Granulated polyamide as external marker to estimate total faecal excretion of grazing cattle in extensive management systems

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Granulated polyamide (PA) was tested for use as an external marker to estimate faecal DM (FDM) excretion of Zebu cattle (*Bos indicus*). The study was conducted in Mali, using seven and eighteen animals respectively in four field trials and six indoor experiments. Cattle ate fresh or dry pasture vegetation and half the animals were additionally supplemented with crop byproducts. Gelatine capsules containing 35, 40 or 45 g PA were administered orally at 12 h intervals. Estimates of FDM were based on the average marker concentration in faeces and were correlated with the actual excretion measured by total faecal collection. The pre-measurement period required to establish equilibrium for regular marker dosing was determined at 4 d. Except for diets with a N content of less than 9·26 g/kg organic matter, marker recovery averaged 98·1 (SE 0·93) % (*n* 62), and was not influenced by diet composition and the quantity of feed ingested (*P* > 0·05). Estimates of FDM based on average PA concentrations in faecal samples were correlated to the actual excretion with *r* 0·98 (*n* 62; *P* ≤ 0·001). Since the PA concentration in individual faecal grab-samples is not correlated with either sample mass or sampling time, accurate estimates of FDM require a grab-sampling schedule that covers the 24 h day. However, estimates of FDM were found to be acceptable if calculations are based on the average PA concentration in the sub-total of samples collected during the day or during night respectively (*r* 0·95, *n* 29; *P* ≤0·001 in both cases). It is concluded that the use of PA marker is a simple and inexpensive method resulting in reliable estimates of FDM. Since sophisticated analytical procedures are not required to recover PA in faecal samples, the marker is particularly suitable for application in extensive grazing systems and in studies conducted in less-developed countries.

Faeces: Polyamide marker: Extensive grazing systems: Cattle

A reliable determination of feed intake is a prerequisite to study the nutritional status of cattle grazing natural pastures. Most commonly, estimates of intake are based on the determination of *in vitro* ingesta digestibility and faecal output (Mayes *et al.* 1995). Although faeces can be collected quantitatively using collection bags, the application of faecal markers is an advantage under field conditions because this approach has very little influence on the animal's grazing behaviour. It is easily applicable to relatively large numbers of animals and is suitable for studies on females (Reid *et al.* 1952; Kotb & Luckey, 1972; Langlands, 1974). Using markers, the accuracy of the estimated faecal excretion depends greatly on a representative collection of faecal samples (Whittington & Hansen, 1985), especially when there are distinct variations in the animal's diurnal patterns

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of feed intake and when the pasture shows heterogeneous species distribution or contains both herbaceous and ligneous vegetation.

The different types of markers as well as the principles, advantages and problems of their application have been thoroughly discussed (Kotb & Luckey, 1972; Langlands, 1974; Cordova *et al.* 1978; Holechek *et al.* 1982; Meuret *et al.* 1985; Cochran *et al.* 1986, 1988; Mayes *et al.* 1995). However, for most markers the sophisticated analytical procedures required for their measurement are often not applicable in less-developed countries (Dicko-Touré, 1980). Small plastic particles that have been used frequently for studies on ruminal and post-ruminal rates of particulate passage have the advantage that only simple laboratory equipment is necessary to measure them gravimetrically in faecal samples. Plastic particles of 1–2 mm diameter and up to 4 mm length and having a specific gravity of 1·10–1·28 showed maximal gastrointestinal rates of passage in cattle and small ruminants (King & Moore, 1957; Campling & Freer, 1962; Katoh *et al.* 1988; Murphy *et al.* 1989; Prigge *et al.* 1990; Welch, 1990). The rapid rate of passage combined with easy recovery suggests that small plastic particles should be a suitable marker substance for the quantification of faecal excretion. The present study, therefore, investigates the potential of small polyamide (PA) particles for use as an external faecal marker to accurately estimate DM excretion of free-ranging cattle. Easy handling of the marker and quick and easy analysis of faecal samples were considered to be important factors when only minimal laboratory facilities are available.

MATERIALS AND METHODS

The marker tested was granulated PA (PA-6®; Friesetta GmbH Kunststoffwerk, D-79677 Schoenau, Germany), a hard, transparent and physiologically-inert plastic. The particles of 2–3 mm diameter have a specific gravity of 1·12–1·15 and a ball indentation hardness of 50–80 N/mm². Studies on the suitability of PA as an external marker to estimate total faecal excretion of grazing cattle were conducted at the Station des Recherches Zootechniques du Sahel at Niono, Mali, from March 1989 to June 1992. The site is in the southern Sahelian zone and has a semi-arid climate with a single rainy season from June to October. Long-term annual precipitation averages 487 mm (years 1958–1988). Cattle production mainly depends on feed supply from natural pastures and harvested millet (*Pennisetum glaucum*) fields. During the dry season, animals are occasionally supplemented with locally-available crop byproducts.

The rate of passage of PA particles was studied in an initial experiment with five housed Zebu oxen (*Bos indicus*). Four field trials were carried out on a total of seven oesophageally-fistulated oxen. Additionally, five indoor experiments were conducted on a total of eighteen oxen to determine the influence of ingesta quantity and quality on marker recovery.

Patterns of marker excretion

To determine the rate of passage and the recovery of the marker, an initial trial was carried out on five animals (live weight 293 (SD 43·6) kg) kept indoors. The diet consisted of bush hay harvested on natural pasture, cowpea (*Vigna unguiculata*) hay and wheat meal offered as one ration in the proportions 1·0 : 1·0 : 0·5 (on fresh mass basis; average DM content 920 g/kg) and was distributed at levels of 29·6, 34·1, 43·5, 50·1 and 92·1 g DM/kg live weight$^{0·75}$ per d (*n* 1 per level). Each animal was offered its ration in two equal portions at 07.00 and 17.00 hours. After a 10 d adaptation period, the animals received a single dose of 225 g PA. As in all trials, gelatine capsules (30 mm × 95 mm) were filled with 40–45 g

marker particles and administered orally with the help of an applicator. Due to difficulties in handling, the animal which was offered the highest amount of feed received only 175 g PA. Starting at the moment of marker dosing, faecal excretion was measured during six consecutive days with every defaecation collected immediately in a plastic bowl held behind the animal. All defaecations were dried individually and analysed separately as described later (p. 791). Marker concentrations in faecal samples of the five animals were listed in chronological order. Marker excretion patterns were calculated as the moving average of the five animals, using overlapping windows of twenty-one contiguous data points each.

Field trials

Four trials (F1–F4) were carried out during the second half of the dry season when cattle were grazing on communal pastures and harvested millet fields. Three of the seven animals relied exclusively on grazed herbage, while four were additionally supplemented daily with 0·5 kg rice feed meal and 1 kg cowpea hay (on a fresh matter basis). Animals were herded from 07.00 to 18.30 hours and corralled overnight. Water was provided during a 1–2 h rest at approximately 12.00 hours. Supplements were given individually. Cowpea hay was offered overnight and rice feed meal was fed in the morning before the animal left the corral.

The quality of the diet selected on pasture was determined from samples of oesophageal extrusa collected during five consecutive days (Harniss *et al.* 1975). Twice in the morning and twice in the afternoon extrusa was collected from an animal, such that samples from supplemented and unsupplemented animals were collected simultaneously in trials F1 and F2. Each animal was sampled only once daily and the sampling time was limited to 30 min. The order in which the animals were sampled was determined by a Latin square design to account for interactions between animal and sampling times. Approximately 700 g of extrusa fresh matter were collected into water-tight bags (Little, 1972) and kept on ice until drying. To avoid browning effects, samples were dried to constant mass at 49° (Scales *et al.* 1974). They were ground through a 1 mm sieve and stored air-tight.

In all trials, the marker was administered twice daily, at 06.30 and 18.30 hours, at a rate of 35 or 70 g PA per application (Table 1). Continuous marker dosing started 4 d before the 5 to 10 d measurement period. Total faecal excretion was measured using faecal collection bags, with the animals being adapted to the bags 4 d before the measurement period. For each animal, faeces were pooled daily and duplicate sub-samples were taken to determine their marker content as described later (p. 791). In trials F2 and F4, total collection was combined with rectal grab-sampling of faeces at intervals of 5 or 10 h during five consecutive days. In trial F1, faeces were also taken during three fixed intervals of 2 h in the morning, at 12.00 hours and in the evening.

Forage intake on pasture was calculated from the quantity of faeces excreted and the digestibility of the selected diet.

Indoor experiments

To examine the influence of ingesta quantity and quality on the recovery of the PA marker, five indoor experiments (C1–C5) were conducted on a total of eighteen animals. Animals nos. 1–6 were used in trial C1, animals 7–18 participated in trials C2–C5 and were allocated at random to the different treatments in each experiment. In all trials, water was provided *ad libitum* and mineral licks were constantly available. The ration was based on

Table 1. *Experimental design, diet quality and feed intake of Zebu cattle (Bos indicus) in four field studies on marker recovery*

(Mean values and standard deviations)

		F1 March 1989 P Mean	P SD	PS Mean	PS SD	F2 April 1989 P Mean	P SD	PS Mean	PS SD	F3 June 1989 PS Mean	SD	F4 July 1989 PS Mean	SD
Collection period	(d)	10		10		5		5		4		5	
Marker dose	(g PA/d)	70		70		70		70		140		140	
Faecal collection: Total		x		x		x		x		x		x	
Grab sampling†	5h												
	10h					x		x		x		x	
No. of animals		3		4		3		4		3		4	
Live weight	(kg)	251	10.8	296	28.8	256	4.4	308	34.1	305	43.0	287	26.7
Diet quality‡ CP	(g/kg OM)	99	24.6	92	7.5	88	6.7	68	9.7	77	11.9	80	12.1
DOM	(g/kg OM)	588	4.5	600	27.5	514	31.5	544	14.7	585	20.8	578	21.9
ME	(MJ/kg OM)	8.7	0.12	8.9	0.55	7.4	0.60	8.0	0.29	8.4	0.44	8.2	0.50
Intake: Pasture§	(kg OM/kg live weight$^{0.75}$ per d)	93	9.5	94	9.7	77	6.6	86	9.2	105	15.0	100	16.1
Supplement	(kg OM/kg live weight$^{0.75}$ per d)			19	1.5			18	1.6	19	2.0	19	1.4

* For details, see p. 787. P, pasture; PS, pasture and supplement; CP, crude protein (N × 6·25); DOM, digestible organic matter; ME, metabolizable energy; OM, organic matter; x, type of collection made.

† Grab-samples were taken at 5 h and/or 10 h intervals throughout the collection period.

‡ Quality of oesophageal extrusa collected on pasture during five consecutive days (one sample per animal per day).

§ Intake from pasture calculated from total faecal excretion and DOM content of extrusa samples, taking into account supplement intake and digestibility.

Table 2. *Feed quality in indoor experiments on marker recovery in Zebu cattle* (Bos indicus)

(Values are averages)

Trial	Feed	Type of feed	n	DM (g/kg FM)	OM (g/kg DM)	CP (g/kg OM)	DOM (g/kg OM)	ME (MJ/kg OM)
C1	Rice straw	B	5	950	853	19	550	7·3
	Cowpea hay	S	8	940	895	124	690	9·4
C2	Rice straw	B	8	914	851	28	482	6·9
	Cowpea hay	S	5	890	906	189	711	10·8
C3	Bush hay	B	7	920	931	29	441	5·9
	Rice feed meal	S_1†	5	928	898	128	590	11·0
	Cottonseed expeller	S_2†	5	929	940	293	613	9·9
C4	Green pasture veg	B	10	319	888	124	617	9·5
	Rice feed meal	S	5	925	851	105	688	12·4
C5	Green pasture veg	B	9	365	918	100	583	8·7
	Rice feed meal	S	5	920	869	105	600	11·0

B, basic feed; S, supplement; DM, dry matter; FM, fresh matter; OM, organic matter; CP, crude protein (N × 6·25); DOM, digestible organic matter; ME, metabolizable energy; veg, vegetation.
† S_1–S_2 1:1, w/w.

rice straw in trials C1 and C2. In the other trials, the basal diet consisted of either bush hay (C3) or green herbaceous vegetation (C4, C5) harvested on natural pasture. Rations were supplemented with cottonseed expeller, rice feed meal or cowpea hay. The quality of the distributed feeds is shown in Table 2.

Feeding levels were calculated as multiples of the digestible organic matter (DOM) requirement for maintenance (33 g DOM/kg live weight$^{0.75}$ per d; Agricultural Research Council, 1980). Diets were classified as low intake (L) corresponding to maintenance, moderate intake (M) corresponding to 1·3 × maintenance and high intake (H) corresponding to 1·6 × maintenance; supplement treatments were LS, MS and HS respectively. Collection periods, marker doses, feeding levels and quantities of feed distributed and consumed are shown in Table 3.

The feed was offered individually in two equal meals at 07.00 and 17.00 hours daily. Green feed (C4, C5) and bush hay (C3) were always harvested 18 h before feeding. The hay was stored in sacks, and the green feed was kept in loose stacks on a concrete floor, shaded by a tin roof. In this way a relatively constant DM content for the green feed on offer was maintained.

All trials were preceded by an adaptation period of 10 d. During the measurement period, samples of feed on offer were taken daily. Food refusals were weighed once daily and samples kept for analysis. To be congruous with the field experiments, the marker was administered at 06.30 and 18.30 hours, before the start of the morning meal and towards the end of the evening meal. PA was administered at a rate of 35 g (C1), 40 g (C4, C3 and C5, treatments L) or 45 g (C2, C3 treatment M, C5 treatment H) per application. Continuous marker dosing started 4 d before the measurement period. During the measurement period, each defaecation was collected individually in a plastic bowl held behind the excreting animal (C1–C5). Faeces collected in this way were weighed and pooled for each 24 h period. In addition, grab-sampling of faeces took place every 7 h throughout the measurement period in trials C3, C4 and C5. Marker concentration in faeces was determined in aliquot samples which were sub-sampled daily from the 24 h pool of total excreta of individual animals (C1, C2, C3) or in rectal grab-samples (C3, C4, C5). In

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Table 3. *Collection periods, experimental design, feeding levels and quantities of feed offered and consumed in indoor experiments and marker recovery in Zebu cattle* (Bos indicus)

(Mean values and standard deviations)

Trial	Month and year	Collection period (d)	Sampling technique	Feeding level*	n	Live wt (kg) Mean	Live wt (kg) SD	PA dose (g/d)	Feed offered (kg OM/d) Basic feed Mean	Basic feed SD	Supplement Mean	Supplement SD	Total intake (g OM/kg live wt$^{0.75}$ per d) Mean	SD
C1	Nov 1989	15	Total and aliquots	HS	6	276	24·3	70	4·0	0·55	2·1	0·20	6·0	0·75
C2	Mar 1992	8	Total and aliquots	L	6	351	78·2	90	4·6	0·87	–	–	3·5	0·64
				MS	6	366	76·0	90	4·4	0·40	1·2	0·08	5·4	0·52
C3	Dec 1991	8	Total and aliquots and gs - 7 h	L	3	245	18·6	80	4·1	0·00	–	–	3·4	0·15
				LS	3	274	23·0	80	2·4	0·00	1·7	0·00	4·0	0·04
				M	3	302	9·8	90	5·8	0·00	–	–	4·3	0·28
				MS	3	389	41·9	90	4·1	0·00	2·8	0·00	6·3	0·46
C4	Aug 1990	10	Total and gs - 7 h	L	3	240	47·6	80	3·4	0·21	–	–	3·3	0·21
				LS	3	193	22·7	80	1·8	0·30	0·9	0·16	2·7	0·43
				H	3	207	18·0	80	5·1	0·31	–	–	3·7	1·24
				HS	3	238	79·2	80	2·4	0·11	1·8	0·66	4·0	0·60
C5	Aug 1991	10	Total and gs - 7 h	L	3	229	10·3	80	3·4	0·00	–	–	3·1	0·08
				LS	3	226	10·7	80	2·1	0·00	1·0	0·00	3·0	0·02
				H	2	277	3·5	90	6·7	0·00	–	–	5·9	0·04
				HS	3	290	22·5	90	4·0	0·00	2·1	0·00	5·6	0·32

Total, total faecal collection; aliquots, aliquot sub-sampling from 24 h pools of faeces; gs - 7 h, grab-sampling every 7 h; L, low (maintenance); M, medium (1·3 × maintenance); H, high (1·6 × maintenance); S, supplement (for details, see Table 2); PA, granulated polyamide; OM, organic matter.
* For details of feed offered in each trial, see Table 2.

trial C5, not only grab-samples but every defaecation excreted between 07.00 and 18.00 hours was analysed individually for its PA content.

Analyses of oesophageal extrusa, feed and faecal samples

Residual moisture, crude ash, and crude protein (N × 6·25) contents of roughage feeds, supplements, and oesophageal extrusa were determined according to standard procedures (Naumann *et al.* 1983). DOM and metabolizable energy content were derived from the gas production of approximately 200 mg air-dry sample incubated with rumen contents *in vitro* for 24 h (Menke *et al.* 1979).

The processing of faecal samples was identical for the initial, field and indoor experiments. In trials C1–C5, the average air-dry matter content of a number of faecal samples was determined during the adaptation period and the amount of water needed to dilute faeces to a moisture content of 800–900 g/kg was calculated. In order to sub-sample mass aliquots from the 24 h pools of total excreta during measurement periods, the pooled faeces were weighed to ±0·2 g, placed in a plastic container and diluted with water. The moisture content of the diluted faeces was determined at 820–850 g/kg. Immediately after thorough mixing of the diluted faeces by manual stirring for 5 min, two aliquot sub-samples of 10 g/kg total mass of the homogenized pool were taken.

Grab samples were treated individually. After determination of fresh sample mass, aliquot sub-samples and grab-samples were dried to constant mass at ambient temperatures (29–35°), weighed again and ground in a household blender without damage to the polyamide particles. The marker was recovered from the ground samples by sifting (1 mm sieve). If faecal particles rested on the sieve, they were removed by hand. The quantity of marker recovered was weighed to the nearest milligram. To determine the influence of the grinding procedure on PA recovery, defined amounts of PA (20, 25, 30, 35, 40, 45 g, replicated three times each) were added to a fixed quantity of fresh faeces (equivalent of 1 kg faecal DM (FDM)). On average, 100·7 (SE 0·03) % (*n* 18) of air-dry PA added to fresh faeces was recovered after the drying, grinding and sieving process. The slight increase in PA mass was due to dust particles sticking to the grains. Although washing and drying of the particles brought recovery to 100·6 %, those procedures increase the risk of particle losses. It was decided, therefore, not to wash the recovered PA grains but to accept the small bias originating from dust adhesion. Marker content of samples was expressed as g PA/kg FDM. From the PA concentration in individual grab-samples and in aliquot sub-samples, respectively, the marker concentration for the whole sampling period was calculated as an unweighted average value for each animal. Based on the observation that extremely high or low marker concentrations mainly occur in very small grab-samples, it was decided to apply the following corrections: grab-samples of less than 10 g DM, grab-samples without any PA particles and grab-samples where the PA concentration differed by more than 3 SD from the mean were excluded. Marker recovery was calculated from mean daily marker excretion in faeces divided by the average daily marker intake during the collection period, where faecal marker excretion (g PA/d) was obtained from average daily FDM excretion (g FDM/d, determined by total collections) multiplied by the average marker concentration (g PA/kg FDM) in faecal grab-samples (F2, F4, C3, C4, C5) or in aliquot sub-samples (F1, F3, C1, C2). To estimate average FDM from marker application, the amount of PA administered daily (g PA/d) was divided by the average PA concentration (g PA/kg FDM) of aliquot sub-samples or grab-samples respectively.

Data analysis

To assess the influence of supplementation, marker dose, and sampling procedure on marker recovery in field experiments, factorial ANOVA was performed using the general linear model procedure (GLM) provided by the Statistical Analysis Systems (1989) software package. The 'nested' classification was used to account for correlation between measurements from the same animal (animal nested in supplement treatment; Littell *et al.* 1991). For the comparison of marker recoveries within and between the five indoor experiments, the independent variables 'type of feed' and 'feeding level' were also included in the model. Multiple comparisons of means were computed by 'contrast' statements. Simple linear regression analysis was carried out to determine the correlation between actual faecal excretion and estimated values across experiments. The effects of diet digestibility, N content and organic matter intake on marker recovery were determined using multiple linear regression analysis. The correlation between dietary N content and PA recovery was further analysed using a non-linear segmented regression consisting of two linear sections with one joint point (Statistical Analysis Systems, 1989).

RESULTS

Marker kinetics

The first marker particles appeared in the faeces 13·0 (SE 0·63) h (n 5) after administration of a single pulse dose. Peak concentration was reached 30·6 (SE 2·11) h (n 5) after marker application, and after 134 h 98·5 (SE 1·30) % (n 5) of the administered marker dose was excreted. The marker excretion expected for continuous dosing was determined mathematically from the course of PA concentration in faeces following pulse dose application by superimposing the decay curve at intervals of 12 h. Fig. 1 indicates that with marker dosing at regular intervals, marker concentration in faeces oscillates closely around a plateau value after 102 h following first marker application. The derived result was confirmed by trial F1, where PA recovery reached a plateau value on average 4 d after the start of continuous marker application (Fig. 2). The required period for marker concentrations to reach equilibrium before the collection of faecal samples was therefore fixed at 4 d and was adhered to in further trials.

Marker recovery

Marker recovery determined by grab-sampling was in accordance with the recovery obtained from total faecal collection followed by aliquot sub-sampling ($P > 0·05$, trials F2, F4, C3). In the field, marker recovery did not differ significantly between unsupplemented and supplemented animals (Table 4). The amount of marker administered daily has no influence on marker recovery. With 70 g PA/d being administered to unsupplemented and supplemented animals grazing natural pasture (F1, F2), recovery averaged 99·0 (SE 3·04) % (n 6) and 97·2 (SE 2·81) % (n 8) respectively, and amounted to 96·9 (SE 1·83) % (n 7) when supplemented animals received a dose of 140 g PA/d ($P > 0·05$).

Taking rectal grab-samples at 10 h intervals during five consecutive days (F2), marker recovery was 104·2 (SE 4·05) % (n 3) and 97·1 (SE 4·22) % (n 4) for unsupplemented and supplemented animals respectively ($P > 0·05$), and the estimated quantity of faeces was 96·4 (SE 3·83) % (n 3) and 103·8 (SE 4·72) % (n 4) of the amount actually excreted. Grab-sampling at intervals of 5 h within the measurement period of 5 d (F4) increased the number of grab-samples from twelve to twenty-four. Marker recovery was determined for
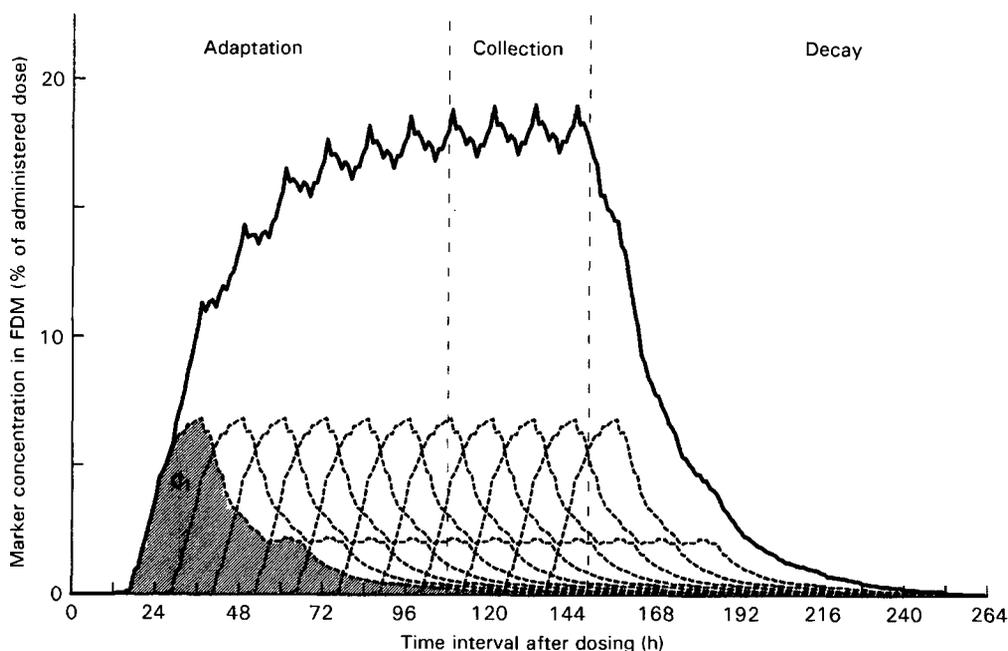
Fig. 1. Granulated polyamide marker concentration in individual grab-samples predicted for continuous dosing in Zebu cattle (*Bos indicus*). Adaptation period, collection and decay phase (——) are determined mathematically from marker excretion patterns following a single pulse dose application (c_1, moving average of five animals), assuming 12 h intervals of dosing during 120 h (- - - -). For details of procedures, see pp. 786–787. FDM, faecal DM.
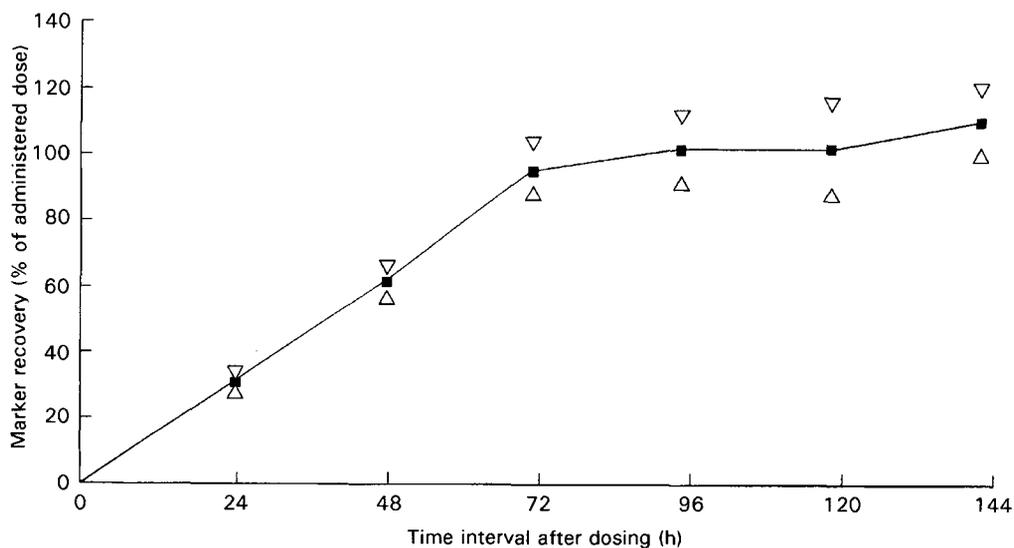

Fig. 2. Daily recovery of granulated polyamide (PA) marker in faeces of Zebu cattle (*Bos indicus*) following continuous dosing of 35 g PA at 12 h intervals. Values are means (■) and standard deviations (△) for four animals.

Table 4. *Recovery of granulated polyamide marker in dry season field experiments with Zebu cattle* (Bos indicus)

(Mean values with their standard errors)

Trial	Treatment*	Sampling technique	Marker recovery (%)		
			Mean	SE	*n*
F1	P	Aliquots	93·7	1·49	3
	PS	Aliquots	97·3	3·72	4
F2	P	gs - 10 h	104·2	4·05	3
	PS	gs - 10 h	97·1	4·22	4
F3	PS	Aliquots	95·3	1·56	3
F4	PS	gs - 5 h	98·1	2·84	4
Mean			97·6	1·52	21

P, pasture; PS, pasture and supplement; Aliquots, aliquot sub-sampling from 24 h pools of faeces; gs - 10 h, grab-sampling every 10 h; gs - 5 h, grab-sampling every 5 h.
* For details, see p. 787.

the 5 h sampling interval as well as for the two intermittent 10 h intervals. The standard error of the relative divergence between estimated FDM and actual excretion calculated for the 5 h interval was 2·84 as compared with values of 5·11 and 3·44 determined for the two 10 h intervals. To obtain about twenty-four faecal samples within measurement periods of 7–10 d, an interval of 7 h was adopted for the collection of grab-samples in trials C3, C4, and C5.

The influence of type of feed, amount of feed ingested and supplement treatment on the recovery of PA marker as determined in trials C1–C5 is illustrated in Table 5. As in the

Table 5. *Influence of forage quality, feeding level and supplementation on recovery of granulated polyamide marker as determined in indoor experiments with Zebu cattle* (Bos indicus)

(Mean values with their standard errors)

Trial	Basic feed	Supplement	Feeding level	Marker recovery (%)*		
				Mean	SE	*n*
C1	Rice straw	Cowpea hay	LS	93·7[a]	1·12	6
C2	Rice straw		L	57·2[d]	4·66	6
	Rice straw	Cowpea hay	MS	94·8[a]	1·95	6
C3	Bush hay		L	72·9[cd]	3·48	3
	Bush hay		M	79·2[c]	2·40	3
	Bush hay	Rice feed meal	LS	91·3[a]	2·36	3
	Bush hay	Rice feed meal	MS	102·2[ab]	0·52	3
C4	Green pasture vegetation		L	98·8[ab]	4·12	3
	Green pasture vegetation		H	100·4[ab]	5·48	3
	Green pasture vegetation	Rice feed meal	LS	97·5[ab]	2·71	3
	Green pasture vegetation	Rice feed meal	HS	110·0[b]	3·58	3
C5	Green pasture vegetation		L	97·8[ab]	4·24	3
	Green pasture vegetation		H	98·4[ab]	0·39	2
	Green pasture vegetation	Rice feed meal	LS	100·2[ab]	6·15	3
	Green pasture vegetation	Rice feed meal	HS	102·6[ab]	2·36	3

[a,b,c,d] Mean values with different superscript letters were significantly different ($P \leq 0.05$).
L, low (maintenance); M, medium (1·3 × maintenance); H, high (1·6 × maintenance); S, supplement.
* Marker recovery determined from aliquot sub-samples (C1, C2) or from grab-samples taken every 7 h (C3, C4, C5).

field trials, differences in marker recovery due to the method of faecal collection (aliquot sub-sampling from a 24 h pool or grab-sampling at 7 h intervals) were not statistically significant. Of a total of 1027 grab-samples collected in trials C3, C4 and C5, only twenty-nine samples were excluded according to the criteria mentioned previously. Among the eighteen samples weighing less than 10 g DM, four contained no PA particles and nine samples showed very high PA concentrations. Of the remaining eleven samples that were excluded, only one was without marker, while the others contained extremely high amounts of PA. Five of these samples had a dry mass of 50–100 g and two samples weighed more than 100 g.

Statistical analyses showed that for diets based on green feed neither the quantity ingested nor the supplement treatment had a measurable influence on marker recovery ($P > 0.05$); thus interactions between diet components could be excluded. For diets based on bush hay or rice straw (straw diets), more feed was ingested by supplemented compared with unsupplemented animals (trials C2, C3, $P \leq 0.001$), so that it was not possible to isolate the impact of food intake and supplement treatment. Marker recovery determined for unsupplemented straw diets (trials C2, C3; treatments L, M) averaged 66·6 (SE 3·78) % (*n* 12), whereas for supplemented straw diets (trials C1 and C2, C3; treatments LS, MS) marker recovery amounted to 95·1 (SE 1·17) % (*n* 18), with the difference being significant at $P \leq 0.001$.

Excluding data obtained for unsupplemented straw diets, the overall average recovery of marker from field and indoor trials was 98·1 (SE 0·93) % (*n* 62). For supplemented straw diets as well as for supplemented and unsupplemented diets of green feed, a linear relationship was established between the excreted FDM and the amount of faeces estimated from the recovered amount of PA marker in faecal samples (Fig. 3). FDM estimated from marker concentration in faeces was correlated with the actual excretion (*r* 0·98, *n* 62; $P \leq 0.001$). Using PA marker overestimated the actual excretion by 2·4 % on average.

Sampling procedures

In trial C5, PA concentrations were determined in grab-samples taken at 7 h intervals and in all defaecations occurring between 07.00 and 18.00 hours during ten consecutive days, which enabled diurnal patterns of marker excretion to be studied. To compare PA excretion among the eleven animals, the marker concentration in individual samples obtained for a particular animal was divided by the average PA concentration of all grab-samples collected for that animal during the trial (standardized marker concentration). Visual inspection of the plotted data indicated a regular accumulation of high marker concentrations between 08.00 and 18.00 hours, whereas low marker concentrations were more equally distributed throughout the 24 h day. To test for the existence of a cyclical variation in standardized PA concentration over time, each of the eleven data sets was fitted to a sine wave function. In all cases, *r* for the function was below 0·21, and for eight animals *r* < 0·06. The irregular patterns of PA excretion over time despite fixed hours of marker application and feed distribution could explain why average PA recovery was less than 50 % for unsupplemented animals and less than 80 % for supplemented cattle when samples were taken during three fixed intervals of 2 h in the morning, at 12.00 hours and in the evening (F1).

Data obtained from grab-samples collected in trials C3, C4 and C5 were compiled after standardizing marker concentration as described previously. Comparing the PA concentration in grab-samples collected during the day (07.00–18.00 hours; maximum fifteen samples per animal and experiment) and the night (19.00–06.00 hours; maximum
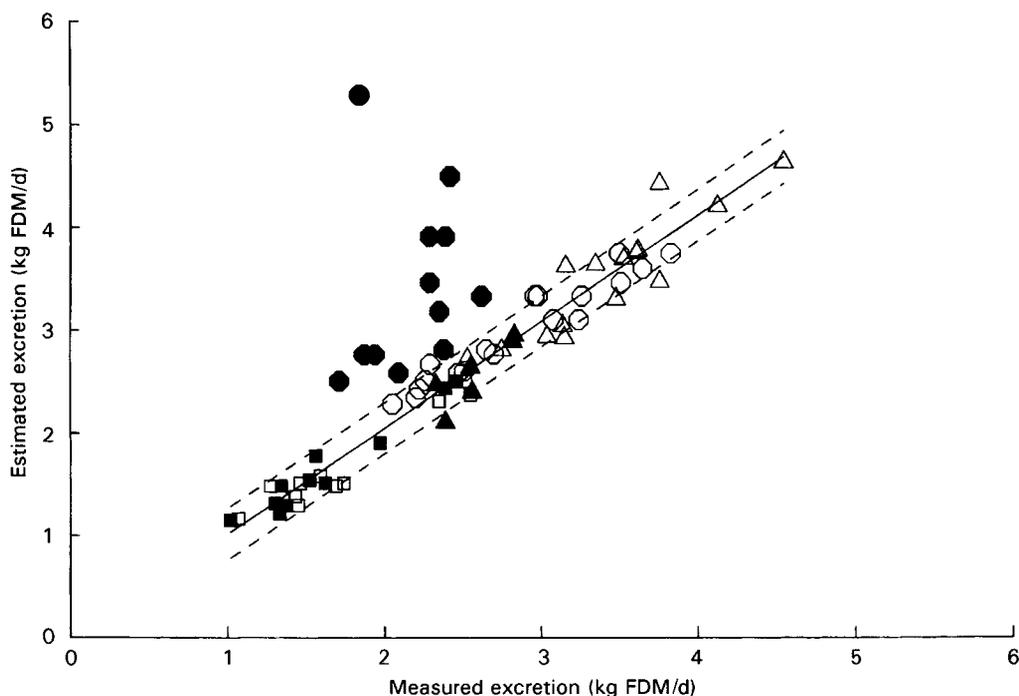
Fig. 3. Relationship between measured faecal excretion and faecal excretion estimated from the application of granulated polyamide marker as determined for unsupplemented and supplemented diets of straw (●, ○), green pasture vegetation (■, □) and unsupplemented and supplemented diets selected on natural pasture during the dry season (▲, △) for Zebu cattle (*Bos indicus*). Values obtained for unsupplemented straw diets were omitted from the calculation of the regression line (—) and its 95 % CI (- - - -). For details of diets and procedures see Tables 2 and 3 and pp. 786–792. FDM, faecal DM. $y = -0.03 + 1.04x$, r^2 0.96, n 62.

thirteen samples per animal and experiment), the standardized marker concentration was 1·03 (SE 0·018; n 541), and 0·96 (SE 0·023; n 486) of the 24 h average respectively with the difference in marker concentration between the day and night grab-samples being significant at $P \leq 0.001$. At the same time, the mass of grab-samples collected during the night was on average 10·8 % higher than the mass of samples collected during the day, i.e. 97·0 (SE 2·52) g DM; (n 486) *v.* 87·6 (SE 2·13) g DM (n 541; $P \leq 0.001$). The correlation between log-transformed sample mass of the day and night grab-samples and their standardized PA concentration was determined at r -0.46 (n 1027; $P \leq 0.001$). Due to the tendencies for higher PA concentrations in smaller grab-samples and lower sample masses during the day, marker recovery determined for individual animals from grab-samples collected during the day (trials C4, C5, and C3 treatments LS, MS) was 104·9 (SE 2·32) % (n 29) compared with a recovery of 93·8 (SE 2·02) % (n 29) calculated for the night values. Faecal excretion estimated from either the day or night PA concentrations (absolute values) was correlated with the measured excretion (24 h average), giving r 0·95 (n 29; $P \leq 0.001$) for both sampling periods. FDM estimated from day and night values respectively was on average 96·8 (SE 2·22) % (n 29) and 108·0 (SE 2·30) % (n 29) of the actual excretion. In trial C5, where all defaecations occurring during the day were analysed for marker concentration, forty to fifty day samples were obtained for each animal during the 10 d measurement period. FDM estimated from their average marker concentration was correlated with the actual faecal excretion (r 0·98, n 11; $P \leq 0.001$). Estimated FDM was

on average 97·9 (SE 3·00) % (*n* 11) of the actual excretion, thus the precision for the estimation of FDM from average PA concentration in up to fifty day samples was not significantly different from estimates based on the average of a maximum of fifteen day samples that were obtained applying the regular 7 h grab-sampling schedule.

To test whether pooling of grab-samples can replace the analysis of single samples, the marker quantities (g PA) determined in individual grab-samples collected for a particular animal and experiment were summed up and then divided by the sum of the masses of all samples (kg FDM), using data from experiments C3, C4 and C5 but excluding values obtained for unsupplemented straw diets (trial C3; treatments L, M). FDM estimated from marker concentration in mathematically pooled grab-samples was correlated with the actual excretion (*r* 0·97, *n* 29; $P \leq 0.001$).

DISCUSSION

Marker adminstration

The oral administration of the PA marker using gelatine capsules is achieved without difficulty or harmful effects on cattle. With a correct positioning of the applicator the capsule is swallowed unchewed. To avoid loss of particles through regurgitation of the capsule, animals should be offered small amounts of feed or should be allowed to graze immediately after marker administration. Furthermore, the distinct rumen contractions accompanying feed intake will lead to a thorough mixing of PA particles with rumen contents and ensure their regular passage through the rumen–omasal orifice (Pond *et al.* 1989).

Gastrointestinal marker passage

Peak concentration of PA marker in faeces was reached between 23·0 and 34·6 h after a single oral pulse dose application. This is similar to the result obtained by Des Bordes & Welch (1984) using plastic particles of the same specific gravity. The quantitative recovery of the amount of PA administered about 6 d after pulse dose application demonstrates that marker administration and analysis of faecal samples can be carried out without particle losses. Complete recovery of the marker also indicates quick and thorough marker passage through the gastrointestinal tract, which would be expected from rumen kinetics and passage rates described for small plastic particles with a specific gravity of 1·1–1·2 (King & Moore, 1957; Campling & Freer, 1962; Des Bordes & Welch, 1984; Lechner-Doll & von Engelhardt, 1989; Kaske *et al.* 1989; Welch, 1990). According to Katoh *et al.* (1988) and Welch (1990) the low proportion of particles with teethprints (i.e. ruminated particles) indicates a short rumen retention time of the marker. Administration of PA at regular intervals of 12 h leads to a plateau concentration of marker in faeces on the fourth day after the start of the marker application. The period of pre-experimental marker dosing is thus shorter for PA than for powdered polyethylene, where the plateau value is reached after 3–10 d (Chandler *et al.* 1966; Baran & Boda, 1982), while continuous intraruminal release of Cr_2O_3 leads to plateau concentrations in faeces at 5–6 d after the start of dosing (Laby *et al.* 1984).

Marker recovery

In the field, 97·6 % of administered PA was recovered in faeces and the correlation (*r* 0·94, *n* 21; $P \leq 0.001$) between the actual faecal excretion and the values estimated from faecal

PA concentrations demonstrates the marker's suitability for studies carried out in extensively-managed grazing systems. In the indoor experiments, the recovery of PA averaged 98·3 (SE 1·18) % (*n* 41) for diets based on green-pasture vegetation and supplemented straw diets. The recovery of 98·1 %, which was determined as an overall average for indoor and field experiments with a CV of 7·51 %, indicates that the recovery of PA is less dependent on experimental circumstances than the recoveries of Cr_2O_3 and Yb, which are reported to vary in the range 75–117 % and 93–125 % respectively (Brisson *et al.* 1957; Corbett *et al.* 1958; Knapka *et al.* 1967; Hatfield *et al.* 1990). However, when cattle consumed unsupplemented straw diets, PA recovery varied from 34·4 to 84·4 %, leading to an overestimation of the actual faecal excretion by 18–187 %. Although with these diets the daily excretion of PA reached equilibrium about 5–9 d after the start of marker dosing, the plateau values were low and relatively inconsistent. Similar observations were reported by Murphy *et al.* (1989) for cattle on a low-N diet. To determine the factors influencing PA recovery, a multiple linear regression analysis was carried out on seventy-four data sets (trials F1–F4, C1–C5) combining organic matter intake (related to metabolic body mass), *in vivo* organic matter digestibility and N content of the diet. While digestibility did not attain a significance level of 15 % and was excluded from the model, 68·8 % ($P \leq 0.001$) and 25·1 % ($P \leq 0.01$) of the variation in PA recovery was explained by the N content and the intake of organic matter respectively, the two variables being correlated (*r* 0·21). The N content of unsupplemented straw diets averaged 5·7 g N/kg OM compared with N contents of 12–17 g/kg OM in supplemented straw diets (C1, C2 treatment MS, C3 treatments LS, MS), diets that were selected from pasture during the dry season (F1–F4) and diets based on green pasture vegetation (C4, C5). The correlation (*r* 0·84) between N content and PA recovery supports the conclusion that the poor performance of the marker with unsupplemented straw diets may be caused by a low N content in the diet. This observation is congruous with the results of Murphy *et al.* (1989). They gave a single pulse dose of small plastic particles of a specific gravity of 1·10 and 1·34 to two cattle fed on a ration of rice straw (950 g/kg) and Leucaena leaf (50 g/kg; N content 6·1 g/kg DM) and recovered 79·9 and 80·1 % of the marker after 12 d. The incomplete marker recovery might be explained by a delayed rate of particulate passage due to slow microbial breakdown of the ingesta caused by sub-optimal rumen NH_3 concentrations (Moran *et al.* 1983; Oosting & Waanders, 1993). Incomplete particle recovery has been ascribed also to the properties of the rumen mat, the relative rumen fill and rumen ingesta consistency (Welch, 1990). Particles with higher density than the rumen mat have a better chance of sedimenting into the lower region of the reticulum, from where the probability of passing the reticulo-rumen omasal orifice is highest (Lechner-Doll & von Engelhardt, 1989). Reviewing literature, Murphy & Kennedy (1993) pointed out that the characteristics of buoyancy and entanglement of particles which determine their sequestration in the rumen mat are quite variable. The extent to which the mat hinders particulate passage from the rumen depends on its density and on the relationship between concentrations of particles in the rumen mat and the ventral sac of the rumen. As losses of marker particles through regurgitation and rumination could be excluded for the present study, it is assumed that with feeds of low N content the PA particles accumulate in the rumen.

To determine the minimum N content (*N*; g/kg OM) required to obtain high PA recoveries (*R*; %), a non-linear segmented regression consisting of two linear sections with one common point was fitted to the seventy-four observations. The line representing the equation for the lower section ($R = 19.632 + 8.185 N$) and the line described by the equation for the higher section ($R = 91.028 + 0.473 N$) intersected at *N* 9·26 g/kg OM and *R*

95·4 %. With respect to the applicability of PA marker under field conditions, this result indicates that reliable estimates of FDM can be expected where heterogeneous botanical composition of pastures enables cattle to select a diet containing more than 9·26 g N/kg OM. In the present study, the field trials were carried out at the end of the dry season when the vegetation of Sahelian rangeland is of very poor quality (Le Houérou, 1980), but even then unsupplemented cattle were able to select a diet with a N content above the required minimum value. However, on semi-arid pastures with reduced species diversity or in systems where cattle graze exclusively on cereal stover for several months during the dry season (e.g. Dicko *et al.* 1983; Powell & Mohamed-Saleem, 1987), the PA marker must be applied with caution.

Sampling techniques

A strongly varying and incomplete recovery of PA was obtained when faecal samples were taken at one to three fixed time intervals during the day. Similar problems have been experienced with Cr_2O_3 (Raymond & Minson, 1955; Putnam *et al.* 1958; Hayes *et al.* 1964; Kiesling *et al.* 1969; Rittenhouse *et al.* 1970; Whittington *et al.* 1978) and Yb (Hatfield *et al.* 1990). However the correlation (r 0·95) between the actual excretion and the FDM estimated from the average PA concentration in grab-samples collected during either the day or night indicates that limiting the collection period to 11 or 13 h instead of 24 h also results in reliable FDM estimates, as long as sampling is equally distributed over the chosen time interval. If it is decided to limit faeces collection to either the day or the night, the marker recovery for the particular sampling period must be determined before routine measurements are carried out. Increasing the number of samples will improve the accuracy with which the average marker concentration occurring during the chosen time interval is determined. To avoid a negative impact on the grazing animal (Fisher *et al.* 1986; Hatfield *et al.* 1990) and to obtain sufficiently large samples, the interval between grab-sampling times should be at least 3–4 h. Altogether, twenty to twenty-four grab-samples should be taken over 5–8 d. A longer collection period improves the precision with which average daily excretion is estimated, because variations between days in feed intake and faecal excretion are at least in part accounted for.

When grab-samples are collected during a 24 h period and analysed individually, marker recovery can exceed 100 % (e.g. trial C4, treatment HS). This is because average marker concentration is derived from the PA concentration in single grab-samples, whose marker concentration is weighted by the factor 1. If several samples with high marker concentrations are obtained, average marker concentration and consequently marker recovery increase. This bias is largely reduced if the corrections described previously are applied, and in most cases only a few small samples will have to be excluded, which at the time often contain extremely high or low amounts of PA. The poor correlation between sample mass and marker concentration in individual grab-samples and the irregular variations in faecal PA concentrations throughout the day indicate that the amount of PA in contributory grab-samples is not proportional to the rate of faecal excretion at the time the sample was excreted. It might be possible, therefore, that pooling of grab-samples and sub-sampling mass aliquots from that pool leads to a more biased estimate of faecal excretion than calculations based on the unweighted mean of the PA concentration in individual grab-samples. If grab-samples are pooled, bias due to extreme PA concentrations in very small samples will not be significant because those concentrations are weighted by a small factor. Furthermore, this bias can be reduced if samples equivalent to less than 10 g DM are not added to the pool. Pooling of grab-samples may however lead to erroneous estimates if

extreme marker concentrations occur in heavy samples. This bias decreases when the total mass of the pool increases, i.e. when a sufficient number of grab-samples is available. The close correlation between FDM estimates from mathematically pooled samples and the actual excretion that was found in the present study indicates that reliable estimates of faecal output can be obtained from the PA concentration determined in pooled grab-samples. A thorough homogenization of the pooled samples is a prerequisite for correct marker determination in aliquot sub-samples. Good results were obtained when pooled faeces were diluted with water to a moisture content of 820–880 g/kg. To reduce analytical errors, it seems appropriate to sub-sample in total six to eight aliquots with an air-dry mass of approximately 200–250 g.

Assessment of optimal marker dose rate

Results showed that marker recovery is independent of the quantity of feed ingested and of the quantity of marker administered. As model calculations illustrate (Fig. 4), a linear increase in PA concentration in faeces is accompanied by an exponential decrease in the extent to which faecal output is under- or overestimated due to analytical errors. On the basis of this correlation, the quantity of PA to be administered daily can easily be calculated for a particular experiment. Marker dose will depend on the required accuracy of FDM estimates and the feed intake and diet digestibility expected for individuals or a group of cattle. For example, if one decides to accept an overestimation of actual FDM by 5 % and expects that losses of PA due to analytical problems will not exceed 1 g PA/kg FDM, an average marker concentration in faeces of 24 g PA/kg FDM should be obtained. For animals consuming about 5 kg DM/d with a digestibility of 46 %, the marker dose would then be 65 g PA/d, whereas for animals consuming the same amount of feed with a digestibility of 68 %, only 39 g PA/d would have to be administered.

Conclusions

The application of PA as an external marker is a simple and precise method for the indirect quantification of faecal excretion of cattle and appears to be particularly suited for intake studies in extensive grazing systems. The short pre-experimental period of continuous dosing reduces costs and labour required for marker application and increases the practicability of the method under field conditions. Irrespective of the quantity of feed ingested, under- or overestimation of faecal excretion can be excluded if the botanical composition of the pasture is such that the N content of the diet is at least 9·26 g/kg OM. Instead of taking rectal grab-samples every 7 h during seven consecutive days, limitation of the grab-sampling period to approximately 12 h during the 24 h day is possible, but marker recovery must then be established for the particular experimental circumstances (diurnal rhythm of feed intake, timing of marker application, duration of the grab-sampling period) before routine measurements are carried out. Irrespective of the sampling schedule adopted, the period of grab-sampling should last 5–8 d and at least twenty to twenty-four samples should be collected. The laborious analysis of marker concentration in individual grab-samples can be replaced by pooling the samples and sub-sampling mass aliquots from that pool.
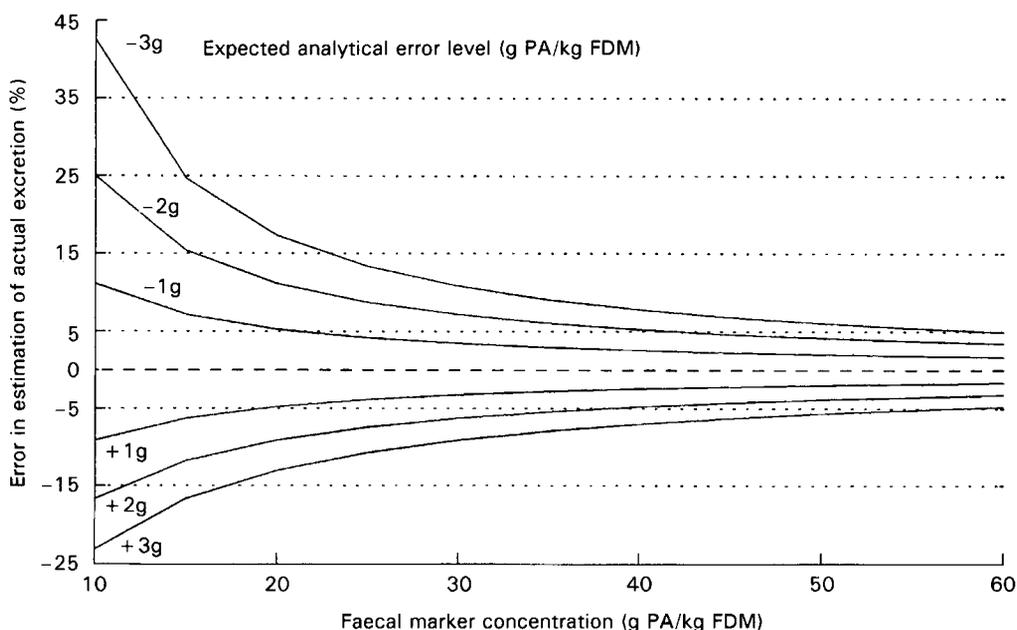
Fig. 4. Granulated polyamide (PA) marker concentration required in faeces (g PA/kg faecal DM (FDM)) of Zebu cattle (*Bos indicus*) to estimate total FDM excretion with an accepted level of accuracy, taking into account the error expected for the determination of marker concentration in faecal grab-samples (± 1 g– ± 3 g PA/kg FDM) due to analytical problems. For details of procedures, see pp. 786–792.

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