Comparing feed intake, utilization of protein and energy for growth and body composition in *S. solea* fed natural and commercial diets

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Report number C045/09

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Publication Date: 04-05-2009

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Amsterdam nr. 34135929,
BTW nr. NL 811383696B04.

A_4_3_2-V6.2

Contents

1 Introduction

Until today formulated diets for sole (*Solea solea* and *Solea senegalensis*) do not support growth rates comparable to those achieved with natural diets (Fonds et al 1989, Day *et al.* 1997). Despite the identified compounds such glycine, betaine and certain amino acids, on-growing fish poorly accept formulated feeds mainly because it lacks unidentified compounds to complement the whole range of feed stimulants (Knutsen 1992, Mackie *et al.* 1980, Reig *et al.* 2003, Velez *et al.* 2009). Nutrient efficiencies obtained, indicate that sole is limited to utilize ingredients commonly used in commercially dry feeds (Day *et al.* 1997, Dias *et al.* 2004).

Despite the slow progress towards a suitable commercial feed for sole the possibility of using natural diets for commercial sole culture has never been comprehensively studied, mainly because the use of live feed on a commercial scale is restricted by its availability and high price (Day *et al.* 1997). Consequently only few studies have investigated the utilization of natural diets in sole and comprehensive information is limited (Irvin, 1973, Fonds *et al.* 1989). With the development of commercial polychaete aquaculture in the Netherlands the opportunity arises to utilize *Nereis virens* as a natural diet for sole. To date no published information exists on growth and utilization in *S. solea* fed with *Nereis virens*. The nutritional composition of natural diets differs considerably and it is not clear whether ragworm supports growth rates comparable to those previously obtained for sole fed oligochaete worm, *Lumbricillus riualis* or mussel, *Mytilus edulis* (Irvin, 1973, Fonds *et al.* 1989, respectively). Moreover, cultured polychaetes are being fed on commercial polychaete feed and may exhibit nutritional properties different to those of wild worm. On the other hand composition of diets collected from the wild as used in previous studies may be more negatively effected by changes in food availability than the composition of cultured worm. For example, the protein content of mussels used in a study by Fonds *et al.* (1989) varied between 54 to 64% (dry weight base) between summer and winter respectively. Apart from covering the species' specific nutritional requirements a suitable diet needs to be readily accepted and efficiently utilized to sustain high growth rates. Amino acids and other non amino acid compounds of ragworm *Hediste diversicolor* have shown to stimulate the olfactory system in sole (Velez *et al.* 2007) suggesting that ragworm appear attractive to sole and stimulate feed intake. Utilization efficiencies commonly being used to assess the suitability of formulated diets in fish aquaculture (Lupatsch *et al.* 1997) have rarely been described for sole (Dias *et al.* 2004). Polychaetes contain high levels free amino acids and a complement of digestive enzymes present in the polychaete body fluid which likely facilitates their uptake and digestion (Fyhn 1989).

The present work was carried out to study the effect of polychaete *Nereis virens* on feed intake, utilization of protein and energy for growth and body composition in sole (*S. solea*). It is hypothesized that intake, efficiencies of protein utilization and growth rates obtained for sole fed ragworm are comparable to those previously reported for *S. solea*. The mussel *M. edulis* was used as a reference diet to allow comparisons of present results with those obtained previously, which until today serve as reference for optimum growth in sole. Intake, efficiencies of utilization for protein and energy for growth and growth rates obtained for sole fed ragworm are expected to be higher compared to commercial feed. A commercial feed, commonly used for turbot but also in sole culture served as a second reference diet to discuss differences between natural and commercial feeds.

2 Materials and Methods

2.1 Experimental animals and design

Experiments were conducted at the facilities of Wageningen IMARES, Yerseke, the Netherland with approval from the "Dieren Experimenten Commissie (DEC)", the Dutch commission for animal experiments from March until December 2008. All fish were supplied from Solea BV, IJmuiden, the Netherlands. Fish in the present study originated from wild caught captive broodstock naturally mating in groups.

Two experiments, an *ad libitum* and a requirements by ration levels (RRL) experiment were conducted to study feed intake, growth and feed efficiencies of sole. Experiments were carried out in recirculation aquaculture systems consisting of a water treatment unit including mechanical filtration (sandfilter), biological filtration (beadfilter) and bacterial disinfection (UV unit). Experiments were carried out using one initial size class of approximately 40g (ad libitum) or two initial size classes of approximately 40g and 100g (RRL). Fish were randomly assigned to the experimental tanks in groups of 10 per tank at initial densities of 1.1 ± 0.1kg/m^2. Fish were adopted to laboratory conditions for two weeks and fed a commercial diet at maintenance level. All fish were weighed individually at the start and at the end of the experiment. Both experiments lasted 56 days (54 feeding days). Fish were fed either *ad libitum* (*ad libitum* study) or at feeding levels of approximately 0.2, 0.4, 0.6, 0.8 and 1% DM feed/body weight (RRL study). Feed was provided in one (0.2, 0.4%), two (0.6, 0.8, 1.0%) or three (*ad libitum*) meals a day. Feed spill was collected after each meal and daily feed intake was estimated after subtracting feed spill from total feed load. Experiments were carried out under artificial light (12L:12D). Light cycle was reversed and fish were fed at their most active nocturnal period during working hours (Lagardere, 1987). During this dark period a low radiation red light was used to provide sufficient light source for fish feeding. Mean water temperature was 19.9±0.3 ºC and 19.3±0.4ºC for the *ad libitum* and RRL study respectively. Water quality ($D0_2$, pH, NH_3, NO_2, NO_3, salinity) were monitored daily during the experimental period. Flow rates of the inlet of each aquaria were kept at 5-6L/min 8-10 L/min for small and large tanks respectively. Mean daily water discharge was 350 l/kg^1 feed and 625 l/kg^1 feed.

2.2 Experimental diets

Three diets, i.e. ragworm, mussel and a commercial feed were used in the first experiment. In the second experiment only ragworm and the commercial feed were used. The commercial feed was DAN- EX 1562 (DANA FEED A/S, Denmark, sinking pellet, 2.00 mm). Worms with individual weight ranging from 0.5g -2.0g were supplied twice a week from a commercial ragworm producer (Topsy Baits, Wilhelminadorp, the Netherlands). After grading worms were kept in a flow through system without feeding till being fed to fish. Mussels were supplied twice a week from a local producer. The mussels were kept in an outdoor flow through system, using unfiltered Oosterschelde water comprising phytoplankton. Both fresh diets were not longer stored than 4 days till consumption. Ragworm and mussel were chopped into small pieces (approx. <5mm) to minimize the effect of prey size on feed intake. Ragworm were rinsed with system water for one minute and drip dried for one minute. Mussels were opened, rinsed with system water for 1 minute, and left to drip dry for 15 minutes followed the protocol of Fonds (1989)

Table I: Proximate composition of the experimental diets: crude protein, crude fat, ash and phosphor in g/kg dry matter, energy values in MJ/g. daily samples of diets were taken as % of total amount of feed calculated for 54 days. Data shown as means ± S.D for the ad libitum study. In case of the RRL study data show a single measurement

Study	Diet	Dry matter	Crude protein	Crude fat	Ash	Phosphor	Energy
ad libitum	Dry Feed	930.0 ± 0.5	620.4 ± 2.8	177.1 ± 2.5	119.4 ± 0.2	16.4 ± 0.7	18.3 ± 0.1
	Mussel	189.7 ± 0.6	702.1 ± 3.6	69.7 ± 1.7	106.4 ± 5.0	11.0 ± 1.3	16.2 ± 0.1
	Worm	181.2 ± 1.0	739.2 ± 13.6	82.7 ± 4.2	112.7 ± 2.3	8.9 ± 1.1	16.4 ± 0.1
RRL	Dry feed	916.0	622.2	189.2	113.9	16.5	21.5
	Ragworm	190.0	736.8	100.0	92.1	7.6	21.0

2.3 Sample preparation

Dry matter and ash free dry matter content of ragworm and dry feed were determined bi-weeky. Dry matter and ash free dry matter of daily feed refusals were measured to calculate daily feed intake on DM basis. Samples of ragworm and the commercial feed were taken daily as the percentage of total amount of feed calculated for 54 days stored at -20°C, pooled after determination of the experiment and subsequently analyzed for proximate composition. Whole fish (n=5) were randomly selected and sacrificed at the beginning (fish were kept in a separate non treatment tank at same conditions as experimental animals) and at the end of each experiment with an overdose of TMS 222, stored at -20°C and subsequently analyzed for proximate body composition.

2.4 Analytical procedures

Dry matter (DM) and ash free dry matter (AFDM) were determined according to ISO standard 6496 and ISO standard 5984 respectively. Diets and fish were analyzed for crude protein (Kjeldahl method), ash (gravimetrically 550°C incineration), crude fat (gravimetrically after extraction with petroleum ether), carbohydrate, energy and phosphorus (calorimetrically after destruction). Proximate analysis was performed by SGS (Spijkenisse, the Netherlands).

2.5 Calculations and statistical procedures

Metabolic feed intake (FI_{MBW}, g/kg$^{0.8}$/day^{1})

$$FI_{MBW} = \frac{FI_D}{MBW}$$

Where *MBW* is the metabolic body weight and FI_D is daily the daily feed intake.

Feed Conversion Ratio was calculated based on the equation:

$$FCR = \frac{FI_T}{(W_F - W_I)}$$

Where W_F is final body weight (g) and W_I initial body weight (g) of fish, FI_T is the total feed intake (g) on DM basis.

Geometric Body Weight (GBW, g)

$$GBW = (W_F \times W_I)^{0.5}$$

Where W_F is final body weight and W_I initial body weight (g) of fish.

Metabolic Body Weight (MBW, kg$^{-0.8}$)

$$MBW = (GBW/1000)^{0.8}$$

Where *GBW* is the geometric bodyweight of the fish
Relative Growth Rate (RGR, g/kg$^{0.8}$/d^{1}) quantifies gain of body mass (g) in relation to metabolic body weight (g/kg$^{0.8}$) of fish and feeding days.

$$RGR = \frac{(W_F - W_I)}{MBW \times d}$$

Gross nutrient uptake ($FI_{MBW}{}^{N}$, g/kg/$^{0.8}$/d^{1})

$$FI_{MBW}{}^{N} = FI_{MBW} \times (N_D/1000)$$

Where N_D is the nutrient content of the diet.
Gross nutrient retention (RET_N, g/kg$^{0.8}$/d^{1})

$$RET_N = [(W_F \times dm_F \times N_F) - (W_I \times dm_I \times N_I)]d/MBW$$

Where *W* is body weight, *dm* is dry matter content of fish and *N* is nutrient content of fish. Subscript indices give $_F$ for final and $_I$ for initial fish sample.

Linear regression equations

$Y = a + bw + cx + dz + dwxz + \varepsilon$ or $Y = a + bx + \varepsilon$

If only a pure dietary but no interactive effect was observed. Where Y is the response variable (e.g. $k_{GE\ growth}$), w is the nutrient intake, x is the diet, z is fish size. The model was chosen based on best fit and biologically meaningful considerations. Linear relationships between energy intake and energy deposition have also been described in Atlantic cod (for example Lupatsch *et al.* 1998, Hatlen *et al.* 2007)

Possible differences between diets in the *ad libitum* study were assessed by one-way ANOVA (SPSS 15.0). Differences were considered significant when p<0.05. Differences between diets in the RRL study were analyzed by comparing general linear regression models (GLM) using by (SAS 9.1). Differences in protein and energy requirements for maintenance and utilization efficiency of protein and energy for growth above maintenance between diets are expressed by the linear relationship between nutrient intake and gain. Efficiency of utilization of energy and protein for growth above maintenance ($k_{GE\ growth}$ and $k_{GCP\ grpwth}$) are described by the slopes of these linear relationship between nutrient intake and gain. Differences in efficiencies between the diets were considered significant when p<0.05.

3 Results

No treatment related mortalities were recorded. Some fish (mortalities n=4) swam upwards along the tank wall and jumped out despite all tanks being covered with lids or net. Results of the *ad libitum* and the RRL study are shown in table II and III and figure 1.

Table II: Performance of S. solea in ad libitum and RRL study: Initial and final body weight ($W_{Initial}$ and W_{final} in g), metabolic feed intake (FI_{MBW} in $g/kg^{0.8}/d^{1}$), relative growth rate (RGR in $g/kg^{0.8}/d^{1}$) and survival (in %). Data show as means ± S.D. (n=3 tanks/treatment). Means within a row lacking a common superscript letter differ significantly (p<0.05).

Experiment	Diet	$W_{initial}$	W_{final}	FI_{MBW}	FCR	RGR	Survival
Ad libitum	Dry Feed	43.3 ± 0.9^{a}	71.4 ± 5.6^{a}	5.7 ± 0.2^{a}	1.11 ± 0.11^{a}	5.22 ± 0.67^{a}	97^{a}
	Mussel	44.7 ± 3.1^{a}	96.2 ± 4.1^{b}	8.0 ± 0.3^{b}	0.94 ± 0.01^{a,b}	8.43 ± 0.24^{b}	100^{a}
	Worm	46.7 ± 1.3^{a}	107.2 ± 0.4^{b}	7.3 ± 0.2^{c}	0.78 ± 0.02^{b}	9.34 ± 0.28^{b}	100^{a}
	p- value	0.208	0.000	0.000	0.002	0.000	ns
	SE	0.752	5.425	0.337	0.051	0.638	1.11
RRL (40g)	DRY FEED	45.5 ± 1.7	47.3± 2.4	1.1 ± 0.0	3.99 ± 2.78	0.37 ± 0.20	100
		46.8 ± 2.9	56.6 ± 3.1	2.2 ± 0.0	1.15 ± 0.15	1.96 ± 0.21	100
		46.5 ± 2.0	64.5 ± 3.4	3.2 ± 0.1	0.96 ± 0.06	3.39 ± 0.14	100
		45.5 ± 2.0	70.9 ± 3.4	4.4 ± 0.1	0.93 ± 0.04	4.66 ± 0.09	100
	WORM	46.6 ± 1.6	49.7 ± 1.5	1.1 ± 0.0	1.94 ± 0.11	0.59 ± 0.03	100
		45.0 ± 4.0	58.6 ± 5.2	2.2 ± 0.0	0.81 ± 0.04	2.70 ± 0.13	100
		46.3 ± 1.4	72.1 ± 1.6	3.3 ± 0.1	0.71 ± 0.02	4.66 ± 0.07	100
		45.2 ± 1.3	82.8 ± 2.1	4.4 ± 0.1	0.67 ± 0.04	6.51 ± 0.21	100
		44.2 ± 2.6	93.7 ± 4.7	5.4 ± 0.1	0.65 ± 0.02	8.25 ± 0.26	100
RRL (100g)	DRY FEED	115.3 ± 2.0	118.7 ± 2.9	1.3 ± 0.0	1.88 ± n. a.	0.35 ± 0.5	100
		112.9 ± 3.7	133.9 ± 7.6	2.7 ± 0.0	1.32 ± 0.21	2.07 ± 0.32	100
		112.4 ± 6.5	149.5 ± 10.6	3.9 ± 0.1	1.12 ± 0.09	3.52 ± 0.21	100
		108.6 ± 1.2	149.5 ± 1.7	5.3 ± 0.0	1.35 ± 0.10	3.94 ± 0.28	100
	WORM	109.8 ± 7.6	120.5 ± 8.1	1.3 ± 0.0	1.20 ± 0.02	1.12 ± 0.02	90
		114.5 ± 3.7	145.4 ± 1.2	2.6 ± 0.0	0.90 ±0.09	2.96 ± 0.28	100
		104.8 ± 0.7	153.2 ± 1.1	3.9 ± 0.1	0.83 ± 0.01	4.68 ± 0.01	95
		110.3 ± 1.5	186.6 ± 2.1	4.9 ± 0.4	0.73 ± 0.05	6.68 ± 0.02	100
		112.4 ± 1.7	190.7 ± 3.3	5.5 ± 0.1	0.81 ± 0.04	6.74 ± 0.42	100

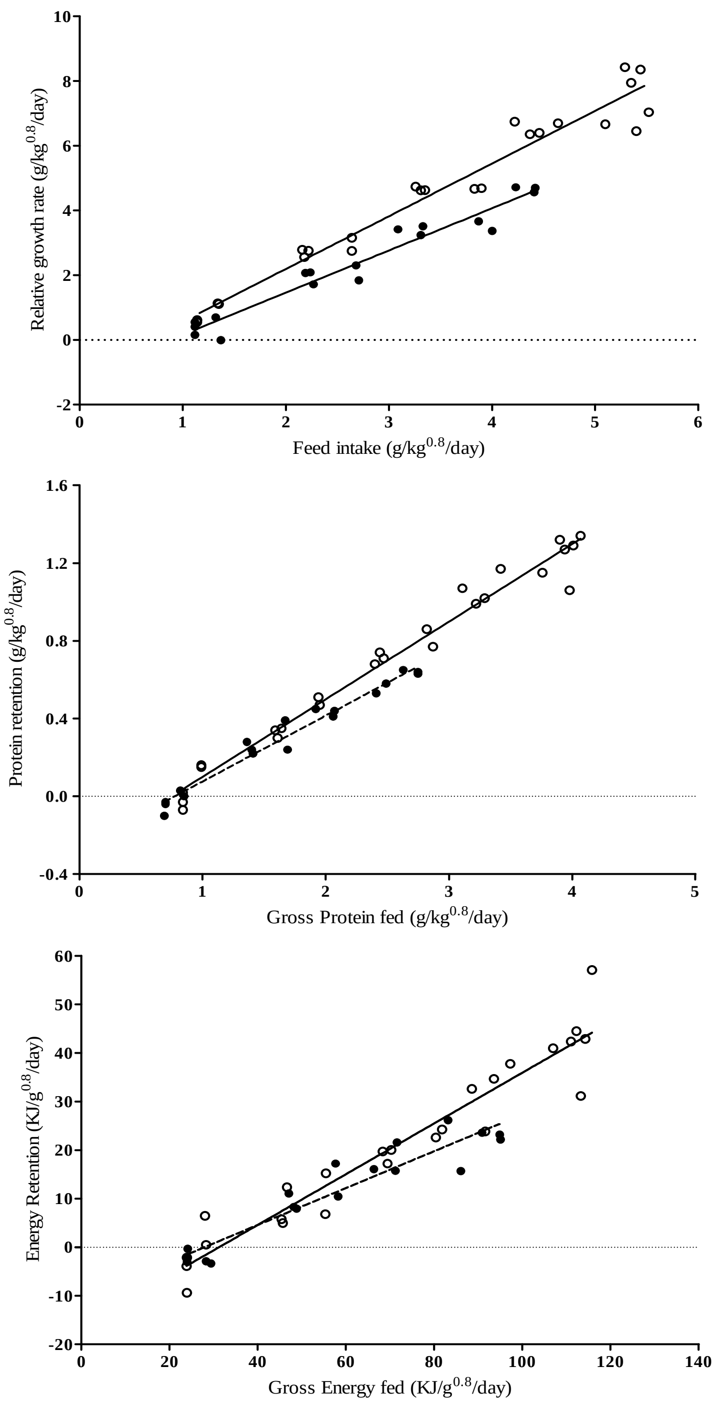

Figure 1: Relative growth (a), protein (b) and energy (c) retention in S. solea *of combined size classes of approximately 40 and 100g IBW fed different diets in relation to nutrient intake. Open circles solid line show data points and regression for worm treatment; solid circles and broken lines show data points and regression for dry feed treatment.*

*Table III: Regression coefficients (± standard error) in the equation y=a + b*x describing growth parameters (RGR and $RET_{protein}$ in $g/kg^{0.8}/d^{1}$ and RET_{Energy} in $KJ/g^{0.8}/d^{1}$) a function of nutritional intake $g/kg^{0.8}/d^{1}$. Data corresponds to regression lines in Fig. 1.*

Dependent var.	Diet	Intercept[1]	Slope	Model	Significance level [2]			
		a	b	R^2	FI_{MBW}	FI_{MBW}*Diet	FI_{MBW}*fish size	FI_{MBW}*Diet*fish size
RGR	worm	-1.1 ± 0.2	1.620 ± 0.065	0.96				
RGR	dry feed	-1.2 ± 0.3	1.273 ± 0.095	0.95	***	***	**	ns
$RET_{Protein}$	worm	-0.3 ± 0.1	0.394 ± 0.020	0.98				
$RET_{Protein}$	dry feed	-0.3 ± 0.1	0.340 ± 0.030	0.97	***	*	ns	ns
RET_{Energy}	worm	-15.7 ± 3.4	0.518 ± 0.050	0.92				
RET_{Energy}	dry feed	-11.6 ± 4.3	0.394 ± 0.070	0.89	***	*	ns	ns

The model R^2 includes the combined effects of FI_{MBW} with Diet and fish size, when significant. The slope b *describes the efficiency of utilization of energy and protein for growth above maintenance. (k_{En}. and k_{Prot}).*

[1]theoretical value from extrapolation of linear function when X=0

*[2]ns, not significant (P ≥ 0.05), * P < 0.05, ** P < 0.01, *** P < 0.001.*

4 Discussion

4.1 Growth performance, feed intake and utilization efficiencies

Growth rates and high efficiencies of utilization for growth in sole fed ragworm indicate that N. virens is a suitable diet for S. solea for the size range used in the present study. Present growth rates for fish fed ragworm (and mussel, which served as reference diet) were however lower compared to highest reported growth obtained for S. solea fed mussel meat (ragworm 9.3g$*$kg$^{-0.8}$$*$day^{-1} ,mussel 8.4 3g$*kg^{-0.8}$$*$day^{-1} present study, Fonds et al 1989, to 17.1g$*$kg$^{-0.8}$$*$ day^{-1} recalculated data). Efficiencies of protein utilization for growth were lower (0.39 $k_{GCP\ grpwth}$) in the present study but the utilization of energy was slightly better for ragworm (0.52 $k_{GE\ growth}$) compared to the study of Fonds (0.56 $k_{GCP\ grpwth}$ and 0.45 $k_{GE\ growth}$, data recalculated). Differences in growth between the two studies are explained by higher feed intake than what was obtained in the present study. Fonds obtained intake levels 7.5% BDW (fish of 10g mean DW, recalculated data), close to what has previously been estimated to be daily consumption in wild sole with mean DW of 13.7g (approx. 7% DM intake/BDW Lagardere 1987). In contrast, fish of 18.9g mean DW (76.9g mean WW) in the present ad libitum study consumed 4.1% DM/BDW. When assuming intake levels 7% DM intake/BDW for ragworm (corresponding to 11 g/kg0.8/d) growth rates of approximately 17 g/kg0.8/d would be obtained. This is similar to those achieved by Fonds on the mussel diet. Feed intake depends on the respective feeding protocol, size of the fish, temperature and origin of the fish. Fish body weight and temperature were similar in the present and the study by Fonds et al (1989). Unfortunately, Fonds did not provide any information on frequency and duration at which feed was administered to the fish. This makes it impossible to say whether observed differences in feed intake are due to extended access to feed in the Fonds study. Fish in the present study were fed three times a day for periods of 70min. In the present study fish were feed during artificial (reversed light cycle) night hours at times when highest feeding activity was expected. However, feeding activity takes place continuously throughout the night (Lagardere 1987) and even during the day Kruuk (1969). It may therefore be assumed that the current feeding regime did not allow fish to feed on their maximum daily ration. The distinct feeding behaviour in sole can be explained by the simple alimentary tract of sole with fast digestives process and fast evacuation rates from stomach to intestine, which forces the fish to feed “little and often” (de Groot 1971). The relatively low feed intake in the present study and hence hampered growth performance is to be explained also by differences in the genetic background and rearing conditions. Fish in the study of Fonds were wild caught fish which presumably originated from different parental batches, hence growth performance represents a variety of genetic backgrounds. Further it was not possible to conclude from the manuscript whether data presented were based on best performing fish. Fish in the present study originated from a limited number of individuals of captive broodstock naturally mating in groups. The genetic background correlated with growth therefore represents a limited fraction of possible performances available in sole. In contrast to wild sole used by Fonds, fish in the present study are farmed and have been subjected to rearing conditions designed on the basis of current knowledge rather than species specific requirements for their whole life span. In contrast to the study of Fonds, fish in the present study were kept in sand free tanks to facilitate tank cleaning and maintain good water quality. However, sole deprived of sand have shown to increase their metabolic rate which in turn leads to more energy being used for maintenance and less being deposited for growth (Howell and Canario 1987). Elevated stress levels in sand free tanks are likely to have effected feed intake. To summarise; maximum growth rates obtained in the present study may still be below the actual growth potential of S. solea due to reduced feed intake brought about by combined effects of current feeding regimes, laboratory conditions and genetic background of the fish.

Ragworm proved to be superior to the commercial feed supporting higher growth rates, higher feed intake and higher efficiencies in protein and energy for growth. In other words, the commercial feed used here as a reference diet (a feed commonly used for turbot but also in sole culture) does not support growth rates and efficiencies close to those achieved with natural feed. Growth rates for the commercial feed (5.27g$*$kg$^{-0.8}$$*$day^{-1}, Table 2) were significantly lower compared to those obtained with the two natural diets ($p<0.05$). Feed intake was nearly 20% higher for natural diets compared to commercial feed (7.3- 8.0g$*$kg$^{-0.8}$$*$day^{-1} compared to 5.70g$*$kg$^{-0.8}$$*$day^{-1}, respectively, $p<0.0001$). Efficiencies of protein (0.34 $k_{GCP\ grpwth}$) and energy (0.39 $k_{GE\ growth}$) utilized for growth were significantly lower for the commercial feed compared to ragworm ($p<0.05$, table III). This supports our initial hypothesis that despite improvement of fish meal based diets through the inclusion of feed attractants, natural diets such as ragworm and mussel appear more attractive to sole. Moreover, although no information was available on the feed attractants used in the commercial feed used in the present study, the feed was designed for turbot a species that has shown to be attracted by stimulants different to those in sole (Mackie 1980). Amino acids and other non amino acid compounds, coming from intact ragworm Hediste diversicolor have shown to stimulate the olfactory system in sole have not been identified yet (Velez et al. 2007). Present results further support the hypothesis that natural diets are more efficiently being utilized than the reference diet. The observed differences in utilization efficiencies may be explained by differences in apparent digestibility and/or the rate of digestion between the two diets. Diets used in feeding trial on S. solea or S. senegelanensis have not been evaluated based on their digestibility for protein and energy as in other species (Lupatsch et al. 1997). This is probably due to the fact that sole faeces recovery constitutes a problem as they dissolve rapidly when excreted. Sole possess a full complement of protein degrading enzymes and show a relatively high ability to digest and metabolize carbohydrates due to strong amylase and maltase activities (Clark et al. 1985). This suggests that fish meal based protein in the commercial feed used in the present does not constitute a problem to the fish's digestive capacities. This is supported by Day et al (1997) who reports that using hydrolyzed fish protein did not increase growth and survival in on-growing sole. Commercial feed is normally prepared by means of hot extrusion, which leads to protein denaturation and loss of enzyme activity. Heat treatment to 95ºC and reduced dietary protein and amino acid digestibility when fed to rainbow trout (Salmo gairdneri) as compared with the raw fish protein (Opstvedt et al. 1984). Another explanation is based on the differences in digestive physiology and the respective digestive rates in sole and turbot. As mentioned before, the reference commercial feed used in the present study was based on fish meal protein. Fish form a large part of the natural diet in adult turbot, digestion in turbot seems functionally well adapted to process fish protein. Due to large storage and digestive capacities of the stomach ingested feed will remain long enough in the digestive tract to be absorbed. A complete clearing of the alimentary tracts was reported to take at least 96h at 10°C. This is contrary to digestion in sole, which possess a relatively small stomach, a long intestine and high digestion rates, suggesting that the feeding of sole is based on the principle of "little and often". Such quick turn over rate can only be meet by readily assimilated nutrients such as free amino acids, high levels of essential polyunsaturated fatty acids (PUFA) and glycogen and a complement of digestive enzymes present in the polychaete body fluid (Fyhn 1989, Luis & Passos, 1995). These differences in digestion rate especially effect efficiencies in utilization at higher feeding levels where the passage rate of ingested feed through the digestive tract is thought to be higher (Henken et al. 1985).

5 Conclusions

The present study strongly indicates that the ragworm *N. virens* is a suitable diet for *S. solea* and may even have the potential to support highest growth rates reported so far for *S. solea*. This implies further studies. It is therefore recommended to focus future research initiatives towards factors effecting feed intake, i.e. feeding regime, genetic background and domestication of sole, laboratory conditions. The study shows that commercial feed used here does not support growth rates and efficiencies close to those achieved with natural feed. Feed intake and efficiencies of this commercial feed need to be considerably improved

6 Quality Assurance

IMARES utilises an ISO 9001:2000 certified quality management system (certificate number: 08602-2004-AQ-ROT-RvA). This certificate is valid until 15 December 2009. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. Furthermore, the chemical laboratory of the Environmental Division has NEN-AND-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 27 March 2009 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation, with the last inspection being held on the 5th of October 2007.

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Justification

Rapport C045/09
Project Number: 439.41028.04

The scientific quality of this report has been peer reviewed by the a colleague scientist and the head of the department of Wageningen IMARES.

Approved:	Oliver Schneider Researcher
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Number of copies:	50
Number of pages	23
Number of tables:	3
Number of graphs:	1