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Digestibility of chitin in cod, *Gadus morhua*, in vivo

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ABSTRACT: Sixteen cod, *Gadus morhua* (L.), were individually fed a single ration of shrimps, *Crangon allmanni*. Four fish were killed and examined 6, 12, 24 and 48 h after the fish had been fed. Chitinase activities were measured in the extracts of stomach contents, stomach tissue, pyloric caecae, intestinal contents and intestinal tissue. The level of enzyme activity in different parts of the digestive tract was shown to be dependent on the phase of the digestive process. High concentrations of the chitin degradation product N-acetyl-D-glucosamine were determined in the stomach and in the intestinal contents. Based on the chitin concentration in the food organisms and the individual food uptake, the amount of chitin consumed by each fish could be calculated. Only up to 9 % of the ingested chitin was recovered from the intestinal contents of the fish at any given time after feeding (6, 12, 24 and 48 h). In addition, only 2.4 % of the chitin consumed with the food could be recovered in the collected faeces of the fish. The 4 cod killed 48 h after feeding had completely emptied their stomach. Chitin digestion in these fish was calculated to have been 90 %.

INTRODUCTION

Early life stages of the majority of fishes depend on planktonic crustaceans as initial food. Insects and crustaceans may also constitute an important part of the diet of adult fish, e. g. members of the Cetorhinidae, Clupeidae, Salmonidae and Gadidae and other families. The major component in the exoskeletons of arthropods is the carbohydrate chitin (Muzzarelli, 1977) which may well be the dominant carbohydrate in the natural diet of fish (Richards, 1951). The biological degradation of chitin is carried out by a system of exocellular enzymes (Jeuniaux, 1963), known as chitinases (EC 3.2.1.14) and chitobiases (EC 3.2.1.29, N-acetyl-glucosaminidases). Chitinases hydrolyze the polymer chitin to the level of di- and trisaccharides which in turn are decomposed by chitobiases to the sugar monomer beta-N-acetyl-D-glucosamine (NAG).

Over the past 25 years, the presence of chitinases in the guts of fishes has been demonstrated in many studies (Jeuniaux, 1961, 1963; Okutani, 1966; Colin, 1972; Micha et al., 1973; Pérès et al., 1973; Goodrich & Morita, 1977 a,b; King et al., 1977; Fänge et al., 1979; Lindsay, 1984; Rehbein et al., 1986). In fact, all but two of approximately 150 fish species tested for gut chitinases, gave positive results, the exceptions being the cyprinid *Abramis brama* and the African lungfish *Protopterus aethiopicus* (Micha et al., 1973).

The origin of chitinolytic enzymes in fish has been discussed quite controversially: Some authors have claimed that chitinase activities measured in the gut contents of the

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fish species under study were of bacterial origin only (Goodrich & Morita, 1977b), which was generally accepted until Jeuniaux (1961) described chitinases as hydrolases that can also be synthesized in vertebrate tissues. Dandrifosse et al. (1965) were able to show that in vitro chitinases could indeed be synthesized and secreted by the gastric mucosa of *Anguilla vulgaris* (Cuv.) and *Salmo irideus* (L.). The mechanism of chitinase secretion in vertebrates could be clarified in another study (Dandrifosse & Schoffeniels, 1967). Most searchers working on "fish gut chitinases" found indications of both chitinase originating from fish tissues and bacterial chitinase. The enzyme produced by chitinolytic bacteria is characteristically different from "fish own" chitinase, for example with regard to the optimum pH-range for its activity (Jeuniaux, 1963; Okutani, 1966; Lindsay et al., 1984; Danulat, 1986a). Considering the extreme abundance and the major contribution of chitin to the diet of many fishes, it is surprising that only a few publications exist on its digestibility in vivo (Geyer, 1940; Mann, 1948; Buddington, 1980; Lindsay et al., 1984). In contrast to the well documented fact that the guts of many fish constitute a rich source of chitinases, chitin is most often referred to as very poorly digestible or indigestible by fish (Rösch, 1985). Especially in the case of *Salmo gairdneri,* reports of the very poor digestibility of chitin (Buddington, 1980; Lindsay et al., 1984) seem puzzling since this species has been shown to have strong chitinolytic potential (Jeuniaux, 1963; Micha et al., 1973; Lindsay et al., 1984).

This paper describes experiments to determine the digestibility of natural chitin in cod, *Gadus morhua* (L.). Cod are omnivorous marine fish, crustaceans being part of the diet during their whole life cycle. The extent to which cod feed on crustaceans is variable, depending on factors such as the age of fish and the local and seasonal food availability. *G. morhua* does not grind the prey mechanically but swallows it whole. This is not only true for smaller organisms like shrimps (*Crangon* sp.) but also for large specimen of the brachyura *(Liocarnicus,* syn. *Macropipus,* and *Cancer).* Nevertheless, no macroscopic parts of the exoskeletons of the prey can be found in the hind gut of cod. In previous studies, strong chitinolytic activities have been measured, originating predominantly in the stomach of *G. morhua* (Danulat & Kausch, 1984; Lindsay & Gooday, 1985; Danulat, 1986a). Often, chitinase activities in the pyloric caecae and the intestine of cod seemed to be substantial as well (Danulat, 1986b). The study of the optimum pH-range of chitinase showed that the enzyme is active under physiological conditions in the cod: its optimum was determined at pH 5.1. The enzyme in the stomach extracts was very active also at pH values lower than 5.1 while the one in extracts of the intestine showed high activity at pH 7.5, but rapid decrease of activity at pH values below the optimum (Danulat & Kausch, 1984).

Now, additional information is presented on the time course of chitin digestion and the digestibility rate, following one meal of a natural chitinous food organism of cod, i.e. shrimps *Crangon allmanni.*

MATERIAL AND METHODS

Fish material

Cod of age group I (22–40 cm) were caught around the island of Helgoland in the German Bight by means of baited fish traps, and transported live to the Biologische

Anstalt Helgoland. They were kept in aerated 330-l tanks fed continuously with water pumped from the sea. During the period of investigation (August 1985), temperature and salinity in the tank water ranged from 18 to 18.5 °C and from 31.3 to 32.3 ‰ S, respectively.

For two weeks the fish were fed daily ad libitum with frozen crustaceans and sprats, *Sprattus sprattus*. Three days preceding the experiment, feeding was stopped (i.e. the fish were starved in order to empty their digestive tract). For practical reasons two cod were put in each tank and separated from one another by a sheet of Plexiglas. Before the experimental feeding, the water flow into the tanks was stopped to avoid loss of food or faeces by circulation. The tanks were supplied with additional air stones, and although the water was cut off, the oxygen concentration in the tanks remained close to saturation for at least 48 h. Each fish was offered a single meal (15 g) of shrimps, *Crangon allmanni*. Before weighing the food portions, the shrimps had been thawed, immersed in sea water and blotted on filter paper. The fish immediately swallowed most of the shrimps. After 5 min, food leftovers were hosed from the tank bottom and weighed. Thus, for every fish the exact amount of food and the time of uptake were known. Faeces were collected by hose every 6 to 8 h. After centrifugation, the sediments were frozen (−25 °C).

In total, 16 cod were treated as described above. Four fish were killed at each of the sampling times (6, 12, 24, 48 h; fish groups I to IV) after receiving the single meal of shrimps (Table 1).

Table 1. Length/weight characteristics of the four groups of cod under study. Also, the amount of food (g wet weight) and chitin (g) consumed by each fish in a single meal of *Crangon allmanni* are presented. The fish were examined 6, 12, 24 or 48 h after food uptake (fish groups I to IV)

Group No. (exp. dur.)	Fish No.	Length (cm)	Weight (g)	Food (g)	Chitin* (g)
	1	40	569	15.50	1.30
I	2	26	162	4.46	0.37
(6 h)	3	31	345	10.60	0.89
	4	29	287	10.10	0.85
	5	29	273	13.22	1.11
II	6	28	231	8.15	0.68
(12 h)	7	32	369	13.90	1.17
	8	37	483	15.00	1.26
	9	30	322	7.40	0.62
III	10	29	265	6.53	0.55
(24 h)	11	27	171	11.27	0.93
	12	35	423	14.80	1.24
	13	27	183	6.98	0.59
IV	14	28	242	8.50	0.71
(48 h)	15	36	475	15.00	1.26
	16	34	416	15.00	1.26

* Calculation of chitin concentration in food based on values in Table 2

Sample preparation

The preparation of enzyme extracts has been described previously in detail (Danulat & Kausch, 1984; Danulat, 1986b). Extracts of stomach contents, stomach tissue, pyloric caecae, intestinal contents and intestinal tissue were frozen at – 70 °C for a maximum period of 4 weeks after the addition of 1 % toluene.

Chitinase assay

Chitinase activity was assayed according to the end product measurement of Jeuniaux (1966), using native chitin (Serva, FRG) as enzyme substrate. It was modified so that 4000 µl of test mixture contained 100 µl of toluene as antibacterial agent; a beta-glucosidase (Sigma, FRG) concentration of 1.5 units per ml distilled water was used. Prior to use, the substrate, 5 mg chitin × ml^{-1} dist. water incl. 2 % toluene, was stirred on a magnetic stirrer for 48 h, resulting in a homogenous suspension of chitin particles ranging from 30 to 60 µm.

After incubation for 180 min at 37 °C, the concentration of end product (NAG) in the test mixtures was determined by the method of Reissig et al. (1955), modified as in Danulat (1986b). The samples were read at 585 nm in a spectrophotometer (Uvikon 810; Kontron, Switzerland). A standard curve was used to convert OD readings to µg N-acetyl-D-glucosamine. The correlation coefficient is 0.9996, m = 0.0129 and b = 0.0009. Readings of two blanks were subtracted from the sample readings (Jeuniaux, 1966): replacing the chitin suspension by distilled water, the concentration of end product (NAG) in the fish extract could be determined (Blank 1). Furthermore, breakdown of chitin due to some reason other than the action of chitinase during the incubation period could be ruled out by analysing a test mixture in which the enzyme extract had been replaced by distilled water (Blank 2). Chitinase activities are reported as µg NAG × h^{-1} × g^{-1} wet weight of sample.

Quantitative determination of chitin

Preference was given to the micro-method of Bishop et al. (1982) which was applied in a slightly modified version in order to determine chitin concentrations in the mg-range. The assay is based on the complete chemical breakdown of chitin to an aldehyde which can be measured colorimetrically. This procedure rules out the difficulties encountered with other methods, most of which are based on acid-base-purification followed by gravimetric determination of chitin. The samples (shrimps; faeces; intestinal contents) were dried to constant weight at 70 °C. By means of a mortar, very fine particles were obtained, 50 to 100 mg of which were then suspended in 10 ml bidistilled water. Subsequently, the samples were treated with a Sonifier B-12 (Branson; Danbury, USA) for 5 min, resulting in a homogenous suspension. Aliquots of 50 to 1000 µl were then treated as described by Bishop et al. (1982). A standard curve was used to convert OD readings to chitin. The correlation coefficient was 0.9959, m = 0.0117, b = – 0.0353, when chitin from Serva, FRG (cat. no. 16620) was used as standard.

Combustion loss

The percentage of organic material was determined by combustion of dried *Crangon* shells at 470 °C for 2 h. Since some dark organic rests could be found after this procedure, the combustion was repeated once after adding 25 µl 10 % ammoniumnitrate solution (DEV, 1981) to the samples in order to obtain inorganic materials only.

RESULTS

Table 1 shows the length-weight characteristics of the 4 groups of cod, examined 6 (I), 12 (II), 24 (III) and 48 h (IV) after the experimental feeding. The mean length and weight of the fish in each of the 4 groups, as well as the average consumption of *Crangon allmanni*, were very similar. The chitin content of the food was calculated on the basis of the food analysis. Table 2 gives average dry weight (%), chitin content (% w.wt; % d.wt) and combustion loss (%) of 5 samples of *C. allmanni*. At a chitin content of 8.37 % (w.wt shrimps) and an average consumption of 11.0 +/− 3.7 g shrimps, each cod had consumed 923 +/− 309 mg chitin (n=16) in a single meal.

Table 3 presents the results of the analysis of the stomach and intestinal contents of *Gadus morhua* 6, 12, 24 and 48 h after food uptake. The data show that the digestion process and evacuation of the stomach had proceeded very rapidly. As early as 6 h after the feeding, relatively high concentrations of the chitin degradation end product N-Acetyl-D-glucosamine (NAG) could be measured in extracts of the stomach and intestinal contents. At the time of examination, the amount of chitin in the intestinal contents varied between an average of 18.08 mg (group I) and 42.66 mg (group IV). Chitin accounted for 9.28 % (group I) to 30.28 % (group IV) of the dry weight of the intestinal contents. These results show that some chitinous material had been evacuated from the stomach at a very early stage (i.e. 6 h after the feeding). The average percentage of chitin in the dried intestinal contents (groups I to IV) is highest after the stomachs had been completely emptied, demonstrating that chitin is degraded at a slower rate than other food components. The amounts of chitin determined in the intestinal contents represent only very small portions of the total chitin ingested with the food: Only 0.58 % (fish no. 1) to 9.10 % (no. 11) of the *Crangon*-chitin were recovered in the cod intestines (compare Tables 1 and 3).

Figures 1a-d present the average chitinase activities as µg NAG × h^{-1} × g^{-1} wet weight in the guts of each 4 fish that were killed 6, 12, 24 and 48 h after the feeding. No matter how much time had elapsed since food uptake, activities were always highest in

Table 2. Mean dry weight (% w. wt.), chitin content (% w. wt.; % d. wt.) and loss of dry weight during combustion (%) of *Crangon allmanni*. Means ($\bar{x}$) and standard deviation (± s.d.) based on 5 determinations. (Chitin assay according to Bishop et al. 1982)

Mean ± s.d.	Dry weight (% w. wt.)	Chitin (% w. wt.)	Chitin (% d. wt.)	Combustion loss (%)
$\bar{x}$	24.44	8.37	34.25	70.82
± s	0.53	0.75	2.83	1.79

Table 3. Stomach and intestinal content of 4 groups of *Gadus morhua*, examined 6, 12, 24 and 48 h after food uptake. Wet weights (g w. wt.) and content of N-Acetyl-glucosamine (mg NAG) are presented, in the case of the intestinal contens also dry weight (g d. wt.) and chitin content (mg)

Fish group	Fish No.	Stomach contents		Intestinal contents			
		g w. wt.	mg NAG*	g w. wt.	g d. wt.	mg chitin**	mg NAG*
	1	12.88	6.74	2.60	0.135	7.50	5.95
I	2	3.86	2.53	1.63	0.171	15.13	6.77
(6 h)	3	8.47	6.81	2.04	0.191	29.22	6.73
	4	8.74	6.70	2.57	0.277	20.47	7.50
	5	7.60	13.34	2.55	0.209	41.49	7.90
II	6	2.50	3.51	1.23	0.076	22.13	0.56
(12 h)	7	8.10	23.90	2.75	0.292	56.31	9.25
	8	9.73	24.53	2.99	0.284	79.86	2.50
	9	0.95	0.62	1.15	0.188	23.65	1.61
III	10	–	–	1.33	0.119	40.10	1.76
(24 h)	11	3.15	3.24	1.99	0.323	84.73	6.01
	12	2.86	2.61	2.36	0.202	44.08	2.13
	13	–	–	0.77	0.080	29.67	1.59
IV	14	–	–	1.05	0.100	28.29	2.78
(48 h)	15	–	–	–	–	–	–
	16	–	–	2.72	0.275	70.02	3.23

– no stomach or intestinal contents present when examined
* NAG-content determined according to Reissig et al. (1955)
** Chitin-content determined by the method of Bishop et al. (1982)

extracts of the stomach tissue. A comparison of the results for the 4 fish groups reveals that chitinase activities varied according to the time elapsed since the food uptake (i.e. the stage of digestion), as well as within the different parts of the guts. In the stomach contents, maximum activities were obtained 12 h after the feeding, while in the stomach tissue the highest activities were found after 24 h. In extracts of the intestinal contents, chitinase results 48 h after feeding were almost twice as high as 6 h after feeding, ranges of standard deviation not overlapping (due to the small size, statistical significance of these results was not tested). Overall there appears to be a definite interdependence between chitinase secretion and the stage of digestion.

Table 4 shows the results of the chitin analysis in the pooled faeces samples. Unfortunately, chitin in the stomach contents was not determined quantitatively, but it

Table 4. Dry weight (mg) and chitin content (mg) of pooled faeces of 3 groups of *Gadus morhua* (n = 4, each), after a single meal of *C. allmanni*.

Fish group	Faeces (mg)	Chitin content (mg)
II (12 h)	65	20
III (24 h)	202	77
IV (48 h)	517	252

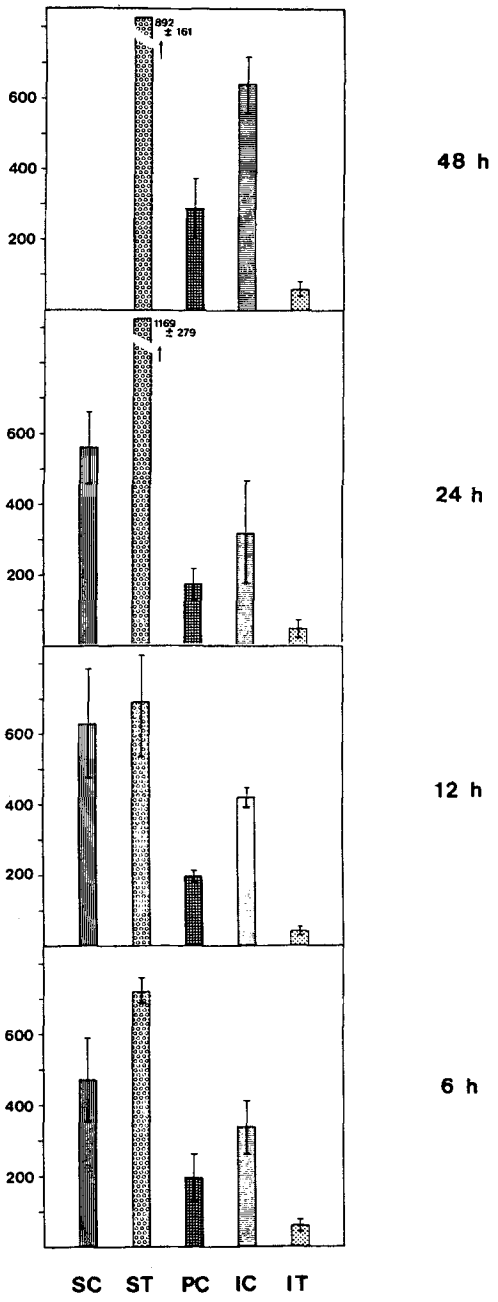

Fig. 1. Average chitinase activity (μg NAG × h^{-1} × g^{-1} wet wt of sample) ± S.D. of each 4 *Gadus morhua* that were examined 6, 12, 24 and 48 h after a single meal of *Crangon allmanni* (chitinase assay according to Jeuniaux, 1966). SC = stomach contents; ST = stomach tissue; PC = pyloric caecae; IC = intestinal contents; IT = intestinal tissue

can be assumed that some chitin was left in the fish that were killed within 24 h after the feeding. However, cod examined 48 h after food uptake had completely evacuated their stomachs. Here, the sum of chitin in the intestine and in the faeces reflects the amount of nonhydrolyzed carbohydrate:

Group IV: 128 mg (faeces) + 252 mg (guts) = 380 mg non-degraded chitin.

In total, 3806 mg chitin had been consumed by this fish group (Table 1). Subtracting 380 mg non-hydrolyzed chitin from this value, a mean digestibility of 90 % can be calculated for chitin. Considering the sampling technique of the faeces material, it seems likely that some of the faeces could be missed when hosing the tank bottoms, resulting in an incorrectly high digestibility value for chitin. Nevertheless, the small percentages of chitin that were recovered from the intestinal contents (Table 3) confirm that the digestibility of chitin in *G. morhua* must have been very high indeed.

DISCUSSION AND CONCLUSIONS

Long before samples of vertebrates were tested for chitinolytic enzyme activity, Geyer (1940) and Mann (1948) investigated the digestibility of the "animal fiber chitin" in fish. Having fed chironomids, gammarids and *Daphnia* to *Umbra lacustris* (Salmoniformes), Geyer (1940) determined digestibility rates between 12 and 16 %, while Mann (1948) found 15 % digestibility in a similar study with *Cyprinus carpio.* Mann (1948) remained convinced that chitin was completely indigestible and accounted his results to difficulties with the chitin determination. In case of *C. carpio,* chitinase tests were performed by Micha et al. (1973): Compared to most other fish species examined in the same study, *C. carpio* had very weak chitinase activities, and the same was true for other members of the Cyprinidae. Thus the low chitin digestibility found for *C. carpio* (Mann, 1948) might be accounted to weak enzyme activities in this species.

Recently, Buddington (1980) performed studies on the digestion of chitin and cellulose in *Salmo gairdneri* and three species of *Tilapia (T. aurea, T. mossambica* and *T. nilotica).* The author fed the fish with pellets containing 2 to 10 % "pure chitin". The amounts of carbohydrates recovered from the intestine and the faeces of the fish were not significantly reduced compared to the pellet mix. From these results, Buddington (1980) concluded that neither of the two polysaccharides can be digested by any of the 4 species, and called chitin and cellulose "hydrolysis resistant matter". The apparent indigestibility of chitin appears contradictory to the very high level of chitinase in the stomach tissue of *S. gairdneri,* reported previously by Micha et al. (1973): in vitro, the stomach mucosa of *S. gairdneri* released 16290 μg NAG $\times$ h^{-1} $\times$ g^{-1} (chitin being used as enzyme substrate).

Lindsay et al. (1984), conducting very extensive feeding studies with *S. gairdneri,* confirmed Buddington's result regarding the "hydrolysis resistance" of chitin: The digestibility of chitin, added at 10 and 30 % to the food of *S. gairdneri,* was determined to be close to zero. Having fed diets containing 4 to 25 % chitin over a 12-week period, the growth of test fish was significantly depressed when compared to control fish which received a mixture containing 25 % starch. In high contrast, enzymatic measurements by the same authors revealed that very strong activities of both chitinase and chitobiase of endogenous origin were present in the guts of the rainbow trout. Moreover, Lindsay et al. (1984) were able to show that amino-sugars, i.e. end products of chitin degradation, could be utilized as an energy source by the trout.

Results obtained in the present study show that chitinases in the guts of cod hydrolyze chitin not only in vitro but also in vivo: (a) Strong chitinase activities were measured in gut contents and tissues of the cod, showing great variations during the course of the digestive process. (b) In extracts of the stomach as well as the intestinal contents of the cod, high concentrations of the chitin degradation product NAG were measured. (c) Feeding a single meal of shrimps, only up to 9 % of the chitin from the food could be recovered from the intestinal contents and the faeces of the fish, chitin from *Crangon allmanni* was determined to be 90 % digestible in *Gadus morhua*. Even though further, more extensive studies with a more elaborate system for the collection of the fish faeces may show that the present results slightly overestimate the true digestibility of chitin, it has become clear that in cod, chitin is digestible to a very large extent.

It has to be taken into account that the digestibility will likely vary depending on the prey organisms that are taken: the chitin of shrimp shells may be digestible to a greater extent than that of a large specimen of *Cancer*, unless the digestion period is much longer in the latter case, i.e. the stomach evacuation is slowed down considerably. Stomachs of fish that had been caught for earlier studies, often still contained considerable amounts of food (crustaceans; fish) after 3 days of maintenance, and stomach evacuation was never obtained in less than 48 h. One cod of the present study had emptied its stomach completely within only 24 h. The difference may be explained by the fact that previously frozen shrimps are more readily digested than fresh shrimps. Other results agree well with earlier experimental findings: For example, when a group of cod was fed exclusively on crustacean shells for 3 weeks, their condition factor was significantly higher than the one of the control group (starved over the same period), indicating that the shells were degraded and the digestive products were absorbed by the intestine (Danulat, 1986b).

So, what are possible explanations for the conflicting results obtained for cod and for rainbow trout (Lindsay et al., 1984), both fish species being equipped with enzymes that are highly specific for hydrolyzing chitin?

In an attempt to explain the fact of very strong chitinolytic potential on one hand, and practically zero digestibility of chitin on the other hand, Lindsay et al. (1984) pointed out that fish in nature do not ingest purified chitin; they assumed, chitinase in trout is "non-functional" since protein covalently bound to chitin and other materials intimately associated with chitin, may present an obstacle to its hydrolysis. (If this assumption is correct, why then were the rainbow trout unable to digest the highly purified chitin, fed with the experimental diet?) Indeed pure chitin so far has only been found in diatoms (Herth & Barthlott, 1979), while in invertebrates chitin seems to be always linked with proteins, such as conchiolin and sclerotin (Muzzarelli, 1977). Interestingly, experimental data indicate stimulatory effects of protein on chitinase rather than support the hypothesis that the linkage of chitin to proteins presents an obstacle to chitinases (Jeuniaux, 1963; Danulat, 1986b). Acidic secretions in the fish stomach do not only promote the action of chitinases and proteases (Jeuniaux, 1963) but may well enhance the destruction of structural components of the prey such as calcified layers (Barnard, 1973). Crustacean shells are known to contain considerable amounts of calcium carbonate (Muzzarelli, 1977; Brine & Austin, 1981). Lindsay et al. (1984) also pointed out that at natural water temperatures for fish (15 °C), chitinase activities may be four- to five-fold lower than at 37 °C, which has been chosen for most chitinase tests. While the calculation is correct regarding the Vant Hoff' temperature law, it remains to be shown whether it is

indeed applicable in the case of *S. gairdneri*. Concerning *G. morhua*, chitinase tests were performed simultaneously at 6 different temperatures between 5° and 55 °C. Surprisingly, testing extracts of stomach and intestinal contents of a cod that was caught at 4.4 °C water temperature, chitinase activities measured at 5 °C reached up to 45–50 % of the maximum activity determined at 37 °C (Danulat, unpublished data). Since chitinase activities are even considerably higher in *S. gairdneri* (Micha et al., 1973) than in *G. morhua*, one should assume that chitin can be digested even better by the salmonid species. Possibly, structural changes in the chitin molecule during the purification process are the reason why chitinase could not attack the purified chitin which was included in the trout feed. It cannot be excluded that there are certain factors or activators in chitinous prey organisms which strongly enhance their digestion in the fish stomach. Also Lindsay et al. (1984) report chitinolytic activities of t i s s u e extracts only, not of extracts of stomach and intestinal c o n t e n t s , so for some reason the enzyme may not have been secreted into the gut lumen.

Without doubt certain fish species do exist that are unable to degrade natural chitin from natural organisms: For example, complete exoskeletons of gammarids could be found in the faeces of the flounder *Platichthys flesus* (Karpevitch & Bokoff, 1937), and even very fine extremities of *Daphnia pulicaria* could be recovered from the intestine of the salmonid *Coregonus lavaretus* (Rösch, 1985; Kausch, pers. comm.). This may be the case where there is a lack of chitinolytic enzymes, and/or specific conditions in the digestive tract of the fish. In general, "There is, however, no ready explanation for the failure of animals possessing chitinase to digest chitin" (citing from: Lindsay et al., 1984).

Future experiments on the digestibility of chitin should be based on the feeding of raw chitinous material instead of purified chitin: for example, adding certain percentages of ground crab shells to the pellet mixture of *S. gairdneri* may lead to very different results concerning the digestibility of chitin than the ones obtained with the same species adding highly purified chitin to the feed (Buddington, 1980; Lindsay et al., 1984). Results of experiments with *G. morhua* are promising and should encourage further, large scale studies of the digestibility of chitin in fish. Provided that certain fish species (or other organisms) that are of interest for aquaculture have the ability to degrade chitin, feeding chitinous wastes from the crab and shrimp industries may prove valuable in several ways: Along with chitin, proteins in the waste material (Knorr, 1984) might function as an energy source. The carotinoid astaxanthine could play a role as a precursor of vitamin A in fish (Love, 1970). In Norwegian aquaculture, astaxanthine has been utilized for a long time to give colour to the flesh of salmonids. Additionally, there may be surprising indirect effects when some chitin is included in the food, an example of which is reported by Austin et al. (1981): They found in feeding experiments that chitin at 2 % in the food enhances the development of bifido-bacteria in the intestines of chickens, which in turn synthesize the enzyme lactase. Thus, the lactose in the food mixture becomes digestible and the growth of the chickens is encouraged.

The utilization of inexpensive "chitinous waste" could be of great interest to aquaculture. At the same time, a solution to a potential waste disposal problem (Senstad & Almas, 1986), associated with the processing of crabs and shrimps, may be an additional benefit.

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