served after 5 minutes.

Seven test persons were chosen for a blinded paired comparison test. Filets were encoded with the number of three digits referring to DIN 10954.

2.5. Chemical analysis

Dry matter (DM) content of fish body samples was determined by drying for 4 hours at 105°C (ED 53, Binder GmbH, Tuttlingen, Germany) and crude ash (CA) content in an combustion oven (P300, Nabertherm, Lilienthal, Germany) at 550°C for 4 hours. Crude protein (CP) was determined using the Kjeldahl-method (N×6.25) (InKjel 1225 M, WD 30, Behr, Düsseldorf, Germany) and crude lipid (CL) via Soxhlet-method (Soxtherm, C. Gerhardt GmbH & Co. KG, Königswinter, Germany). Crude fiber (CF) content was determined in an external lab (LUFA-ITL GmbH, Kiel, Germany) via method VO(EG) 152/2009, III, I. Nitrogen-free extracts (NFE) were calculated as 100 - (moisture-XA+CP+CL+CF).

Amino acids, calcium and phosphorus contents of experimental diets were determined via methods VO(EG) 152/2009, III, F/G and VDLUFA III, 10.8.2, respectively.

Phytic acid content of experimental diets was determined via method SAA A 006 (ÖHMI Analytik GmbH, Magdeburg, Germany).

Glucosinolates were analysed by Eurofins WEJ Agro Nutrition GmbH (Bremen, Germany) in accordance with EN ISO 9167-1, HPLC.

2.6. Calculations and statistic

Specific growth rate (SGR in % per day) = [ln (final body weight (g)) - ln (initial body weight (g))] × 100 / feeding days
Feed conversion ratio (FCR) = g feed intake / g body weight gain
Daily feed intake (DFI in % per day) = SGR × FCR
Protein efficiency ratio (PER) = g body weight gain / g crude protein intake
Fulton's condition factor (FCF) = (body weight in g / body length in cm^3) × 100
Hepatosomatic Index (HSI) = (g liver weight / g body weight) × 100
Splenic Index (SI) = (g spleen weight / g body weight) × 100
Mortality (%) = (1- (final number of fish / initial number of fish)) × 100

Statistical analyses were conducted with SPSS Statistics 21 program (SPSS Inc., IBM, Chicago, USA). Normal distribution of data was checked with Kolmogorov-Smirnov test and homogeneity of variances using Levene's test. To identify significant differences in-between dietary treatments a one-way ANOVA was executed. Homogenous subsets were identified using Tukey-HSD test and in case of inhomogeneity of variances Dunnett-T3 test was used. Data of the sensory panel was analysed with a binomial distribution. The p-value in all statistical applications was set to 0.05. Data shown are mean ± standard deviation among triplicates within each experimental group.

3. Results

3.1. Growth performance

Total fish meal replacement (FM0) did not lead to adverse effects on final body weight or specific growth rate in comparison with fish fed diets based on conventional feed ingredients (FM30) (Table 3).

Table 3. Growth performance of turbot fed with three different diets in the long-term study

	FM30	FM15	FM0
IBW[1]	21.7 ± 0.0	21.6 ± 0.2	21.6 ± 0.2
FBW[2]	622.6 ± 22.7[a]	712.5 ± 17.3[b]	606.4 ± 44.0[a]
SGR (%)	1.13 ± 0.01[a]	1.18 ± 0.01[b]	1.12 ± 0.02[a]
FCR	0.89 ± 0.02[a]	0.90 ± 0.01[a]	0.96 ± 0.03[b]
DFI (%)	1.01 ± 0.03[a]	1.06 ± 0.01[b]	1.08 ± 0.02[b]
PER	1.94 ± 0.04[ab]	1.99 ± 0.02[b]	1.88 ± 0.06[a]
FCF	2.09 ± 0.21	2.07 ± 0.27	2.11 ± 0.24

[1]initial body weight (g); [2]final body weight (g); different letters in the same line show significant differences ($p<0.05$)

However, highest growth performance was observed in dietary treatment FM15. The feed conversion ratio remained unaffected for partly fish meal and soy protein replacement, but was significantly increased in experimental group FM0. The daily feed intake increased in both groups, FM15 and FM0, in comparison to FM30. No significant difference in the protein efficiency ratio (PER) was observed

between the control fish (FM30) and fish fed other diets, but PER was higher in FM15 than in group FM0. In addition no significant differences in-between dietary treatments were observed with regards to the condition factor (FCF).

3.2. Physiological status and mortality

The hepatosomatic index (HSI) increased significantly in fish fed FM0 in comparison to FM30 and FM15. No significant differences in-between dietary treatments were observed with regards to splenic index (SI) and haematocrit. Furthermore mortality during the long-term study was on a very low level (<1% in total) and did not differ significantly between dietary groups (Table 4).

Table 4. Physiological status of turbot fed with three different diets in the long-term study

	FM30	FM15	FM0
HSI	2.09 ± 0.52[a]	2.21 ± 0.49[a]	2.83 ± 0.54[b]
SI	0.09 ± 0.02	0.11 ± 0.04	0.13 ± 0.09
Haematocrit (%)	25.4 ± 6.4	26.2 ± 6.8	23.6 ± 4.5
Mortality (%)	1.08 ± 0.74	0.22 ± 0.37	0.43 ± 0.37

Different letters in the same line show significant differences (p<0.05)

3.3. Sensory panel with fish filets

Results of the sensory panel are presented in Table 5 and 6. It was shown, that no significant differences in filet quality in-between dietary treatments could be noticed with regards to odour, colour, firmness and flavor of turbot filets.

Table 5. Results of sensory panel: identified differences and preferences from panelists (%);

Filets from group FM30 vs. FM0

	FM30	no difference	FM0	Significance[1]
more intensive odour	28.6	57.1	14.3	n.s.
preferred odour	7.1	57.1	35.7	n.s.
more intensive colour	7.1	85.7	7.1	n.s.
preferred colour	14.3	85.7	0.0	n.s.
more intensive firmness	7.1	57.1	35.7	n.s.
preferred firmness	14.3	57.1	28.6	n.s.
more intensive flavour	14.3	71.4	14.3	n.s.
preferred flavour	21.4	71.4	7.1	n.s.
More intensive in total	**14.3**	**67.9**	**17.9**	
Preferred in total	**14.3**	**67.9**	**17.9**	

[1] p<0.05

Table 6. Results of sensory panel: identified differences and preferences from panelists (%);

Filets from group FM30 vs. FM15

	FM30	no difference	FM15	Significance[1]
more intensive odour	14.3	78.6	7.1	n.s.
preferred odour	7.1	78.6	14.3	n.s.
more intensive colour	0.0	78.6	21.4	n.s.
preferred colour	14.3	78.6	7.1	n.s.
more intensive firmness	21.4	57.1	21.4	n.s.
preferred firmness	28.6	57.1	14.3	n.s.
more intensive flavour	21.4	50.0	28.6	n.s.
preferred flavour	28.6	50.0	21.4	n.s.
More intensive in total	**14.3**	**66.1**	**19.6**	
Preferred in total	**19.6**	**66.1**	**14.3**	

[1] $p<0.05$

4. Discussion

Overall, the observed high growth performance of turbot during the investigated growth-phase was in line with other studies and proved excellent environmental condition in applied pilot scale RAS system (Imsland et al. 2008; Person-Le Ruyet 2002). Water temperature was adapted to achieve optimal fish growth according to Imsland et al. (2008). Measured TAN-N and NO_2–N levels were low and should not negatively affect the growth performance of fish. Nitrate level was also low (peak 57 mg l^{-1} NO_3-N) and thus below harmful concentrations for turbot according to van Bussel et al. (2012). Summarised, all water parameters monitored in the pilot system were in an optimal range throughout the trial and could provide a viable code of practice for commercial farms.

The observed increase of daily feed intake in fish fed diets FM15 and FM0 are obviously correlated with mussel meal inclusion into diets. Different studies showed that mussel meal acts as a feed attractant in flatfish nutrition. For example Bonaldo et al. (2012) observed increased feed intake and even better growth performance in comparison with fish meal based diets by dietary mussel meal incorporation in diets for Common Sole. Additionally, Nagel et al. (2013) showed that feed intake increased with dietary incorporation of mussel meal in diets for turbot. Consequently the highest DFI was observed by highest inclusion level of mussel meal in group FM0.

In contrast, a total replacement of fish meal and soy protein concentrate with regional ingredients led to a slightly inferior feed conversion ratio. Various studies have shown that a higher dietary phytic acid content negatively influenced protein and mineral digestibility and as a consequence reduced feed conversion ratios could be observed (Denstadli et al. 2006; Satoh 1989; Spinelli et al. 1983). Consequently, we assume that higher dietary inclusion levels of phytic acid (>10g kg^{-1}) in diet FM0 influenced FCR and PER negatively. Additionally slightly higher dietary carbohydrates (NFE) content in diet FM0 may result in reduced digestibilities which potentially supported the observed effect (Munilla-Moran & Saborido-Rey 1996).

The overall best growth performance in group FM15 was probably a result of both higher feed intake and a feed conversion ratio similar to group FM30. This increased feed intake seemed to be promoted by mussel meal inclusion.

The increased hepatosomatic index (HSI) in group FM0 was suggested to be a result of decreasing digestible and utilisable protein sources. The protein level was slightly lowered in diet FM0 in comparison to FM30. But more important seemed to be the obviously reduced digestibility induced by higher dietary phytic acid levels. The reduced protein efficiency ratio of group FM0 supported this hypothesis. Consequently the available energy excess from feed was mainly stored as fat in hepatocytes.

In contrast to that, other investigated physiological parameters, as splenic index and haematocrit showed no differences between all dietary treatments. As the physiological status of fish represents the stress level of fish (Barton 2002; Roche & Boge 1995) it can be concluded that the different dietary treatments did not affect stress level, fish health or animal welfare in farmed turbot. This conclusion was supported by very low and not significantly different mortalities in all dietary treatments.

As dietary composition could greatly influence fish body composition and flesh quality (de Francesco et al. 2004) differences in fish filet sensory was evaluated by a panel. The results led to the conclusion that the investigated protein sources did neither negatively nor positively influence product quality of turbot filets.

5. Conclusion

Turbot production based on alternative feed ingredients could be viable without negative effects on growth performance and flesh quality. However, further improvements in treating raw materials or enzyme supplementations are useful and necessary to ensure feed utlization similar to conventionally designed diets. Therefore, subsequent efforts in research activities for optimized processing of alternative feed sources should be focused in near future.

Additionally, further improvements in mussel farming management and processing are necessary to make this protein source economically viable. Nevertheless, tested feed ingredients proved their huge potential as regionally available feed source in turbot nutrition although further improvements should enhance nutrient purity and lower production costs.

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