

***In vitro* digestibility of different prey species of minke whales (*Balaenoptera acutorostrata*)**

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Information on diet composition, daily energy expenditure, energy storage and the utilization of energy in the prey are important factors when evaluating the food consumption of minke whales (*Balaenoptera acutorostrata*) during their summer stay in northern waters. The purpose of the present study was in this context to obtain information on the digestible energy (DE) of different prey selected by minke whales. An *in vitro* three-stage digestion technique, simulating the different compartments of the digestive system, has been developed. The initial step simulated the anaerobic microbial fermentation of substrate in the forestomach. The next stage included the addition of pepsin (*EC* 3.4.23.1)–HCl, simulating ventricle enzymic decomposition, and finally, in the third step, fresh extract from duodenal contents was used to simulate enzymic intestinal degradation of the remaining components of the food. The inoculum was normally obtained from animals which had recently eaten the prey to be tested. In such tests we obtained a dry matter disappearance (DMD) and a DE for herring (*Clupea harengus*) of 80.4 (SD 5.0)% (*n* 18) and 92.1 (SD 3.7)% (*n* 16) respectively, and a DMD of krill (*Thysanoessa* sp.) of 83.4 (SD 4.9)% (*n* 6). The DMD of krill was reduced to 73.8 (SD 7.3)% (*n* 8) while the DE was 70.6 (SD 10.4)% (*n* 7) when inoculum from whales which had recently eaten cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) was used. These results indicate a high digestibility of the most common species of prey in these animals, and also that the whales have little difficulty in changing from one prey species to another.

Digestible energy: Dry matter disappearance: Minke whales

Assuming a population of about 80000 minke whales (*Balaenoptera acutorostrata*) in Norwegian waters (Øien, 1991; Schweder *et al.* 1991) it is important to know what and how much all these animals eat for proper management of both fish and whales. In evaluating the food requirements of the whales it is, among other things, necessary to know the digestibility of the different prey they choose to eat (Nordøy & Blix, 1992). Harp seals (*Phoca groenlandica*), which inhabit much the same waters and eat much the same food, have been extensively studied. Their ability to digest a variety of food is well known, but seals are single-stomached animals, while minke whales depend to a great extent on microbial fermentation in a multi-stomach system (Mathiesen *et al.* 1990; Olsen *et al.* 1993), and findings from seals, therefore, have little application for baleen whales. This probably is particularly true when it comes to digestion of crustaceans, since we have shown that minke whales are rich in chitinase (*EC* 3.2.1.14)-producing microbes in their forestomachs (Mathiesen *et al.* 1990).

Unlike seals and small cetaceans, baleen whales are difficult to keep in captivity for experimental purposes. Based on anatomical and bacteriological examinations of the digestive system of minke whales (Mathiesen *et al.* 1990) we have consequently developed a three-stage *in vitro* technique which is, in principle, a combination of the two-stage procedure of Tilley & Terry (1963) for ruminants and the two-stage *in vitro* technique for

the single-stomached pig (Furaya *et al.* 1979), for evaluation of food digestibility in minke whales. First, fresh inoculum from the forestomach of minke whales is used to mimic the initial bacterial degradation of the substrate. Second, pepsin (*EC* 3.4.23.1)–HCl is added to simulate the digestion in the fundic chamber (ventricle), and finally, extract of duodenal contents is added to imitate intestinal enzymic decomposition of the remaining organic matter. The dry matter is then isolated and compared with the initial dry matter content of the substrate for estimation of dry matter disappearance (DMD), and subsequently the energy contents of the substrate and the residual dry matter are determined by bomb calorimetry for estimation of digestible energy (DE).

MATERIALS AND METHODS

A total of six mature minke whales of both sexes from the north-eastern Atlantic stock were used in the present study.

A laboratory fully equipped with centrifuges, incubators, warm baths, coolers, freezers and other necessary equipment was established aboard a whaling vessel as part of the Norwegian scientific whaling programme in 1989 and 1990. A series of experiments was performed on samples from an animal which had recently eaten krill (*Thysanoessa* sp.) off Spitsbergen and on animals which had recently eaten herring (*Clupea harengus*), cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) off Northern Norway. Fresh samples of krill and herring were obtained by trawling at the same locations.

Preparation of forestomach inoculum

Fresh forestomach contents of recently caught minke whales were collected, filtered through doubly layered gauze cloth, and diluted 1:4 (v/v) with a physiological buffer (Løwgren *et al.* 1989) giving a concentration of culturable viable bacteria in the order of 10^9 cells/ml in the final inoculum. Before use the inoculum was gassed with CO₂ to obtain a pH between 6.7 and 6.9.

Preparation of duodenum extract

About 1 litre fresh duodenal contents was collected from the same minke whale, and diluted 1:1 (v/v) with a physiological buffer (Løwgren *et al.* 1989) and mixed by shaking. The solution was then poured into 50 ml polyethylene vials and centrifuged for 10 min at 2500 g. The supernatant fraction was collected in an Erlenmeyer flask and stored at 5° until use.

In vitro digestion procedure

Between 4 and 10 g freshly thawed krill or cut herring was weighed (accuracy ± 0.1 mg) into 250 ml Erlenmeyer flasks and 50 ml forestomach inoculum from a whale which had recently eaten the prey to be tested was added to each flask. The pH of the inoculum before incubation, ranging between 6.7 and 6.9, was within the range of that measured in the forestomach content (pH 5.36–6.99). In two separate cases inoculum from whales which had recently eaten cod and haddock, respectively, were used with krill as substrate.

The flasks were equipped with one-way valves to ensure anaerobic conditions (Tilley & Terry, 1963). A number of flasks were filled only with inoculum fluid for later analysis of background levels of dry matter. All flasks were then incubated in the dark for either 12 or 24 h to test the effect of different incubation periods. All incubations took place at 34°, which is the deep body temperature of minke whales (Folkow & Blix, 1992). Occasionally, all vials were gently shaken during incubation. At the end of incubation the number of culturable viable bacteria was of the same order of magnitude (10^9 cells/ml) as that found in fresh forestomach fluid.

After incubation with forestomach inoculum the pH was measured to confirm that pH was maintained within the initial range during the first phase of digestion. HCl (2 M) was then added to each flask to obtain a final concentration of 0.075 M-HCl, resulting in a pH of 4.0–5.0, which is the pH measured in the ventricle of minke whales (Mathiesen *et al.* 1990). Pepsin (Sigma; 2900 U/mg) was now added to give a final concentration of 0.33 g/l (Furaya *et al.* 1979) before all the flasks were incubated for another 12 h at 34°. Strictly anaerobic conditions are not necessary during this stage.

Finally, concentrated NaOH was added to give a pH of 6.9–7.0, which is the pH measured in fresh duodenal contents (Mathiesen *et al.* 1990), and 27 ml duodenal extract added. The flasks were now all incubated at 34° for another 12 h, and then frozen at –20° until analysis of dry matter content was made.

Insoluble dry matter isolation

The flasks were later thawed in water (5°) and the contents of each poured into three 50 ml vials. The Erlenmeyer flasks were carefully flushed with cold, distilled water to ensure that no dry matter remained. Experience has shown that it is difficult to filter the samples without previous centrifugation; therefore, the vials were centrifuged for 10 min at 2500 *g* and the supernatant fraction discarded. The precipitate was transferred onto a preweighed glass microfibre filter (Whatman GF/C d: 100 mm) in a Büchner funnel connected to a vacuum pump. After filtration the filter with its precipitate was put into a small preweighed Erlenmeyer flask and dried at 95° for 17 h. Thereafter, the flasks were cooled in a desiccator before being weighed on a calibrated scale (accuracy ±0.1 mg). The dry matter value was then corrected for background and compared with the dry matter content of the substrate before incubation. The following formula was used for estimation of DMD:

$$\% \text{ DMD} = 100 - \left(\frac{\text{DM}_r - \text{DM}_b}{\text{DM}_s} \right) \times 100,$$

where DM_r is the residual dry matter content after incubation, DM_b is the dry matter content of background and DM_s is the dry matter content of fresh substrate at the start of the experiment. DM_s was calculated by drying a number of preweighed substrate samples at 95° for 17 h and expressing dry matter weight as a proportion of fresh 'wet' substrate.

Digestible energy

The samples of the fresh dried substrate and residual dry matter (after the three-stage incubation) had their energy contents (J/g dry matter) measured using a Gallenkamp automatic adiabatic bomb calorimeter. DE was then calculated as follows:

$$\% \text{ DE} = 100 - \left(\frac{(\text{DM}_r - \text{DM}_b) E_r}{(\text{DM}_s) E_s} \right) \times 100,$$

where E_r is the energy content of residual dry matter and E_s is the energy content of the substrate.

Statistical analysis

All values are presented as means and standard deviations, and *n* is the number of parallels. A Student's *t* test was used to test the difference between means.

RESULTS

Most of the dry matter (about 70% of initial dry matter) of the substrate disappeared into solution by bacterial degradation, during the first phase of the *in vitro* digestion procedure (Fig. 1), indicating the importance of the forestomach microbial population in energy

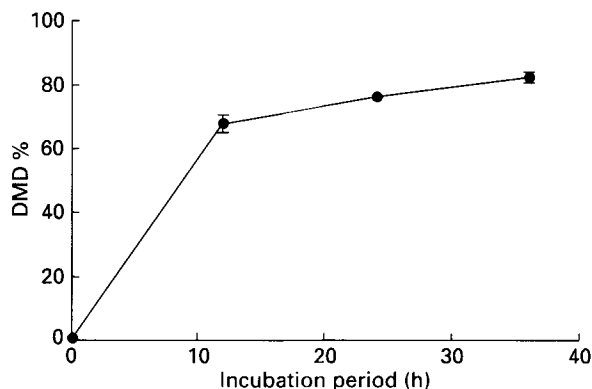


Fig. 1. Typical experiment showing *in vitro* dry matter disappearance (DMD; %) of herring (*Clupea harengus*) by minke whale in relation to the period of incubation (h). A three-stage *in vitro* test was used to simulate (1) the forestomach microbial fermentation, (2) the enzymic breakdown with pepsin (*EC* 3.4.23.1)-HCl in the fundic chamber and (3) the duodenal enzymic decomposition of the substrate. Incubation period was 12 h at each stage.

uptake from prey in minke whales. Changing the incubation period from 12 to 24 h during the first phase did not increase DMD ($P > 0.05$). The residual material from phase 1 was then further digested using added pepsin and duodenal contents containing enzymes during phases 2 and 3 respectively, further increasing the DMD of the prey (Fig. 1).

DMD of krill based on fresh forestomach and duodenal contents from a whale which had recently eaten krill was 83.4 (SD 4.9)% (n 6). In this case DE could not be determined due to lack of residual dry matter. The DMD and DE of krill did not differ significantly ($P > 0.05$) when inoculum from whales which had recently eaten cod and haddock was used; therefore, the results of these experiments were pooled. Thus, the DMD of krill was reduced to 73.5 (SD 7.4)% (n 8), while DE was 70.6 (SD 10.4)% (n 7). The DMD and DE of herring (Fig. 1) were 80.4 (SD 5.0)% (n 18) and 92.1 (SD 3.7)% (n 16) respectively, when fresh forestomach and duodenal contents from three whales which had recently eaten herring were used.

The energy content of residual dry matter of herring was 11.9 (SD 3.3) kJ/g (n 16) compared with 12.1 (SD 2.4) kJ/g (n 10) in the faeces of whales which had eaten herring.

DISCUSSION

The three-stage approach to the measurement of the digestibility of different food eaten by minke whales (Nordøy & Blix, 1992) presented here is derived from the two-stage method of Tilley & Terry (1963) for ruminants, and the two-stage method of Furaya *et al.* (1979) and Graham *et al.* (1989) for pigs. Results obtained by use of these techniques on ruminants and pigs respectively correspond well with those of *in vivo* tests. *In vivo* verification of our method in the minke whale is not feasible for obvious technical and logistical reasons; therefore, we have to rely on indirect evidence. First, the appearance and the smell of the krill in the different stages of decomposition during *in vitro* digestion compare strongly with those of the contents of the corresponding parts of the digestive system of the whale. Second, the energy content of the residual dry matter in our herring experiment was rather similar to that of the faeces of whales which had recently eaten herring.

Our results suggest, therefore, that our measurements are reliable and that the DMD of both krill and herring is about 80%, while DE is in the order of 90%. There are no other digestibility values available for whales, but Ronald *et al.* (1984) reported a DE of 93% in

grey seals (*Halichoerus grypus*) fed on herring, and Parsons (1977) found a DE of above 90% in ringed seal (*Phoca hispida*). Keiver *et al.* (1984), in the hitherto only study of digestibility of crustaceans in seals, found that the DE of *Pandalus borealis* in harp seals was less than 72%. This is likely to be lower than the DE of krill in minke whales, and suggests that the microbial fermentation in the forestomach of the whales (Herwig *et al.* 1984; Herwig & Staley, 1986; Mathiesen *et al.* 1990; Olsen *et al.* 1993) increases the utilization of particularly resistant components such as the chitin skeletons of the krill.

It is also intriguing that the minke whales appear to be able to adapt readily to different types of prey, although the utilization of the new prey at first seems to be somewhat reduced, probably being due to the time taken for the microbial population in the forestomach to adjust to the new substrate.

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