# Efficacy of $^{22}Na$ turnover in ecophysiological studies of carnivores

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$^{22}Na$ turnover rates gave overestimates of mean $^{23}Na$ intake in both brown hyaenas (449,9%, range 54,7–991,2 ) and aardwolf (336,0%, range 118,4–776,0 ). There was also no significant relationship between $^{22}Na$ turned over and $^{23}Na$ ingested in either species. It would therefore be impossible to utilize this technique to determine food intake in these species. Further uncertainties pertaining to the dietary content of the predator and the distribution of body sodium in the prey, cast doubts on the applicability of the technique for use in carnivore studies.

$^{22}Na$-omsettempo's het die gemiddelde $^{23}Na$-inname in beide bruin hiënas (449,9%, perke 54,7–991,2 ) en aardwolwe (331,9%, perke 118,4–776,0) oorskat. Daar was ook geen betekenisvolle verwantskap tussen $^{22}Na$-omsettempo en $^{23}Na$ ingeneem in beide spesies nie. Dit sal dus onmoontlik wees om hierdie tegniek te gebruik om die voedselinname in hierdie spesies te bepaal. Verdere onsekerhede aangaande die aard van die dieet van karnivore, sowel as die verspreiding van liggaamsnatrium in prooidiere, lei tot bedenkinge oor die toepaslikheid van die tegniek vir die evaluering van karnivoor-energetika.

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Quantitative investigation of the bioenergetics of free-living animals became feasible after a technique was developed to measure $CO_2$ production by quantifying the turnover of $^{18}O$ (Mullen 1970; Mullen & Chew 1973). This method is , however, too expensive to use on large mammals.

Research into the bioenergetics of larger species was stimulated by the discovery of $^{22}Na$ as a less costly alternative for quantifying energy relations in these species (Green 1978; Green, Anderson & Whateley 1984). By relating $^{22}Na$ turnover to food consumption and subsequently to food and energy assimilation Green (1978) found it possible to establish the energy intake of free-living animals.

The present study was designed to test the feasibility of using the $^{22}Na$ technique to estimate food, and hence energy intake in brown hyaenas, *Hyaena brunnea*, and aardwolves, *Proteles cristatus*, and to obtain under captive conditions values of digestibility and assimilation required to calculate energy intake in these individuals. Brown hyaenas are generalistic feeders, eating anything from small mammals to fruit and insects (Mills 1977, 1978), while aardwolves are very specialist in their feeding habits, feeding almost entirely on termites, *Trinervitermes* sp., (Cooper & Skinner 1979).

## Materials and methods

Feeding trials were carried out at the De Wildt Cheetah Research Centre (25°37'S / 27°58'E), Transvaal, South Africa. Four brown hyaenas were confined for four days before the trials, to the den areas of their respective enclosures. They were then fed exclusively on beef from which all bone and fat had been removed. The meat was given in this form to enable more accurate measurement of $^{23}Na$ intake and energy content of the ration.

At the start of the trial the animals were immobilized by an intramuscular injection of Ketamine hydrochloride (600 mg) (Ketalar, Parke Davis Laboratories (Pty) Ltd, Isando). Following immobilization each animal was weighed, a blood sample (5 ml) taken (without the use of an anticoagulant) and injected intravenously with 20 $\mu$Ci $^{22}NaCl$ (37 MBq / ml, sp. act. 1,37 mCi / ml, Amersham International PLC, Amersham, U.K.) in 4 ml distilled water. Six hours later, after allowing equilibration with the exchangeable sodium pool, another blood sample was taken. Serum fractions were kept at –20°C until analysed.

Each hyaena was given a known mass of food daily. Uneaten food remains were collected, weighed and stored in a freezer. All faeces were collected from the cages and kept frozen until analysed.

The first trial was carried out over nine days after which final blood samples were taken and body masses measured. The second trial was over five days when the amount of food was reduced to less than half that of the first trial. Other procedures remained the same. Two animals were used in each trial.

The procedure used on aardwolf differed slightly from that for the hyaenas. Aardwolves ($n = 4$) were individually housed in enclosures in which they remained for the duration of the trial. They were fed a mixture of Pro-Nutro (Cerebos Food Corporation Ltd., Randburg, — contents: protein 22%, fat 5,9%, carbohydrate 59% & minerals 5,5%), sand, eggs, milk and minced meat bound with water. Faeces were collected daily from established middens within the enclosures (the middens were sieved clean of all faecal matter before the trial began). Aardwolves were injected intravenously with 10 $\mu$Ci $^{22}NaCl$ in 2 ml distilled water at the onset of the trial. Blood samples were collected at the same intervals as those for hyaenas. The aardwolves were not immobilized.

All faecal and food samples were dried at 75°C in an air stream until no further loss in mass occurred. They were then ground to a powder.

## Analytical methods

### Energy content

Duplicate aliquots (0,5 g) of dried food and ground faeces were combusted in a ballistic bomb calorimeter (Coallab CP400) to determine energy values.

### $^{22}$Na activity

Serum samples of 0,5 ml were placed in dry plastic scintillation vials with 0,5 ml hydrogen peroxide. When bleaching was complete the vials were heated to dryness, upon which 0,5 ml distilled water was added followed by 5 ml of scintillation cocktail. All samples were counted in a Packard liquid scintillation counter (Model 2002), (Green & Eberhard 1979). Sodium pool sizes (ES) were calculated from dilution of the isotope (Forbes & Perley 1951):

$$ES = \frac{^{22}Na \text{ injected} - ^{22}Na \text{ excreted}}{\text{serum } ^{22}Na \text{ / serum } ^{23}Na}$$

### Sodium determination

Food samples (1 g) ashed at 550°C for 48 h were dissolved in 5 ml 6N HCl and diluted in 100 ml de-ionized water. Sodium concentrations were measured using a Varion Techtron atomic absorption spectrophotometer (Model AA-175) with an air-acetylene flame. Serum samples (0,1 ml) were diluted in 5 ml de-ionized water and sodium levels estimated as above.

### $^{22}$Sodium turnover calculations

Turnover of $^{22}$Na was calculated using the formula:

$$\text{Turnover (meq)} = ES\ (\ln I - \ln F)$$

where $I$ and $F$ are the initial and final serum activities of $^{22}$Na respectively (Green 1978).

## Results

Information obtained during feeding trials is presented in Table 1. Mean dry matter digestibility (DMD) for brown hyaenas (89,6 ± 8,1%; $n = 4$) differs significantly (Mann Whitney; $U = 0$; $p < 0,05$) from that of aardwolf (54,2 ± 13,7%; $n = 4$). Apparent digestible energy (ADE) for brown hyaenas (96,3 ± 2,7%; $n = 4$) was similar to that estimated for aardwolf (93,7 ± 2,0%; $n = 4$). Mean sodium pool size in brown hyaenas was 44,9 ± 5,1 meq/kg ($n = 4$) and for aardwolf 34,1 ± 2,7 meq/kg ($n = 4$) and serum sodium 146 ± 6 meq/l and 145 ± 8 meq/l respectively (Table 2). Sodium intake and $^{22}$Na turnover was not related in either brown hyaenas ($r = -0,76$; $df = 3$; $p > 0,05$) nor aardwolf ($r = 0,45$; $df = 3$; $p > 0,05$).

The exchangeable sodium pool sizes of brown hyaena and aardwolf differ significantly ($t_6 = 3,72$; $p < 0,01$; $n = 8$), while the sodium levels in the serum show no significant difference. When compared with data on spotted hyaenas, *Crocuta crocuta*, (Green, Anderson & Whateley 1984) it is seen that exchangeable sodium pool size differs significantly between brown and spotted hyaena ($t_8 = 3,31$; $p < 0,05$; $n = 10$), and aardwolf and spotted hyaena ($t_8 = 6,02$; $p < 0,001$; $n = 10$). There were no significant differences between serum sodium levels in aardwolf, brown and spotted hyaenas.

$^{22}$Na turnover rates resulted in overestimates of sodium intake in all experimental animals (Table 2). In

**Table 1** Body weights, food consumption, faecal production, dry matter digestibility (DMD) and apparent digestible energy (ADE) determined for captive brown hyaenas and aardwolves

| | Initial | Weight | Food consumption | | Faecal production | | | |
|---|---|---|---|---|---|---|---|---|
| Animal No. | weight kg | change %* | Dry wt. g* | Energy kJ* | Dry wt. g* | Energy kJ* | DMD % | ADE % |
| **Hyaenas** | | | | | | | | |
| 1 | 53,5 | +3,3 | 2307 | 50770 | 362,8 | 2307 | 84,3 | 95,5 |
| 2 | 44,5 | 0,0 | 2247 | 49440 | 10,9 | 56 | 99,5 | 99,9 |
| 3 | 44,0 | -1,1 | 848 | 20250 | 60,0 | 784 | 92,9 | 96,1 |
| 4 | 48,0 | -0,2 | 852 | 20360 | 154,2 | 1326 | 81,9 | 93,5 |
| Mean | 47,5 | +0,5 | | | | | 89,6 | 96,3 |
| *S.D.* | 4,4 | 1,9 | | | | | 8,1 | 2,7 |
| **Aardwolves** | | | | | | | | |
| 1 | 10,0 | 0,0 | 1491 | 24120 | 507,0 | 1427 | 66,0 | 94,1 |
| 2 | 9,3 | 0,0 | 1257 | 20690 | 823,0 | 1886 | 34,5 | 90,9 |
| 3 | 13,3 | -3,8 | 513 | 9810 | 209,7 | 524 | 59,2 | 94,6 |
| 4 | 9,3 | -2,8 | 513 | 9810 | 221,2 | 457 | 56,9 | 95,3 |
| Mean | 10,4 | -1,7 | | | | | 54,2 | 93,7 |
| *S.D.* | 1,9 | 2,0 | | | | | 13,7 | 2,0 |

* = All values calculated over the period of the trial(s).

brown hyaenas the differences between actual and estimated intakes varied from 54,7 to 991,2% ($\bar{x}$ = 449,9; $n$ = 4) and in aardwolf from 118,4 to 776,0% ($\bar{x}$ = 336,0; $n$ = 4).

### Example of calculation of $^{22}$Na turnover and $^{23}$Na intake values

In this example the data for hyaena 2 in Tables 1 and 2 are used.
The injected solution of $^{22}$NaCl was diluted 1000 times to give a standard solution to test samples against.
All samples counted were 100 $\mu$l in volume.

Counts per minute (CPM) of standard solution = 245,0625
CPM of blood sample taken after equilibration period = 62,3725
CPM of final blood sample = 55,0575
$^{23}$Na content of 100 $\mu$l of serum = 0,014103 meq

Firstly the exchangeable sodium pool size of the animal must be calculated:

$$ES = \frac{^{22}\text{Na injected} - ^{22}\text{Na excreted}}{\text{Serum } ^{22}\text{Na} / \text{Serum } ^{23}\text{Na}}$$

As 4 ml of $^{22}$NaCl were injected:

$$\text{CPMs in injected solution} = 245{,}0625 \times 10 \times 1000 \times 4 = 9802500 \text{ CPM}$$

$^{22}$Na excreted during the equilibration period = 0

$$\text{Thus ES} = \frac{9802500 - 0}{62{,}3725 \times 0{,}014103} \text{ (meq Na)} = 2216{,}436 \text{ meq Na}$$

Once the ES is known the turnover of $^{22}$Na can be calculated:

$$\text{Turnover (meq)} = ES(\ln I - \ln F) = 2216{,}4 (\ln 62{,}3725 - \ln 55{,}0575) = 2216{,}4 (4{,}133 - 4{,}008) = 277 \text{ meq}$$

The $^{23}$Na intake of the animal was calculated as follows:

$^{23}$Na content of food provided = 0,0795 meq / g
Animal consumed 2247,5g of food

Therefore 179 meq of $^{23}$Na was ingested by the individual.

### Discussion

Compared with the published data, 87% for *Vulpes vulpes* feeding on snowshoe hare (Litvaitis & Mautz 1976) and 71–80% in *Canis familiaris dingo* (Green in Green & Eberhard 1979) the DMD value of brown hyaena appears rather high. Diets in the published data, however, contained bone which may constitute a component more difficult to digest. In the present study the animals were fed on flesh only and this ration was potentially easily digestible. The DMD of aardwolf is much lower than recorded in other carnivores (Litvaitis & Mautz 1976; Green in Green & Eberhard 1979, present study). This may be due to the sand component of the aardwolves' diet, thus rendering part of the ration indigestible. Furthermore the stools of aardwolf are covered in a layer of sand when collected and it is often difficult to distinguish the actual voided waste from the surrounding layer of sand. Too much sand was thus possibly included in the samples.

ADE values in both species correspond to data published on other carnivores, 91% in *Vulpes fulva* (Vogtsberger & Barrett 1973), 85% in *V. vulpes* (Litvaitis & Mautz 1976) and 91% in *Lynx rufus* (Golley, Petrides, Rauber & Jenkins 1965). These high values infer an extremely efficient digestive system.

In the present study we could not confirm the close relation found between actual sodium intake and $^{22}$Na turnover by other authors (Green 1978; Green & Eberhard 1979). The coefficient of determination ($r^2$) indicates that in the case of brown hyaenas 42% (it should also be noted that the correlation in this species was negative and not positive) of observed variation is due to a factor other than the influence of $^{23}$Na intake to the body, and in aardwolf 80% of the variation, and that these other factors were not considered in the present study. $^{22}$Na would thus not be a reliable indicator of sodium input in these species.

The overestimates of sodium intake obtained from the $^{22}$Na values differ from the close relationship in earlier reports (*Canis familiaris dingo,* 13,7 ± 5,6%, $n$ = 10 (Green 1978), *Sarcophilus harrissii,* 6,6 ± 7,2%, $n$ = 6

**Table 2** Exchangeable sodium (ES) pool size, serum sodium levels, $^{23}$sodium intake, $^{22}$sodium turnover and percentage differences in $^{22}$sodium and $^{23}$sodium, determined for captive brown hyaenas and aardwolves. All values are for whole trial period(s)

| Animal No. | ES pool meq /kg | Serum Na meq / l | $^{23}$Na intake meq | $^{22}$Na turnover meq | % Difference $^{22}$Na : $^{23}$Na |
|---|---|---|---|---|---|
| **Hyaenas** | | | | | |
| 1 | 38,3 | 141 | 182 | 388 | 113,2 |
| 2 | 49,8 | 141 | 179 | 277 | 54,7 |
| 3 | 43,6 | 147 | 57 | 422 | 640,4 |
| 4 | 48,0 | 154 | 57 | 622 | 991,2 |
| Mean | 44,9 | 146 | | | 449,9 |
| *S.D.* | 5,1 | 6 | | | |
| **Aardwolves** | | | | | |
| 1 | 31,2 | 146 | 87 | 190 | 118,4 |
| 2 | 32,9 | 154 | 71 | 231 | 225,4 |
| 3 | 37,5 | 146 | 25 | 81 | 224,0 |
| 4 | 34,9 | 134 | 25 | 219 | 776,0 |
| Mean | 34,1 | 145 | | | 336,0 |
| *S.D.* | 2,7 | 8 | | | |

and *Dasyurus viverrinus,* 15,7 ± 7,4%, $n = 4$ (Green & Eberhard 1979). At present the discrepancy in hyaenas can not be explained. In aardwolf it is possible that the animals found a natural food source within the enclosure where they were housed and thus ingested sodium in addition to the ration provided.

### Comments on the $^{22}$Na technique

The theoretical basis of the $^{22}$Na technique and some of its shortcomings have been discussed by Green (1978). Despite such shortcomings, previous research (Green 1978; Green & Eberhard 1979) indicated a correlation between sodium intake and $^{22}$Na turnover. Green (1978) points out that food intake in free-living animals can be calculated by the formula:

$$\text{Food intake (kg/day)} = \frac{k\ [\text{Na turnover (meq)}]}{t\ (\text{days}) \times \text{Na}_d\ (\text{meq / kg})}$$

where $k$ = a rate constant which refers to the conversion of $^{22}$Na turned over to $^{23}$Na ingested by the animal. $Na_d$ = total sodium content of the prey.

Determination of $Na_d$ in species which feed on more than one prey species causes problems. First, the dietary components are likely to vary in sodium content, and therefore their sodium content must be known. Secondly the proportional contribution of each of these to the predator's diet must be known. This will vary, and indeed, if known would remove the need for using the technique.

If the predator eats only one species of prey, problems remain in that sodium is not distributed evenly throughout the body (Edelman & Liebman 1959; Forbes & Lewis 1956) (Table 3). Consider a brown hyaena feeding on the remains of a herbivore. It may feed on muscle, viscera or skin and bone, all of which differ in sodium content (Table 3). Unless the proportion of each in the diet is known it is not possible to use the technique. Since the most reliable method of obtaining this information is by direct observation, the technique is rendered obsolete.

With regard to the application of this technique to the bioenergetics of free-living animals, it is important to stress the need for comprehensive captive feeding trials, as the digestibility and assimilation of different dietary components may vary (Litvaitis & Mautz 1976). Before an accurate estimate can be made of energy intake and expenditure such variations must be determined.

**Table 3** The distribution of sodium in the human body (modified from Forbes & Lewis (1956))

| Component of body | Weight kg | Na content meq | Na per unit weight meq / kg |
|---|---|---|---|
| Skin | 3,372 | 232,0 | 68,80 |
| Skeleton | 9,223 | 1544,0 | 167,41 |
| Muscle | 20,857 | 968,0 | 46,41 |
| G.I. tract & organs | 4,475 | 254,0 | 56,76 |
| Remainder | 14,931 | 577,0 | 38,64 |
| Total | 52,858 | 3575,0 | |

In summary, even if there is a correlation between sodium intake and $^{22}$Na turnover, the technique has many shortcomings. The most important factor is that the reasons Green (1978) gave for not using the technique on herbivores apply equally to most carnivores (i.e. the variation of sodium distribution in the body and between bodies is analogous to the differing sodium content of plants within a given habitat). If the technique does have an application it would only be for species which consume whole items of vertebrate prey.

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