Environmental factors that affect the concentration of P and N in faecal samples collected for the determination of nutritional status

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Faecal samples have been proven to be valuable in determining the nutritional status of wild herbivores. It is often, however, difficult and impractical to collect dung as it is dropped. In this study, the effect of various environmental factors that may influence P and N concentrations in faeces were investigated. Factors examined were rain, age of samples, presence of dung beetles, method of storage, length of storage, the effect of fungal growth (mould) on poorly dried samples, and drying period of samples. Results indicate that samples should only be collected from fresh dung pads that are still wet and which show no signs of dung beetle activity. Collecting of samples shortly after a rain storm should be avoided. Samples can be air-dried in a ventilated room or oven-dried at 60 °C without any effect on the nutrient concentrations, but care must be taken to avoid fungal growth during the drying period. Dried samples can be stored in paper bags for up to 1 year before analysis. The individual variation in P concentration is larger in browsers than in grazers and more samples should thus be collected from browsers to be representative of the herd when samples are pooled. During the period of this study, no difference between males and females was found and samples representing the herd can therefore be collected randomly. Faeces from juvenile impala are only representative of the herd once they have been weaned.

Key words: nutritional status, faecal analysis, environmental factors.

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Introduction

Collection of samples for the determination of nutritional status of wild herbivores can be prohibitively expensive and time-consuming. Using faecal samples for this purpose may be an inexpensive and relatively easy way to overcome this problem (Grant *et al.* 1985; Mould & Robbins 1981; Wofford *et al.* 1985). The question is, however, how and when to collect samples to obtain reliable results, as the nutrient content of samples may be influenced by various environmental factors (Howery & Pfister 1990). The influence of the following environmental factors were considered in this study: recent rain on the samples, age of samples, dung beetle utilisation, method of storage, length of storage, fungal growth (mould), and the drying period. In addition, the effect of sex and age on N and P concentrations in faecal samples were examined, to determine the influence of variable metabolic states. Individual variation was noted for the recommendation of suitable numbers of samples to be collected if pooled samples are to be representative.

Methods

Preparation of samples

Faecal samples from impala (*Aepyceros melampus*) grazing on the artificial grass lawns of the Golf Course at Skukuza, Kruger National Park, were used to limit soil contamination and to ensure that trial animals had a fairly uniform diet. Samples of 10 pellets each were collected from about 50 dung heaps. Only adult animals were sampled (Cook *et al.* 1994). Samples were thoroughly mixed by gently shaking them in a brown paper bag to allow a random distribution of pellets and to avoid breaking the pellets. The mixed sample was then divided into two equal parts. The fresh control was placed into small white paper bags (ash free) and dried at 60 °C to constant weight. The experimental sample was dried as above after treatment. Both samples were milled in a Tecator mill with a 1-mm sieve and analysed for N and P.

Environmental factors

Rain reportedly has a leaching effect on faecal minerals due to erosion of pellets (Wigley & Johnson 1981). The effect of rain on pellets was investigated by dividing a mixed sample into four equal parts. The first served as control. Clean water was administered artificially and in different quantities to the other three groups in the form of a mist rain. The samples treated with 10 mm and 20 mm rain were cracked and broken, whereas those receiving 5 mm were still intact. After allowing samples to dry off, grab samples were taken randomly from each of the three treatments as well as from the control and dried as described above.

To determine the properties of the ideal fresh sample which can act as a reliable indicator of P and N status, fresh samples were exposed to direct sunlight. This was done in February when the average minimum temperature was 23.2 °C and the average maximum temperature 32 °C. From the second day onward, a representative sample was taken daily and dried in the oven at 60 °C. Changes were observed on the second day when samples were lighter in weight and some of the pellets had started to crack. Sampling continued until the sample disintegrated after four days.

Dung beetles are very active in summer and it is often difficult to find even fresh samples without dung beetles. To examine the effect of dung beetles in a dung heap or on a ball of dung, fresh samples were collected from heaps where small dung beetles could be seen on the samples. Samples were also obtained by removing the dung ball from a dung beetle and by collecting pellets that had been obviously broken up by dung beetles. The control samples were collected in the same area but were free of dung beetles or had not been broken up by dung beetles.

Laboratory factors

When samples are collected in the veld it is not always possible to dry the wet samples in the oven immediately. The possibility of drying samples in a dry, well-ventilated room was therefore investigated. The effect of drying samples in bags in the sun was also examined. The fresh mixed samples were divided equally into ash-free paper bags, six samples were left out of the sun in a ventilated room to dry, and three were dried in the sun. When samples were air-dry after six days, they were placed in the oven. The fresh control samples were immediately placed in the oven after collection.

To examine the effect of storage on dried samples, samples were oven dried and stored in paper bags in a closed cupboard for one year before analysing them. The control samples were analysed immediately after drying.

Fungal growth often occurs on dung samples even when fresh samples are oven dried. To test the effect of mould on P and N concentrations in faecal samples, samples were divided into two equal groups. The control groups were placed into paper bags, with the thickness of the sample in the bag being approximately 1cm, to ensure good air flow throughout the sample, and dried in the oven until constant weight. The samples for the experimental group were packed to a thickness of approximately 4 cm to produce conditions favourable for fungal growth. Samples were dried in a protected area away from sunlight. Once fungal growth had occurred, samples were dried in an oven at 60 °C to constant weight.

The effect of leaving samples for a prolonged period in the drying oven was examined by dividing a fresh mixed sample into two equal groups, both of which were dried in the oven at 60 °C . The control samples were taken out after four days once a constant weight had been reached, whereas the other samples were taken out after 14 days.

Animal factors

Grasman & Heelgren (1993) and Ozoga & Verme (1970) found that male deer in rut voluntary reduced food intake. To examine the effect of sex on the P

and N content of faecal samples, samples from male and female impala were collected separately in May, when the males were in rut.

According to Cook *et al.* (1994) faecal samples from suckling juveniles can not be used to represent nutritional status. To examine the possibility of collecting unrepresentative samples from juvenile animals, faecal samples were collected from heaps where pellets were visibly smaller and thus represented juvenile animals. Samples were collected in April and May when the young animals were already weaned and samples should thus be representative of nutrient status. Control samples of bigger pellets were collected from the same herd.

To determine the nutrient status of a herd or group of animals, without analysing individual samples, it is important to know the number of samples needed to be representative of a herd. To determine how many samples are needed to be representative for different herbivore species, samples from 20 impala, 15 buffalo (*Syncerus caffer*), 16 blue wildebeest (*Connochaetes taurinus*), 8 kudu (*Tragelaphus strepsiceros*) and 8 giraffe (*Giraffa camelopardalis*) were collected and analysed separately. These samples were collected from animals in the same herd in the same area and on the same day during May.

Methods used for analyses

Dried samples were mixed thoroughly and pellets were ground with a pestle and mortar and passed through a Tecator mill with a 1-mm sieve. Nitrogen analysis was done according to the Kjeldahl method (AOAC 1984). For P analysis, the samples were digested using wet digestion and then read in a spectrophotometer (Heckman 1968).

Statistical analyses

Differences between control and treatment sample means were determined by using Student's *t*-test (Zar 1974).

Results and Discussion

Environmental factors

Up to 20 mm simulated rain had no significant influence on N levels in faeces, whereas more than 5 mm of rain decreased P concentrations significantly (Table1). Similarly, Jenks *et al.* (1990) found that N concentrations in deer faeces were not affected by rain. However, in their case the faeces were covered when rainfall exceeded 1 mm and the samples thus received little direct precipitation and were not eroded. We suggest that collection of samples after a rain storm be avoided, as it is difficult to determine the exact amount of rain on faeces in the veld, but samples can be collected in a drizzle.

Samples that were left open in the sun for two days or longer had significantly lower P and N concentrations when compared to control samples (Table 1). This is contrary to the findings of Jenks *et al.* (1990) who reported that faecal N levels in deer faeces under natural conditions were not affected by exposure. The difference could be explained by the fact that Jenks *et al.* (1990) only collected whole pellets, whereas in the present investigation pellets were collected even if they were cracked. The higher ambient temperatures in this area probably also increased the rate of decomposition of faecal samples. These results indicate that only fresh samples that have not been exposed for more than one day should be collected for assessment of nutrient status.

Samples that had been processed by dung beetles had a significantly higher N ($P = 0.0001$) content than those which were not used, while P concentration was unaffected (Table 1). The increase in N content could be due to the excretions from the dung beetle or from flies or mites which are often present in samples that have been processed by dung beetles. The presence of dung beetles on samples can quite easily be recognised and these samples should be avoided.

Laboratory factors

Samples that were air-dried in a ventilated room did not differ significantly from samples dried in an oven. This corresponds with the findings of Jenks *et al.* (1990) who found

that there was no effect on faecal N when samples were exposed to laboratory conditions for less than 12 days (Table 1). Samples that were dried in paper bags in the sun, however, had a significantly lower N (P = 0.0008) and P (P = 0.002) level than control samples (Table 1).

The P and N concentration of samples that were stored for a year did not differ significantly from that of the control samples (Table 1).

In samples that developed mould, N content was significantly lower (P = 0.005) and P content significantly higher (P = 0.003) than in control samples (Table 1). To prevent fungal growth, it is important to ensure good ventilation of samples and they should be placed where they can dry quickly.

Drying time did not influence P and N content significantly as there was no significant difference in N or P content of control samples and those that were left in the oven for 14 days (Table 1).

Animal factors

The individual samples collected from males and females showed no significant difference in either N or P (Table 2). This implies that faecal samples can be collected at ran-

Table 1
The effect of different factors on N and P levels in impala dung

Factor	Treatment	N (g/kg D.M.)	P (g/kg O.M.)	*n*
Rain	Control	22.8	3.7	4
	5 mm	23.2	3.7	4
	10 mm	23.0	3.5*	4
	20 mm	21.9	3.5*	4
Age of sample	Control	23.2	3.7	5
	Sun-dried 1 day	22.7	3.6	5
	Sun-dried 2 days	21.4*	3.5*	5
	Sun-dried 3 days	21.6*	3.4*	5
	Sun-dried 4 days	21.3*	3.2*	5
Dung beetle activity	Control	20.3	1.26	7
	Dung beetle	24.6*	1.09	7
Air-dry	Control	22.5	5.77	4
	Air-dry	22.7	6.06	6
Exposure to sun	Control	23.2	3.7	3
	Sun-dried	21.6*	3.1*	3
Storage	Control	12.3	2.29	16
	Stored for 1 year	12.6	1.91	16
Fungal growth	Control	22.9	3.4	4
	Growth	18.5*	3.7*	4
Oven drying	Control	22.5	5.77	4
	14 days dried	22.1	5.66	4

* Significant difference from control ($P < 0.05$)

dom to represent the nutritional status of all mature impala.

Table 2
The difference between sexes and ages of impala in N and P levels in dung

Impala	N (g/kg D.M.)	P (g/kg O.M.)	*n*
Male	19.7	4.2	11
Female	21.1	4	7
Adult	18.83	4.62	4
Juvenile	19.16	4.59	4

The P and N concentration in samples collected from the juveniles after weaning did not differ significantly from those in adults (Table 2). By avoiding obviously smaller pellets representative samples can thus be collected from a herd, as nutrient levels are similar even when pellets are still visibly smaller.

Belonje (1980) and Jenks *et al.* (1989) proved that, to cut costs, pooled samples can be used to determine P and N concentrations. To determine how many samples should be collected to represent a group of wild herbivores in an area, individual variation for N and P in browsers and grazers should be taken into account. In order to detect differences (at the 10% significance level) in the 0.5 g/kg range, the current variances obtained (Table 3) indicate that about 43 samples should be collected for impala, 11 for giraffe, 32 for kudu, 7 for buffalo and 16 for blue wildebeest. However, when statistical resolution need not be as fine, (such as distinguishing differences between area and between season) 20 samples from browsers and 10 from grazers should, as a general rule, be sufficiently representative.

Recommendation

Faecal samples can be collected and stored efficiently by veld staff doing normal duties. Samples up to a day old, showing no signs of dung beetle activity can be collected and air dried in a ventilated room at room temperature or oven-dried at 60 °C, to constant weight. They can also be stored for up to 14 days in paper bags, provided small amounts are stored. Dried samples can be stored for up to 1 year before analysis. Samples should not be collected the first day after heavy rains. Once collected, samples should be dried in thin layers to avoid fungal growth. If samples are to be pooled and a difference of 0.5 g/kg P and 1.0 g/kg N detected, 43 samples are needed for impala, 32 for kudu, 21 for giraffe, 7 for buffalo and 17 for blue wildebeest. By avoiding obviously smaller pellets, samples from suckling animals can be avoided for the determination of the nutrient status of the herd.

Table 3
Variation in faecal N (g/kg D.M) and P (g/kg O.M.) within species

Species	Impala		Buffalo		Blue wildebeest		Giraffe		Kudu	
n	20		15		16		8		8	
	P	N	P	N	P	N	P	N	P	N
Average	4.4	17.2	3.8	10.1	3.9	12.76	2.38	20.43	2.9	20.78
S.D.	1	1.24	0.4	0.45	0.6	1.24	0.51	1.38	0.86	1.04
CV(%)	22.7	7.21	10.5	4.46	15.4	9.72	21.4	6.75	21.7	5

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