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# FEED ATTRACTANTS FOR COD (GADUS MORHUA)

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#### ABSTRACT

The feed attraction properties of squid and prawn for cod were investigated in two growth experiments using a feed based on minced saithe fillet, of which 10% on a protein-energy basis was exchanged for squid or cooked prawn, compared with a control group given minced squid mantle as the protein source.

Both additions increased growth and feed consumption over the basic saithe feed from 60-100%, giving the same effect as the control feed of squid mantle.

A protein digestibility of 85% and fat digestibility of 90% was found. The liver index was between 6.5 and 9.5% with fat energy contents of 13 to 20% in the feeds.

The feed attractants improved nutrient retentions as shown by the values for feed conversion, PER and PPV.

It was concluded that feed attractants are beneficial additions to feeds of marine fish species, and cheap marine products locally available may be preferred over chemicals such as free amino acids and other nitrogenous compounds.

## INTRODUCTION

Feed attractants are added to fish feeds to increase feed consumption and, as a consequence, increasing nutrient retention, i.e. growth. Studies on feed attractants have been concentrated around low-molecular nitrogenous substances, particularly different mixtures of free amino acids, betaines, choline, amines and nucleotides (Mackie and Mitchell, 1985). Mostly feeding experiments have been studied, but recently also the effect on neural responses were reported (Ishida and Hidaka, 1987). The addition of feed attractants seems to be of particular importance in the aquaculture of marine species. This field is presently under development in Norway, focussing on cod, ocean catfish and different species of flatfish. An economically feasible feed should comprise fish industry waste products and other available marine feed ingredients. Such feed mixes may be less palatable for the fish, and the use of feed attractants may then increase acceptability and feed intake. Experience gained from feed optimation studies with cod (Lied et al., 1985; Lie et al., 1988) showed that feeds based on fillets from cod or saithe alone have low acceptance, while feeds containing squid and prawns are highly palatable to the cod. The use of natural feed attractants rather than chemicals may be preferred for many reasons. The present study was designed to test whether additions of small amounts of squid and prawns to fish fillet based wet feed would increase acceptability and nutrient retention in aquaria fed cod.

## MATERIAL AND METHODS

## Experimental design

Cod (Gadus morhua) hatched and reared at the Aquaculture Research Station, Austevoll<sup>1</sup>, were used in two feeding experiments. The first consisted of three groups of fish averaging 280 g, fed for 42 days in a sheltered 25 m<sup>3</sup> tank divided into three compartments and supplied with running sea water. The second experiment comprised four duplicated groups of 35 fish, averaging 60 g, fed for 45 days, five days a week, in 175 L aquaria as described by Lie et al. (1986). The salinity and temperature were kept constant at 35‰ and 8 °C, respectively.

The protein source was based on minced saithe filled, while capelin oil and dextrinized potato starch were used as fat source and carbohydrate source, respectively. In experiment 2, a fourth group was given a feed were squid mantle was substituted for saithe fillet. In each of the two experiments, two groups were given feeds where 10% of the calculated protein energy content was replaced by either whole squid or cooked prawn. The feeds were produced as described by Lie et al. (1986).  $Cr_2O_3$  was added as an indicator of digestibility in the second experiment.

At the end of the feeding period the fish were weighed and liver and intestine (exp. 2) were dissected. The gut content from middle ileum to rectum was squeezed out gently. The samples were stored at -20 °C until analysis.

## Analytical methods

Feed, whole fish, liver and gut content were analysed in pooled samples for water, protein, fat and ash. Protein (N  $\times$  6.25) was determined by the micro-Kjeldahl technique according to Crooke and Simpson (1971). Fat was extracted with ethyl acetate and carbohydrate was calculated by difference. Chromium in the diets and the gut content was determined by atomic spectrophotometry as described by Lied et al. (1982).

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#### **RESULTS AND DISCUSSION**

Table 1 gives the composition, analyses and calculated energy distribution of the three respectively four feeds used in the two experiments.

In the second experiment a group given a feed based on squid mantle as the protein source was added as a comparison to the other groups because of the known high acceptability to cod for this product. The analytical values showed a small difference in water content between the feeds from the two experiments.

The energy distribution was tried kept constant at 70% for protein, 18% for fat and 12% for carbohydrate, with an energy concentration of 4 MJ/Kg. Some variations from this aim are shown in Table 1. Table 2 and 3 show growth values, feed conversions and nutrient retentions from the two feeding experiments.

*	-							
Diet	А		В		С		D	
Experiment	1	2	1	2	1	2	2	
Composition (g)								
Saithe Fillet	932		838		838		-	
Prawn			96		-			
Squid	-		_		101		-	
Squid mantle	and the second se		-		-		932	
Capelin oil	19		17		12		19	
Starch	36		36		36		36	
Vitamin mix. <sup>1</sup>	1.2		1.2		1.2		1.2	
Mineral mix. <sup>1</sup>	1.5		1.5		1.5		1.5	
Guar gum	10		10		10		10	
Analysis (g/Kg)								
Dry matter	247	275	241	273	234	263	256	
Protein	157	175	144	168	146	167	154	
Fat	22	23	22	21	17	15	16	
Ash	12	14	15	16	12	14	16	
Available energy <sup>2</sup> (%)								
Protein	70	70	67	69	66	73	70	
Fat	19	18	20	17	15	13	14	
Carbohydrate	11	12	13	14	19	14	16	
Energy conc. (MJ/Kg)	3.9	4.4	3.8	4.3	3.6	4.0	3.9	

Table 1. Composition of the diets in experiment 1 and 2.

<sup>1</sup> Vitamin and mineral mixtures given by Lie et al. 1986.

<sup>2</sup> Protein: 17.6 MJ/Kg, fat: 33.6 MJ/Kg and carbohydrate: 12.6 MJ/Kg.

The effect of adding prawn and squid at a 10% protein-energy level was clearly demonstrated in experiment 1 by the feed consumption data. Groups B and C increased the feed intake over group A with about 100% and 60%, respectively (Table 2). Although group B (prawn) had the highest feed consumption, groups B and C achieved nearly the same weight gain. Group C therefore had superior feed conversion, PER and PPV values, relative to group B, while group A, having a low feed consumption nevertheless achieved retention values only somewhat lower than group C.

Diet	А	В	С
Start weight, g	281.5	279.2	307.6
S.D. $(n = 40)$	84.0	82,6	85.7
Final weight, g	346.2	388.9	418.7
S.D	110.9	116.9	102.3
Weight gain, %	23	39	36
Liver index, % <sup>1</sup>	8.2	9.5	9.1
Feed consumption, g	232.4	482.6	375.0
Feed conversion <sup>2</sup>	3.6	4.4	3.4
PER <sup>3</sup>	1.78	1.58	2.03
PPV <sup>4</sup>	0.25	0.22	0.30
Retained fat	_	12	5

Table 2. Growth values and feed retention in experiment 1.

<sup>1</sup> Liver index = Liver weight \* 100/Final weight, was 12.0% when the feeding experiment started.

<sup>2</sup> Feed conversion: (g: feed/weight gain).

<sup>3</sup> Protein Efficiency Ratio (PER) = Weight gain/Protein intake.

<sup>4</sup> Productive Protein Value (PPV) = Protein gain/Protein intake.

The extreme feed intake by group B seemed to have overloaded the digestive capacity of the fish, whereby the feed was inadequately utilized. A similar effect was observed in cod fed twice a day (Lied et al., 1985).

Experiment 2 repeated feeds A, B and C, adding diet D based on squid mantle as protein source. The experiment consisted of duplicated groups of smaller cod in aquaria to ensure good feed control. As in experiment 1, the addition of prawn and squid at a 10% level markedly increased the feed consumption and weight gain (Table 3). The very high consumption of feed containing prawns found in experiment 1 was not confirmed by experiment 2, where both groups B and C were comparable to group D in weight gain as well as in feed consumption. The PER and PPV values showed clearly improved protein retentions over group A, but with a further increase in group D, fed squid mantle (Table 3).

	А		В		С		D	
	1	2	1	2	1	2	1	2
Start weight, g	60.2	58.4	60.0	61.7	66.0	66.3	63.3	63.8
S.D. $(n = 35)$	9.4	12.3	14.9	12.3	12.0	11.8	9.9	9.9
Final weight, g	73.0	75.6	93.9	89.1	98.4	95.7	95.9	96.3
S.D	15.5	20.4	24.8	21.5	18.1	19.5	17.0	19.0
Weight gain, %	21	29	55	44	49	44	52	51
Liver index, % <sup>1</sup>	6.4	6.5	8.4	7.5	7.1	6.6	7.6	7.6
Feed consumption	55.2	60.3	88.9	83.7	98.1	89.9	97.2	97.7
Feed conversion <sup>2</sup>	4.3	3.5	2.6	3.1	3.0	3.1	3.0	3.0
Protein:								
Digestibility, %	78	83	86	85	83	81	74	79
PER <sup>3</sup>	1.3	1.6	2.3	1.9	2.0	2.0	2.2	2.2
PPV <sup>4</sup>	0.28	0.28	0.36	0.32	0.32	0.33	0.35	0.35
Fat:								
Digestibility, %	89	94	94	92	94	91		87
Retained fat, %	_	14	63	33	40	23	44	69

Table 3. Growth values and feed retention in experiment 2.

<sup>1</sup> Liver index = Liver weight \* 100/Final weight, was 8.7% when the feeding experiment started.

<sup>2</sup> Feed conversion: (g feed/weight gain).

<sup>3</sup> Protein Efficiency Ratio (PER) = Weight gain/Protein intake.

<sup>4</sup> Productive Protein Value (PPV) = Protein gain/Protein intake.

Differences were observed in digestibilities of protein and fat, but averages of 81% for protein and 92% for fat were within the ranges reported for cod (Lie et al., 1988) and seemed not influenced by the feed composition in this experiment.

Tables 4 and 5 give the gross composition of whole fish and liver in the two experiments, respectively.

No significant differences were found between groups in either experiment. The older fish in experiment 1 had a higher fat content in the liver, but in both experiments the liver index decreased during the feeding period, based on an 18 energy percent fat in the feed. The low feed consumption in group A in both experiments resulted in the lowest liver index. Fat retentions were also very low in experiment 1, 0–12%, but higher in groups B, C and D in experiment 2, 23–69%. Widely varying fat retentions were expected on a fed with less than 20 energy percent from fat, calculated to give a low liver index (Lie et al., 1988).

Most marine prey organisms, particularly molluscs, worms, echinoderms and crustaceans contain numerous non-protein nitrogenous compounds.

According to Ikeda (1980) 95% or more of the non-protein nitrogen is accounted for by: amino acids, histidin dipeptides, guanidine compounds, trimethylamine oxide, urea, betaines, nucleotides and their derivatives. Different combinations of these have been shown to act as feeding stimulants for fish.

Free L-amino acids are present in all animal tissues, and their effect as feed attractants for several fish species has been shown, whereas the corresponding D-amino acids were without effect (Mackie and Mitchell, 1985).

Diet	А	В	С
Carcass			
Dry matter	25.1	25.7	25.4
Protein	14.5	14.5	14.7
Fat	5.6	5.7	5.6
Ash	2.2	2.2	2.3
Liver			
Dry matter	70.7	71.4	72.3
Protein	5.3	5.3	5.3
Fat	61.6	60.7	62,7
Ash	0.4	0.4	0.4

Table 4. Gross composition of carcass and liver (percent of wet weight) in experiment 1.

Values prior to the experiment: carcass; dry matter: 27.1, protein: 14.6, fat: 7.5, ash: 2.4. Liver; dry matter: 70.7, protein: 3.9, fat: 60.9 and ash: 0.4.

Diet	А		В		С		D	
	1	2	1	2	1	2	1	2
Carcass								
Dry matter	24.1	24.1	24.6	24.6	24.2	23.8	24.0	24.1
Protein	15.0	14.4	14.4	14.5	14.4	14.5	14.3	14.1
Fat	4.1	4.6	4.8	4.6	4.4	4.2	4.4	4.8
Ash	2.4	2.3	2.4	2.6	2.5	2.6	2.6	2.5
Liver:								
Dry matter	66.0	65.0	63.2	64,2	63.8	64.6	65.5	63.9
Protein	6.4	5.9	5.5	5.9	5.8	6.3	5.1	6.0
Fat	55.1	53.6	50.4	51.9	52.4	53.6	56.7	52.5
Ash	0.6	0.6	0.5	0.6	0.5	0.5	0.5	0.6

Table 5. Gross composition of carcass and liver (percent of wet weight) in experiment 2.

Values prior to the experiment: whole fish, dry matter: 20.9, protein: 13.5, fat: 5.6, ash: 2.5. Liver: dry matter: 65.2, protein: 6.0, fat: 52.5, ash: 0.6.

Squid mantle and extracts from it are particularly potent feed attractants to different fish species. Mackie and Mitchell (1985) made up a mixture of chemicals to correspond to an analysis of an extract from squid mantle and found it to increase feed consumption of different fish species. The mixture contained 17 amino acids and glycine betaine, TMAO, TMA, hypoxanthine, inosine, AMP and lactic acid. A more simple mixture of glycine betaine and glycine acted as an attractant for Dover sole (Mackie and Mitchell, 1985).

The only major difference in the amino acid composition of saithe fillet and squid mantle is the content of taurine, 9.5 mg/g and 21.6 mg/g, respectively. With only a 10% addition to the feed, no major difference in the amino acid composition was found, and the highly increased feed consumption in this study must be caused by other components than the amino acids.

According to Konosu and Hayashi (1975), squid and prawn contained 733 and 539 mg/100g, respectively, of glycine betaine. Glycine betaine in fish muscle is considered to be low or absent (Love, 1980), but Shewan (1951) reported 102 mg/100g in cod. Values for glycine betaine in saithe fillet were not available to us. In a preliminary experiment with feeds made from shrimp and squid meals, no feed improvement was seen, implying that the feed attractants are volatile.

In conclusion the addition of minced squid or prawn at only 10% of the protein energy level to fish feeds, nearly doubled the feed intake and growth, and also improved nutrient retention as shown by the values feed conversion, PER and PPV.

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