Do minke whales (*Balaenoptera acutorostrata*) digest wax esters?

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(*Received 13 January 1995 – Revised 24 March 1995 – Accepted 29 March 1995*)

Mammals are known to utilize wax esters with an efficiency of less than 50%. The purpose of the present study was to examine whether or not minke whales (*Balaenoptera acutorostrata*), which at times may eat considerable amounts of wax-ester-rich krill, represent an exception to this general pattern. Samples of fresh undigested forestomach, as well as colon, contents were obtained from minke whales (*n* 5) that had been feeding on krill (*Thysanoessa inermis*) for some time. The samples were analysed for dry mass, energy density, lipid content and the major lipid classes, including wax esters. The concentrations of wax esters were compared with previous estimates of dry-matter disappearance of the same type of prey using an *in vitro* technique, to calculate the dry-matter digestibility of wax esters (DMD_{wax}). Wax esters contributed 21% of the energy and 47% of total lipids in the krill diet. The energy density of gut contents decreased by 50% after their passage from forestomach to the end of the colon. The DMD_{wax} was 94·1 (SD 2·8)% (*n* 5). This high DMD_{wax} and the occurrence of fatty alcohols, one of the products of wax-ester hydrolysis, in faeces show that minke whales are very efficient digesters of wax esters and absorb most of the energy-rich products of this process.

Minke whale: Krill: Wax esters: Digestive efficiency

The importance of wax esters at different trophical levels in the marine ecosystem has been recognized for more than two decades (Benson & Lee, 1975; Bauermeister & Sargent, 1979). A number of studies have documented the flux of this energy-rich substrate through the lower levels of the food chain. At higher trophical levels both marine fishes and birds have been shown to be efficient converters of marine waxes to substrates available for energy expenditure and energy deposition (Patton & Benson, 1975; Sargent *et al.* 1979; Jackson & Place, 1990; Place, 1992*a*). However, there exists no information on wax ester assimilation efficiency in marine mammals.

Minke whales (*Balaenoptera acutorostrata*) in the Southern Ocean prey almost exclusively on krill (Ohsumi, 1979), while in the Northern Atlantic they also prey on a variety of fish (Jonsgård, 1982; Nordøy & Blix, 1992). In particular, minke whales that occupy the ice-edge zone of the Northeastern Atlantic in mid-summer appear to feed extensively on krill (Nordøy & Blix, 1992). A large proportion of the dry matter of many polar krill species consists of wax esters (Benson & Lee, 1975), which they accumulate in early summer during the seasonal plankton blooms (Falk-Petersen *et al.* 1981). Waxes are known to be poorly digested by many mammals, including humans, and may even have purgative effects in some cases (Hansen & Mead, 1965; Verbiscar *et al.* 1980).

The present study was undertaken to study the efficiency of wax ester digestion in minke whales and thus examine whether these marine animals have developed an adaptation to utilize an energy source poorly utilized by terrestrial mammals. This was done by measuring the wax ester concentration, along with other lipid classes, of forestomach and

colon content of krill-feeding minke whales, and comparing these measurements with previous estimates of dry matter digestibility (DMD) of krill measured by *in vitro* techniques (Nordøy *et al.* 1993).

MATERIALS AND METHODS

Samples from five minke whales with recently ingested prey in their forestomachs were obtained during the Norwegian scientific whaling programme in July 1989 (Table 1), under licences issued by the Norwegian Government, in accordance with Article VIII of the International Convention for the Regulation of Whaling. Catches were carried out west and north of Spitsbergen. Forestomach and colon contents were collected and frozen within 30 min after death and kept at −20° until analysis. Forestomach and faecal analyses revealed that the animals had, for some time, been eating krill (*Thysanoessa inermis*).

Dry mass of fresh samples and dry mass:wet mass ratio were determined by weighing samples (about 2 g wet mass) before and after drying for 24 h at 60°. Fat was extracted by the following procedure: about 8 g 'wet' sample was weighed into a centrifuge vial. To reduce adhesion of sample to the centrifuge vial, 0·5 g Na_2SO_4 was added. Ethyl acetate (25 ml) was then added and the content homogenized for 60 s using an ultraturrax. The vials were centrifuged at 7000 rev./min for 5 min, whereafter the supernatant fraction was transferred to a round-bottomed flask. The ethyl acetate was evaporated on a rotary evaporator (55°) and the fat content of the dry matter calculated using the following formula:

$$\% \text{ fat of dry mass} = \frac{(25 \times \text{mass of fat} \times 100)}{(\text{volume of supernatant fraction} \times \text{dry mass})}. \quad (1)$$

For determination of the major lipid classes, 15 μg extracted fat from each sample was applied to high-performance TLC (HPTLC) plates, coated with silica gel 60 and developed alongside standards, using hexane–diethyl ether–acetic acid (88:15:1, by vol.) as developing solvent. A duplicate set of samples was analysed by HPTLC using hexane–diethyl ether–acetic acid (70:30:1, by vol.) to confirm identities of components. Developed plates were sprayed with the copper acetate–phosphoric acid reagent of Fewster *et al.* (1969) and the separated components quantified by scanning densitometry as detailed by Sargent *et al.* (1977).

The energy density of dried samples of forestomach and colon contents was determined with a CBA-301 automatic adiabatic bomb calorimeter with a CVM-3000 microprocessor (Gallenkamp, London).

Digestibility of wax esters in minke whales was calculated by combining the values obtained for wax ester concentration in food and faeces with values for DMD for the same food previously published using an *in vitro* three-stage procedure (Nordøy *et al.* 1993). These *in vitro* experiments were performed on material from the same whales from which forestomach and colon samples were obtained and using the same type of prey as these whales had been feeding on.

First, the total wax ester content of the meal (TW_{meal}) was determined as follows:

$$TW_{meal} = (\text{meal size} \times \% \text{ DM}) \times [\text{wax}]_{meal}, \quad (2)$$

where meal size (Table 1) is expressed in g, % DM is the percentage dry matter content and $[\text{wax}]_{meal}$ is the wax ester concentration of the meal, expressed as a percentage of total dry matter. The amount of wax ester assumed to appear in the faeces (TW_{faeces}) from this meal was calculated using the following formula:

$$TW_{faeces} = (\text{meal size} \times \% \text{ DM} \times \% \text{ FDM}) \times [\text{wax}]_{faeces}, \quad (3)$$

Table 1. *Date of capture, sex, length (m) and forestomach volume (litres) of five minke whales from which samples of forestomach and colon content were obtained*

Whale no.	Date of capture	Sex	Length (m)	Forestomach volume (litres)
1	11.07.89	F	7·70	10
2	13.07.89	F	6·90	12
3	14.07.89	F	7·85	12
4	17.07.89	F	7·70	30
5	17.07.89	F	6·20	24

where the dry matter of the meal (meal size × % DM) is multiplied by percentage faecal dry matter (% FDM) assumed to be produced from this meal. This value is obtained from the *in vitro* three-stage procedure, which indicates that 83·4% of the dry matter of krill is digested (Nordøy *et al.* 1993). Thus, % FDM is 100% − 83·4% = 16·6%. $[\text{Wax}]_{\text{faeces}}$ is the wax ester concentration of faeces, expressed as a percentage of total dry matter.

The DMD of wax esters (DMD_{wax}) then becomes:

$$\text{DMD}_{\text{wax}} = \frac{(\text{TW}_{\text{meal}} - \text{TW}_{\text{faeces}})}{\text{TW}_{\text{faeces}}} \times 100\,\%. \quad (4)$$

Similar calculations were also made to determine the digestibility of total lipid and the other classes of lipids present in the prey.

All values are presented as means and standard deviations. The variability of the *in vitro* determinations of DMD (SD 4·9%) (Nordøy *et al.* 1993) gives an uncertainty in the estimate of DMD_{wax} of ±2·0% for individual whales. A Students' *t* test was used to test the difference between means. $P < 0{\cdot}05$ was regarded as significant.

RESULTS

The energy density of the fresh forestomach samples was 24·2 (SD 0·6) kJ/g (*n* 5), which is slightly higher than that obtained for *Thysanoessa* sp. collected by trawling at the same time of year (Mårtensson *et al.* 1994), confirming that the forestomach samples were fresh. After digestion the energy density of the gut contents decreased to 13·0 (SD 1·5) kJ/g (*n* 5) (Fig. 1). There was a close correlation between the lipid content and energy density of the samples (r 0·998, $P < 0{\cdot}001$). Using an energy density value of 39·7 kJ/g for fat (Kleiber, 1975) it was calculated that wax esters contributed about 21% of the energy in the krill diet.

The dry matter content of the fresh krill obtained in the forestomach, 19·8 (SD 3·5)% (*n* 5), was not significantly different ($P > 0{\cdot}05$) from the dry matter content of the faecal samples, which was 20·9 (SD 3·3)% (*n* 5). The lipid content of the dry matter, however, decreased from 26·9 (SD 1·8)% in the forestomach to 11·8 (SD 3·3)% (*n* 5) in the faecal samples ($P < 0{\cdot}001$).

Wax esters were the most important lipid class in fresh *Thysanoessa inermis* constituting 47% of total lipids or 12·6 (SD 1·3)% (*n* 5) of total dry mass (Fig. 2), being similar to that of fresh krill caught by trawling (Falk-Petersen *et al.* 1981). After digestion, wax esters decreased to 4·3 (SD 1·9)% (*n* 5) of total dry mass in the faecal sample (36% of total lipids). The triacylglycerols (TAG), free fatty acids (FFA), sterols and partial acylglycerols all decreased significantly ($P < 0{\cdot}05$) their contribution to total dry matter during digestion in

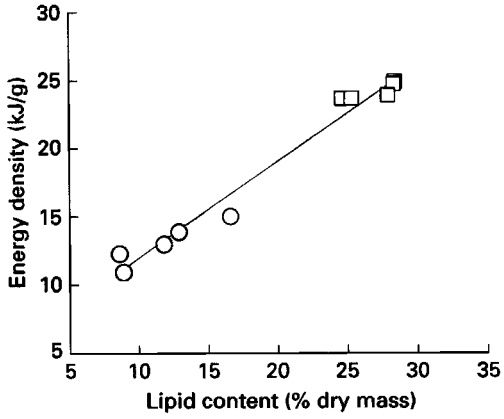

Fig. 1. The energy density (kJ/g dry mass) as a function of lipid content (% dry mass) of colon (○) and forestomach (□) samples from five minke whales (*Balaenoptera acutorostrata*) containing digested and fresh krill (*Thysanoessa inermis*) respectively.

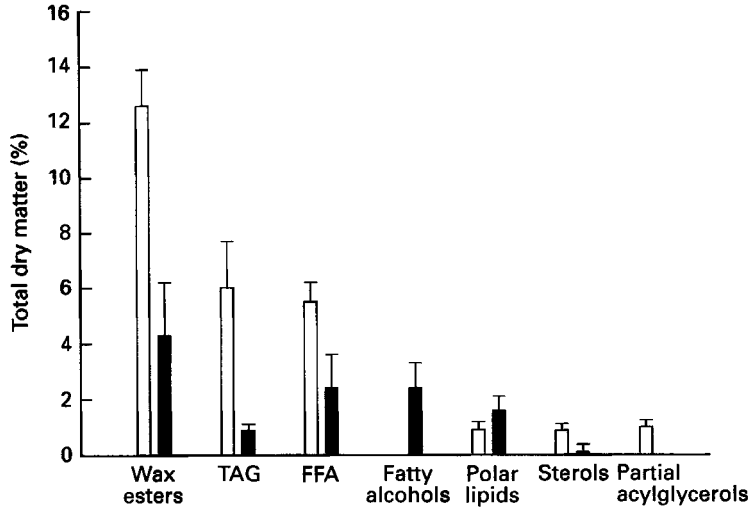

Fig. 2. The concentration (% total dry matter) of wax esters, triacylglycerols (TAG), free fatty acids (FFA), fatty alcohols, polar lipids, sterols and partial acylglycerols of fresh undigested forestomach content (□) and colon content (■) collected from five minke whales (*Balaenoptera acutorostrata*) that had been feeding on krill (*Thysanoessa inermis*) for some time. Values are means with standard deviations indicated by vertical bars.

minke whales (Fig. 2). Interestingly, fatty alcohols, which are absent in fresh krill, appeared in faecal material.

The following example illustrates how the digestibility of wax esters was estimated using equations (2), (3) and (4). The forestomach of whale no. 1 contained a fresh meal of about 10000 g Krill (Table 1). The dry mass of this was 1980 g (10000 × 19·8 %) of which 250 g was wax esters (1980 × 12·6 % (Fig. 2)). From *in vitro* experiments it is assumed that 83·4 (SD 4·9) % of 1980 g dry krill mass is absorbed (Nordøy *et al.* 1993); this produces about 330 g dry faeces, of which 15 g is wax esters (330 × 4·3 %; Fig. 2). According to equation (4) the DMD_{wax} then becomes: $DMD_{wax} = ((250-15) \times 100\,\%)/250 = 94\,\%$.

By combining data from all five whales, the DMD_{wax} in the krill diet of minke whales was calculated to be as high as 94·1 (SD 2·8) % (*n* 5). In comparison, the digestive efficiency of

total lipid in the krill diet of minke whales was about 93%. While TAG, sterols and partial acylglycerols were all almost completely absorbed during digestion, polar lipids were the only class of fat with a rather lower digestibility of about 70%.

DISCUSSION

The values for DMD of wax esters presented here are based on previous estimates of DMD using an *in vitro* three-stage technique (Nordøy *et al.* 1993) and the assumption that these can be used to predict the DMD of various prey of the minke whale *in vivo*. It can thus be argued that the DMD of wax esters obtained in this study may not be representative of the true digestive processes in a live minke whale. However, we have found that the energy density of the residue from the three-stage *in vitro* technique is very similar to the energy density of faeces from the minke whale, when the same prey has been digested (Nordøy *et al.* 1993). Moreover, we have found a very good correlation between the results of this method and the use of an independent Mn-marker method (Mårtensson *et al.* 1994), when estimating the digestive efficiency of herring in minke whales, indicating that the values obtained by use of the three-stage *in vitro* method are reliable.

This study therefore suggests that minke whales are able to absorb about 93% of the lipids and digest about 94% of the wax ester content of *Thysanoessa inermis*. Not all of the energy released from the hydrolysis of wax esters is available for absorption in the minke whale, since fatty alcohols and fatty acids, the products of wax ester hydrolysis, appear in some quantity in the faecal matter of the minke whale (Fig. 2), demonstrating incomplete absorption of these products. By adjusting for this energy loss the digestive efficiency of wax esters in minke whales appears to be about 92%. This is very high compared with other mammals, like the rat and dog, which absorb less than 50% (Hansen & Mead, 1965) and 10% (Place, 1992*b*) of the ingested wax esters in experimental diets respectively. In contrast, many marine species of seabirds, with the interesting exception of the rockhopper penguins (*Eudyptes chrysocome*), have a unique capacity for assimilating (> 90%) marine wax esters (Jackson & Place, 1990; Place, 1992*a*, *b*). This high utilization rate has been attributed to a high concentration of bile salts, a retrograde movement of duodenal contents to the gizzard, prolonging the exposure of wax esters to lipolytic enzymes, and a nearly equivalent hydrolysis of wax esters and TAG promoting the action of lipolytic enzymes.

Whether the minke whale has developed some similar adaptations for improving digestibility of, for example, wax-ester-rich krill is not known. However, extensive studies of the gastrointestinal anatomy of minke whales have been performed (Olsen *et al.* 1994*b*), speculating as to how a high wax ester utilization is achieved. Minke whales have a small intestine only four times their body length, suggesting that the exposure time to bile, lipases and salts is rather short compared with, for example, seals which have a small intestine of sixteen to twenty-five times body length (Helm, 1983). The answer to an efficient wax ester utilization may be the existence of a multi-chambered stomach system, probably favouring retention of foods in this compartment (Olsen *et al.* 1994*b*). A high concentration of anaerobic bacteria has been found in the first chamber of this system, the forestomach (Olsen *et al.* 1994*a*). It may be that these micro-organisms assist in an early hydrolysis of the wax esters of prey. Indirect evidence for this has been obtained by the three-stage *in vitro* technique (Nordøy *et al.* 1993). The first stage, which simulates the bacterial decomposition of food in the forestomach, has indicated that as much as 60–70% of the dry matter of krill is digested there.

In conclusion, this study indicates that the baleen whales, exemplified here by the minke whale, have a unique capacity among mammals to digest wax esters and in this context

resemble other marine inhabitants, like most seabirds and fishes. This high digestive efficiency confirms that previous estimates of digestible energy of whole krill (Nordøy *et al.* 1993; Mårtensson *et al.* 1994) are widely applicable for other crustacean prey with varying seasonal contents of wax esters.

The author thanks the crew on board the whaling vessel M/S 'Båragutt' for cooperation in the field, and Professor A. S. Blix and Dr S. Falk-Petersen for providing helpful comments on this manuscript. This study was supported in part by the Norwegian Fisheries Research Council, Marine Mammal Research Programme, grant no. 408.008.

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Printed in Great Britain