Cholecalciferol supplementation alters gut function and improves digestibility in an underground inhabitant, the naked mole rat (*Heterocephalus glaber*), when fed on a carrot diet

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Naked mole rats (*Heterocephalus glaber*) lead a strictly subterranean existence and appear to be naturally deficient in cholecalciferol (D_3). Oral supplementation with D_3 (D_s) led to a 1·8-fold increase in food intake and the associated enlargement (1·4-fold) of the caecum. The effect of D_s, and the concomitant increase in food intake, on caecal fermentation efficiency when animals were fed on a carrot-based diet was determined by measuring the rate of both gas production and short-chain fatty acid (SCFA) production. Microbial-controlled fermentation processes in the caecum were enhanced with D_s when compared with animals not receiving a D_3 supplement (D_n). Both the rates of gas production (D_n 10·76 (SE 0·77), D_s 15·20 (SE 1·77) ml/g dry matter (DM) per h) and SCFA production (D_n 463·0 (SE 33·7), D_s 684·3 (SE 74·8) μmol/g DM per h) increased more than 1·4-fold per g DM caecal substrate. These factors contributed to the higher digestibility of the food in D_s animals. The larger quantity of energy available to D_3-replete naked mole rats was not used in anabolic processes, for these animals maintained mass. These findings suggest that metabolic rate in D_3-replete animals was elevated. Thus, despite improved gut function, D_3-replete animals may be disadvantaged by their higher energy and food requirements in their natural milieu.

Cholecalciferol: Caecal fermentation: Mole rats

The naked mole rat (*Heterocephalus glaber*; Rodentia; Bathyergidae) lives in an extensive maze of underground burrows in the compacted soils of arid north coast Africa. Here it feeds on a herbivorous diet of roots and tubers (Jarvis & Bennett, 1991) which are scarce, randomly distributed and invariably high in fibre. Digestion of fibre is facilitated by a large population of symbiotic bacteria and protozoa in the hind-gut (Buffenstein & Yahav, 1991*a*). These micro-organisms digest the insoluble plant fibres, via fermentation processes, and in so doing produce gaseous waste products (CO_2, CH_4 and H_2) and short-chain fatty acids (SCFA). The SCFA (i.e. acetic, propionic, butyric acids) are absorbed by the host animal through the caecum and large intestine and are used as an energy source by the host animal (Rechkemmer *et al.* 1988).

Fermentation processes contribute to the digestibility and maximal usage of the available food. Maximal utilization of food is highly advantageous in an underground environment,

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where there is a high energetic cost associated with the location of randomly distributed underground food supplies (Vleck, 1981).

Mole rats, living in an environment devoid of sunlight and consuming only plant material, appear to be naturally cholecalciferol (D_3)-deficient. Serum 25-OH vitamin D_3 levels are undetectable ($< 0{\cdot}004$ nmol/ml; Buffenstein *et al.* 1988, 1991). In addition mole rat mineral absorption when fed on a diet of sweet potato (*Ipomoea batatas* Poir) and apple appears to be independent of D_3 (Buffenstein & Yahav, 1991*b*) and Ca absorption is via non-saturable passive processes (Skinner *et al.* 1991; Buffenstein & Yahav, 1991*b*). Mole rats, however, can metabolize tritium-labelled D_3 to its more polar metabolites and possess calbindins thought to be D_3-dependent (Buffenstein *et al.* 1988). These findings imply that they do not normally require D_3 for mineral metabolism but may employ D_3, if and when it becomes available, in other physiological processes.

Deficiency in D_3 is a complex nutritional state in which most mammals consume less (Chertow *et al.* 1983). A decrease in food intake might influence gut function. We, therefore, investigated the effect of an oral supplement of D_3 on food intake and gut function in these chthonic naturally D_3-deficient rodents when maintained on a carrot-based diet.

In captivity, mole rats, when given a variety of foods, select carrots, sweet potato and apple (Jarvis, 1991). Previous fermentation studies (Buffenstein & Yahav, 1991*a*) have indicated that fermentation efficiency on a carrot diet was superior to that on sweet potato and apple. Furthermore, we have previously found that D_3 supplementation had no effect on the damara mole rat (*Cryptomys damarensis*) when fed on sweet potato and apple (Skinner *et al.* 1991), whereas it had a pronounced effect when animals were fed on carrots (unpublished results). We, therefore, examined whether fermentation efficiency improved with D_3 supplementation.

MATERIALS AND METHODS

Animals and treatment

Non-breeding, adult (1–2-year-old) male naked mole rats were used in the present study. These animals were born in captivity. The progenitors of these animals were collected in Kenya. Captive mole rats were housed in climatically controlled rooms ($30° \pm 2°$; 75% relative humidity) in accordance with the housing protocol suggested by Jarvis (1991) for a minimum period of 6 months before experimentation. These rooms had no access to natural light and animals were housed under dim (20 W) incandescent light. Mole rats show no circadian rhythm in activity and time of feeding and were, therefore, always supplied *ad lib.* with carrots. They were maintained on this diet for 6 weeks before experimentation.

At 10 d before monitoring food intake and excretory losses, animals were housed individually in metabolism cages and given free access to the chosen diet. Two groups of mole rats were used in the present study. Both were fed on the same fresh carrot diet and subjected to the same experimental conditions, the only difference being that an oral supplement of D_3 was given to the second group (D_s).

The D_3 dose was determined from the American Institute of Nutrition (1977) prescribed dose for rats (25 ng D_3/g dry matter food eaten). This was administered orally in an oil vehicle ($0{\cdot}1$ mmol/l) every 3 d during the experimental period, starting 6 d before the commencement of monitoring food intake and faecal output.

Food budget

Over a 12 d period, body mass, food supplied, left-over food and the quantity of faeces produced were monitored daily. Uneaten food, daily faecal output and representative food samples, dried to constant mass at 70°, were weighed.

Table 1. *Change in body mass, food intake and digestibility in the naked mole rat* (Heterocephalus glaber) *when fed on a diet of carrots without* (D_n) *and with* (D_s) *an oral supplement of cholecalciferol**

(Mean values with their standard errors)

No. of animals	D_n 9		D_s 8		Confidence intervals (CI)	Statistical significance of difference: $P =$
	Mean	SE	Mean	SE	95% CI	
Average mass (g)	31·9	1·8	37·9	2·2	—	
Change in mass (%/d)	−0·21	0·11	0·08	0·17	0·29, −0·14, 0·72	0·18
Food intake (g/100 g animal per d)	3·56	0·37	6·21	0·33	2·65, 1·58, 3·72	0·000087
Digestibility (%)	89·4	1·6	94·5	1·5	5·04, 0·35, 9·74	0·037

* For details of animals and treatment, see p. 234.

Table 2. *Caecal measurements for the naked mole rat* (Heterocephalus glaber) *when fed on a diet of carrots without* (D_n) *and with* (D_s) *an oral cholecalciferol supplement**

(Mean values with their standard errors)

	D_n		D_s		Confidence intervals (CI)	Statistical significance of difference: $P =$
	Mean	SE	Mean	SE	95% CI	
Caecal mass (g DM/100 g animal)	0·69	0·10	1·15	0·12	−0·46, −0·82, −0·10	0·016
Caecal wall mass (g DM)	0·24	0·02	0·33	0·04	−0·09, −0·20, 0·01	0·084
Caecal fluid: No. of protozoa ($\times 10^6$/ml)	6·67	1·13	9·34	2·12	1·14, −0·95, 3·23	0·11
No. of bacteria ($\times 10^9$/ml)	7·11	0·36	8·09	3·17	0·98, −1·21, 3·17	0·36
Caecal pH	6·60	0·08	6·70	0·04	−0·1, −0·30, 0·10	0·29

DM, dry matter.

* For details of animals and treatment, see p. 234.

Caecal measurements

At the end of the period of 'food budget' monitoring, unstarved animals were killed, between 08.00 and 10.00 hours, by injecting Euthenase (Centaur Laboratory, Johannesburg) intraperitoneally (200 mg sodium pentobarbitone/ml; 0·01 ml/g body weight). The gastrointestinal tract was immediately removed intact, and the caecum separated from the rest of the gut. This was weighed and the pH measured. Thereafter the caecal contents were mixed to obtain uniform portions. Weighed portions (approximately 0·3 g) were immediately used in fermentation studies and in the determination of dry matter content.

Fermentation capacity

Rates of gas production and short-chain fatty acid (SCFA) production were measured to determine fermentation capacity. This was done by transferring the weighed caecal sample to a 25 ml Warburg flask and rapidly displacing the air within the flask with CO_2. The manometric apparatus was kept open, allowing the displacement of CO_2 by the gases produced during fermentation. Thereafter the flasks were sealed and incubated at 33°, the optimal temperature for caecal function in these animals (Yahav & Buffenstein, 1991). The volume of gas produced in the flasks was monitored every 5 min for 1 h using standard manometric techniques. The gas was released after each period of monitoring. The rate of gas production at the time the caecum was excised was extrapolated from the exponential relationship between gas production and time, in compliance with the methods of Carol & Hungate (1954).

Rate of SCFA production was determined from SCFA concentration in caecal samples taken after excising the caecum and those taken after the completion of gas production measurements (McBee, 1970). Known amounts of NaOH (100 g/l) were added to each sample. These samples were then stored at −20° for later analysis. The concentration of SCFA was determined using gas–liquid chromatography (Carlo Erba Strumentzione 4200, Searle Instruments, Johannesburg) using pivalic acid as an internal standard (Davis, 1988).

The dry matter content was determined by taking weighed portions of caecum content and drying these at 70° to constant mass.

Micro-organism analysis

Bacteria and protozoa present in the caecal fluid were counted directly using a haemocytometer (0·1 mm deep) and light microscopy.

Statistical analyses

Untransformed data were subjected to a Kolmogorov–Smirnov test and the data were found to follow a normal distribution. Thereafter data from the two experimental treatments (with (D_s) and without (D_n) D_3 supplementation) were compared using a two-tailed unpaired Student's t test (Zar, 1974). All values were expressed as means with their standard errors and were considered significantly different at $P \leqslant 0{\cdot}05$.

RESULTS

Body mass

Irrespective of experimental treatment, animal weight remained relatively constant throughout the monitored experimental period (Table 1) and percentage change in mass between the two experimental treatments was not significant ($P = 0{\cdot}18$).

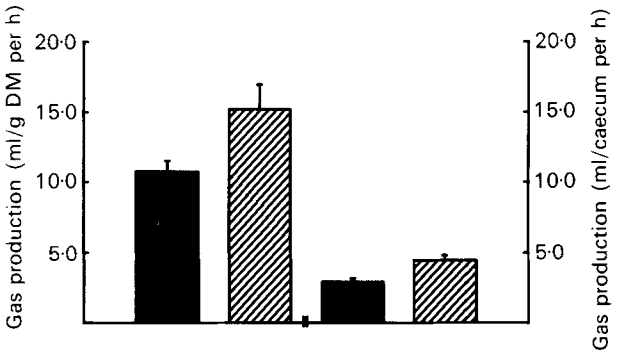

Fig. 1. The effect of cholecalciferol (D_3) on rates of gas production (measured as zero gas production; see p. 236) in the naked mole rat (*Heterocephalus glaber*). Values are means with their standard errors represented by vertical bars. Mean values were significantly increased with cholecalciferol (D_3) supplementation (▨) when compared with that of animals not receiving an oral D_3 supplement (■): on ml/g dry matter (DM) per h basis $P = 0{\cdot}031$; on a ml/caecum per h basis $P = 0{\cdot}028$.

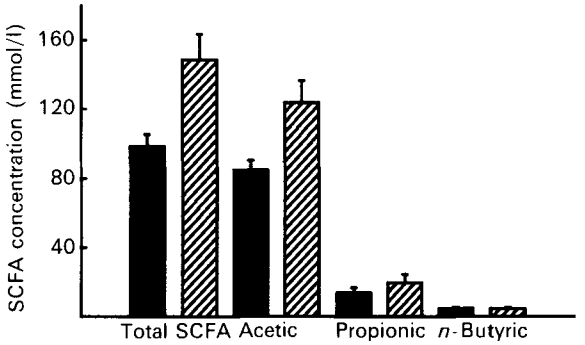

Fig. 2. Initial short-chain fatty acid (SCFA) concentrations in the caecum of the naked mole rat (*Heterocephalus glaber*) when fed on a diet of carrots without (■) and with (▨) an oral cholecalciferol (D_3) supplement. Values are means with their standard errors represented by vertical bars. Mean values were significantly different for total SCFA and acetic acid $P = 0{\cdot}0061$. All other pairs of values were not significantly different. For details of procedures, see pp. 234–236.

Food intake

Oral supplementation of D_3 had a pronounced effect on appetite. Food intake increased 1·8-fold (Table 1). Increase in food intake was accompanied by a 5·1 percentage units increase ($P = 0{\cdot}037$) in digestibility.

Caecal measurements

Caecal mass (expressed relative to final mass, per 100 g animal) increased 1·4-fold with D_3 supplementation (Table 2). Caecal wall mass increased 1·2-fold; however, this trend was not statistically confirmed ($P > 0{\cdot}05$). Supplementation with D_3 had no effect on caecal pH (Table 2).

The average number of protozoa (1·4-fold) and bacteria (1·1-fold) in caecal fluid increased with D_3. However, these trends were not statistically confirmed ($P \geqslant 0{\cdot}10$; Table 2).

Gas production

Gas production per g dry matter increased 1·4-fold ($P \leqslant 0{\cdot}03$) with D_3. When the enlarged caecal mass is taken into account gas production per caecum increased 1·53-fold ($P = 0{\cdot}03$) with D_3 (Fig. 1).

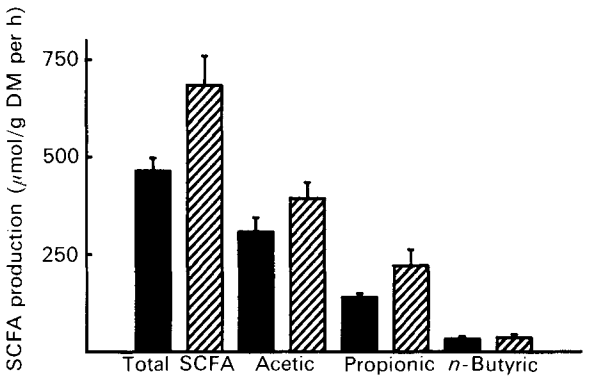

Fig. 3. Rates of short-chain fatty acid (SCFA) production (/g dry matter) in the naked mole rat (*Heterocephalus glaber*) when fed on a diet of carrots without (■) and with (▨) an oral cholecalciferol (D_3) supplement. Values are means with their standard errors represented by vertical bars. Mean values for total SCFA were significantly different: $P = 0{\cdot}03$. All other pairs of values were not significantly different. For details of procedures, see pp. 234–236.

SCFA

Oral supplementation of D_3 resulted in a significant increase in total SCFA concentration and acetic acid concentration ($P \leqslant 0{\cdot}0061$ and $P \leqslant 0{\cdot}0128$ respectively) in the caecum. When values were expressed per g dry matter, a significant increase in the total SCFA production was demonstrated in D_s animals (Fig. 2). The values for SCFA produced in D_3 replete animals:SCFA in deficient animals were 1·27; 1·58; and 1·11 for acetic, propionic and butyric acids respectively. The increases in production rate of individual SCFA were not statistically significant. When the individual increments of each SCFA were compounded, however, total SCFA production was significantly enhanced with D_s (Figs. 2 and 3).

DISCUSSION

Despite the fact that naked mole rats appear to be naturally D_3 deficient (Buffenstein *et al.* 1988), oral supplementation of D_3 led to an increase in appetite, food digestibility and a marked improvement in caecal function.

Food intake followed reported trends (Chertow *et al.* 1983; Kadowaki & Norman, 1984; Nyomba *et al.* 1984; Clark *et al.* 1986) and increased 1·8 times with D_s (Table 1). This could be attributed to either a direct effect on appetite receptors in hypothalamus (Stumpf *et al.* 1982) or an indirect effect via changes in carbohydrate metabolism. Carbohydrate metabolism in D_3-deficient animals is known to be defective (Norman *et al.*1980; Clarke *et al.* 1981). Insulin secretion is particularly affected, in that it is impaired in D_3-deficient animals and enhanced by D_3 supplementation (Norman *et al.* 1980; Kadowaki & Norman, 1984; Clark *et al.* 1986). Changes in blood glucose levels with D_3 supplementation, may in turn lead to an increase in appetite and food intake.

Naked mole rats showed no response to D_3 supplementation when fed on a diet of sweet potato and apple (Buffenstein & Yahav, 1991*b*), yet when the diet was altered to that of carrot (in the present study), appetite was enhanced. Differences between these two studies could possibly be attributed to different dietary fibre, glucose and starch contents (Buffenstein & Yahav, 1991*a*).

Despite the pronounced increase in food intake and digestibility, naked mole rats showed no significant increase in body mass (Table 1). These findings suggest that energy expenditure was raised in response to D_3 supplementation. Clark *et al.* (1986) showed

similar mass constancy despite an increase in food intake and attribute this to increased soft tissue metabolism. Increased food requirements and elevated metabolism in response to D_3 supplementation would disadvantage animals living underground in an environment where food resources are limiting and their underground location is energetically costly (Vleck, 1981). Furthermore an elevation in metabolic rate, with the concomitant increase in heat production, would handicap the ability of these underground inhabitants of warm equatorial Africa to keep body temperature below lethal levels. Mechanisms of heat loss in an underground closed environment are impeded by the high relative humidities which preclude the efficient use of evaporative cooling and also by the absence of air movement for convective cooling. Naked mole rats naturally exhibit a reduced metabolic rate when compared with superterranean rodents and this is thought to be highly adaptive to the prevailing burrow conditions (Buffenstein & Yahav, 1991*c*).

In most hind-gut fermenters, increased food intake is accompanied by a decline in food digestibility (Sibly, 1981; Van Soest, 1982). Mole rats did not conform with this generalization; instead, digestibility improved (Table 1). Increased food intake and digestibility were accompanied by an enlargement in caecal size (Table 2). Similar improvements in food utilization with increased food intake have been reported when D_3-deficient rats were given D_3 supplementation (Clark *et al.* 1986). The mode of action of D_3 supplementation on digestibility is, however, not known. We suggest that improved digestibility is a result of enhanced microbial-controlled fermentation processes in the enlarged caecum. The precise mechanism is unknown. We speculate that the number and efficacy of micro-organisms increases when an oral D_3 supplementation is given.

Evidence supporting an improvement in microbial-controlled fermentation processes with D_s include a 1·4-fold increase in both the rate of gas production (Fig. 1) and the total SCFA production per g substrate dry matter (Fig. 2). Substrate utilization and microbial functional efficiency were, thus, enhanced. Fermentative micro-organisms can metabolize D_3, albeit to unusual derivatives (Sommerfeldt *et al.* 1980; Gardener *et al.* 1988), and it is possible that D_3 directly affects microbial function The mode of action of D_3 on microbial function, however, has to date not been investigated.

Population counts on micro-organisms in the fermentative chamber (caecum/rumen) fluid may only serve as indicators of the true population density. This is because most of the fermentative micro-organisms adhere to plant fibres and are excluded from population assessments (El-Din & El-Shazly, 1969). Trends (whilst not statistically significant) in population density in the caecal fluid showed that the number of bacteria increased with D_s at least 1·1-fold and that the number of protozoa increased 1·4-fold (Table 2). Similar incremental changes (1·4–1·5-fold) in the number of protozoa, gas and total SCFA production per g dry matter suggest that D_3 had a direct effect on microbial proliferation and that the improved microbial function (per g substrate dry matter) is a reflection of, primarily, the change in protozoa population density. Not only does the number of micro-organisms appear to increase with D_s, but protozoal species diversity also appears enhanced. In the absence of a D_3 supplement, only one bi-flagellate protozoal species was seen (Buffenstein & Yahav, 1991*a*). At least three other holotrich ciliate species were observed in caecal fluid samples taken from D_s animals (S. Yahav & R. Buffenstein, unpublished results). The different values for SCFA produced in D_s:SCFA in D_n for the three acids (acetic 1·26; propionic 1·58; butyric 1·11) suggest that increased SCFA production is not merely due to an increase in population density but may be also due to species changes with D_s.

Enhanced fermentation efficiency coupled with the 1·4-fold increase in caecal mass elicited the doubling of the total SCFA available to the naked mole rat (Figs. 2 and 3). These SCFA are an important energy source to the host animal (Van Soest, 1982) as they

are absorbed into the blood and provided an energy-rich substrate for metabolism (Rechkemmer *et al.* 1988). Oral supplementation of D_3 assists in the liberation of a large energy supply that would otherwise be lost to this underground inhabitant. The increase in available energy is, however, not used in anabolic processes by these animals and appears rather to be dissipated by an elevation in metabolic rate.

In conclusion, hind-gut microbial function is enhanced by oral D_3 supplementation. This results in improved food utilization and a concomitant larger energy supply to the host animal. The additional energy is dissipated, with no evidence for anabolic usage, as these animals maintained body mass despite a 1·8-fold increase in food intake by D_3-replete mole rats. As D_3 repletion is not essential for mineral homeostasis (Buffenstein & Yahav, 1991*b*), a D_3-replete status does not afford any obvious advantage to these underground inhabitants.

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