

THE USE OF INERT MARKERS IN THE MEASUREMENT OF THE DIGESTIBILITY OF CUBED CONCENTRATES AND OF HAY GIVEN IN SEVERAL PROPORTIONS TO THE PONY, HORSE AND WHITE RHINOCEROS (*DICEROS SIMUS*)

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Abstract—1. The rate of passage of chromic oxide (Cr) was similar in the pony and rhinoceros.

2. Higher apparent amounts digested were found using 4N-HCl-insoluble ash (AIA) than those determined by Cr, but overall digestibilities were similar for the two species.

3. An abrupt increase in the starch content of the horse diets increased the number of faecal ciliate protozoa. Only when the overall feed intake was increased in horses receiving a high dietary proportion of starch were the numbers depressed.

4. When the rhinoceros received 109 kJ apparent DE per kg bodyweight daily (716 kJ/W^{0.75} daily) it maintained normal condition.

INTRODUCTION

Many animal species do not readily lend themselves to containment in metabolism crates, nor to total collection of their excrement, over extended periods of time. Although many horses accept containment in crates for short periods exercise is essential for others, especially where large amounts of energy-rich feed are given.

The purpose of this investigation was to determine whether the method of measuring the quantity of energy digested, as proposed by Frape *et al.* (1976), could be adapted successfully to the assessment of both hay and cubed concentrate digestibilities when both are fed to the non-ruminant herbivores—horses and ponies (*Equus caballus*) and white rhinoceroses (*Diceros simus*). The method entails the provision of both small and large amounts of digestible energy (DE) so that extensive daily exercise by the equids was deemed essential, and sub-total collection of faeces containing markers was necessary. Chromic oxide (Cr) has been used as an inert marker in horse digestibility studies (Householder *et al.*, 1976), but there is evidence (Sauer *et al.*, 1979) that it yields lower digestibility values than those obtained by complete faecal collection. Sutton *et al.* (1977) found no difference between estimates of digestibility in mature geldings determined by total collection and those derived from the measurement of 4N-HCl-insoluble ash (AIA) in feed and faeces. A comparison was made in this experiment between AIA and Cr, as markers, when the pony, horse and white rhinoceros were subjected to diets containing various proportions of long hay and cubed concentrates.

An approximate estimate of the maintenance requirement for energy was made in the rhinoceros on the assumption that over extended periods of time the

voluntary energy consumption of the sedentary adult will equate with maintenance requirement.

When starch is infused rapidly into the rumen of sheep given a low starch diet there is a rapid fall in the pH of the ruminal fluid causing a loss of ciliate protozoa (Eadie *et al.*, 1970; Hino *et al.*, 1973) and a greater consequential susceptibility to acidosis (Krogh, 1959). The pony caecum has been shown to contain of the order of 6×10^3 protozoa/ml (Kern *et al.*, 1974); but fermentation of large amounts of caecal starch causes laminitis (Moore *et al.*, 1979). Nevertheless the presence of starch in the caecum can increase N retention in horses given NPN (Reitnour, 1979). It was considered that the measurement of changes in the population of ciliate protozoa of equine faeces may reflect changes in the pH of the large intestinal contents subjected to rapid changes in the amount of dietary starch imposed by the experimental design.

MATERIALS AND METHODS

Animals and management

The two horses (520 kg and 670 kg body weight) and two ponies (380 kg and 450 kg body weight) used in Expts 1–4 were held at the Kennett Nutritional Centre, Newmarket, Suffolk.

The two white rhinoceroses, Ben, a male aged 26 years and Beni, a female aged 20 years used in Expts 5–7 were each approximately 1900 kg in body weight. They were held throughout the study in the Elephant House at the Zoological Society of London, Regents Park, but managed similarly to the horses and ponies.

The horses and ponies were worked daily and bedded on peat, but during periods of the day when faeces were collected they were held in stalls with rubber-matted floors. The rhinoceroses were fed individually on a clean concrete floor from which uncontaminated faecal samples were col-

Table 1. Ingredient composition of cubed feeds (g/kg)

	Experiments	
	1 and 5	2, 3, 4, 6 and 7
Wheat offal	357.0	400.0
Barley	—	446.5
Oats	76.1	—
Oatfeed	256.0	—
Extr. soyabeanmeal	15.0	50.0
Ground hay and straw	141.5	—
High protein grass meal	56.1	—
Sucrose	5.0	—
Molasses	69.2	75.0
Sodium chloride	5.0	7.5
Limestone	5.5	10.0
Dicalcium phosphate	8.1	—
Vitamins and trace minerals	2.5	5.0
Chromic oxide	3.0	6.0

lected. They exercised daily in an open earth-based yard free of vegetation.

Diets

The cubed concentrates (Table 1) for the equine and comparable rhinoceros experiments were manufactured on the same day from the same batches of ingredients, but the hay sources were different. The chemical composition of each of the hay sources and that of the two types of cubed concentrate is given in Table 2.

Experimental procedure

Seven experiments were conducted three with pony geldings (Expts 1–3), one with horse geldings (Expt 4) and three with white rhinoceroses (Expts 5–7). In each experiment the treatments consisted of a diet composed of cubes and hay given in several proportions.

In the equine experiments the ratios of (cubes):(cubes + hay) were:

- Expt 1, 0.29, 0.43, 0.71 and 0.86.
- Expt 2, 0.24, 0.38, 0.52, 0.67 and 0.82
- Expt 3, 0.24 and 0.67
- Expt 4, 0.23 and 0.57.

These ratios were allotted at random with the restriction that each ratio was imposed only on each animal. Additionally in Expts 3 and 4 two levels of daily dry matter intake were superimposed in a 2 × 2 factorial arrangement. In the rhinoceros experiments the ratios of (cubes):(cubes + hay) were:

- Expt 5, 0.25, 0.41 and 0.50
- Expt 6, 0.31, 0.40, 0.47 and 0.60
- Expt 7, 0.29, 0.51 and 0.70.

These ratios were allotted randomly in as many periods with the same ratio being used for both rhinoceroses in each period.

In all experiments the dry matter intake was approximately constant between periods. In Expts 1 and 5 a more fibrous cubed concentrate was given than in the remaining experiments (Table 2). The ration of cubed concentrate was given daily in two equal feeds which in the rhinoceros experiments reached the required level of feeding during the first 4–5 days of each 24 day period. The hay allowance in the rhinoceros studies was adjusted during these days to an amount readily cleared up, but for the remainder of each period the allowances of cubes and hay remained constant at the levels achieved on the 5th day. In the equine experiments the hay allowance in each period was fixed by design, and the treatment changes between periods were abrupt.

In a preliminary experiment employing the cubed concentrate of Expts 1 and 5, given at a constant rate with hay, faecal samples were collected daily from two ponies for 30 days from the day Cr was included in the cubes until 8 days after its removal. In Expt 5 faecal samples were also collected daily from each rhinoceros from days 1 to 24 during the first and last periods and in addition for 14 days beyond the last period. These samples were used in a measurement of the change with time in the Cr concentration of faeces (Fig. 1). For the purpose of measuring amounts apparently digested, faecal samples were collected from each animal during 8 of the last 10 days of each period in each of the seven main digestibility experiments. These samples were stored at –20°. Prior to analysis they were bulked in 2 × 4 day sub-periods for each animal, dried at 80° for 24 hr and ground before subsampling for analysis. Feed samples were collected daily from day 10 to day 22 and bulked for each period prior to grinding and subsampling for chemical analysis. Further faecal samples were collected on days 4 and 22 in Expts 2–4 and 20 g placed in 80 ml 10% formaldehyde solution for a determination of ciliate protozoa, according to the method of Adams (1951), for the purpose of assessing whether an abrupt change in dietary starch content had any effect in the short or longer term on their abundance.

Cr was determined in feed and faeces by titration and by atomic absorption spectrophotometry. Acid-insoluble ash was determined by the method of Sutton *et al.* (1977).

RESULTS

Statistical analysis of digestibility data

In general the animal × diet term in the analysis of responses was used as the residual error for testing diet effects, but where the variation amongst sub-periods within animal–diet combinations was not signifi-

Table 2. Average chemical composition (g/kg or MJ/kg) of concentrate cubes and hay

Expt no.	Cubed concentrate		Hay				
	1 & 5	2, 3, 4, 6 & 7	1	2, 3 & 4	5	6	7
Dry matter	905.7	873.0	909.0	858.0	905.0	875.0	903.0
Oil	27.7	23.5	8.8	9.7	—	10.7	—
Crude protein	107.0	134.0	59.2	58.5	78.9	106.3	80.0
MAD fibre	205.1	63.5	332.0	348.6	386.3	361.5	341.0
Calcium	15.8	6.5	4.4	7.0	5.3	6.3	6.4
Phosphorus	6.0	5.9	2.2	2.0	1.8	2.5	2.3
Potassium	10.8	9.6	19.5	16.8	—	20.9	—
Gross energy	16.31	15.99	16.76	15.74	16.79	16.06	16.96
Acid-insoluble ash	17.0	4.4	16.5	21.6	13.3	8.0	12.1
Cr ₂ O ₃	3.36	5.64	—	—	—	—	—

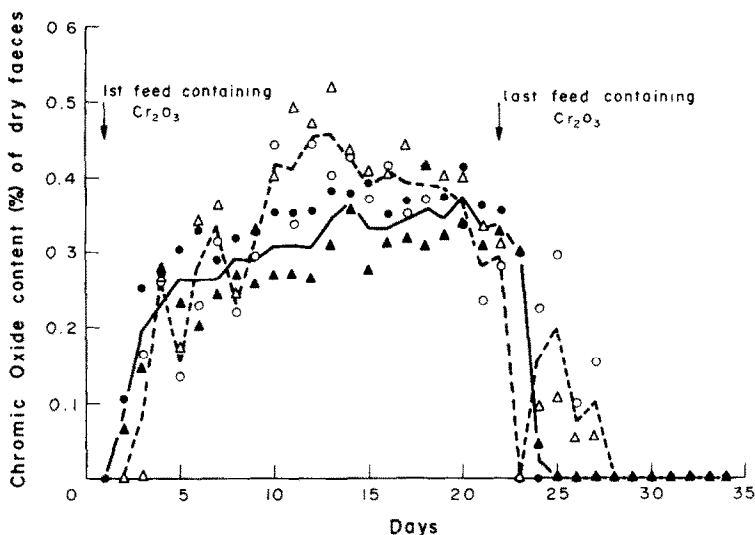


Fig. 1. Chromic oxide content (%) of dry faeces from ponies (●—●; ▲—▲) in a preliminary investigation of 30 days duration and from rhinos (○---○; △---△) all receiving a cubed concentrate of the same composition. The hay source differed for ponies and rhinos. The rhino data are taken from the first (days 1–20) and last (days 21–34) periods of Expt 5 in which different proportions of cubes and hay were used.

cantly less than the animal \times diet effect the two sources of variation were pooled to form the residual error. In Expts 3 and 4 the feeding level \times concentrate:hay ratio term was non-significant for all responses and could therefore also be included in the error. In the other experiments the quadratic and higher order terms of the regression of response on concentrate proportion were absorbed into the error where they were non-significant. Possible period effects could not be allowed for.

Coefficients for amounts of cubes and hay digested were calculated by the method of Frape *et al.* (1976) where deviations from linear regression of digestible nutrient values on proportion of cubes were non-significant.

Rate of passage of chromic oxide

The Cr in faecal samples collected daily (Fig. 1) shows similar rates of increase in concentration for the pony and rhinoceros with a plateau concentration not occurring before the 14th day. For the purpose of measuring amounts digested, therefore, samples were not collected before the 15th day. Following removal of Cr from the feed, Cr disappeared from the faeces more rapidly than its concentration had previously increased.

Amounts digested by horses and ponies

In Expts 1, 2, 3 and 4 the apparent amounts digested, calculated by AIA, tended to be higher than the amounts calculated by the Cr marker (Tables 3 and 4). The coefficients of variation of the apparent amounts digested, calculated by the two methods, were less for AIA in Expt 1, but less for Cr in Expts 2–4. A significant correlation amongst diet mean amounts digested determined by the two methods occurred for protein digested daily in Expts 1, 2 and 4 ($P < 0.1$, 0.01 and 0.1 respectively). Otherwise the

results using the two methods were not well correlated.

No significant regression of DM, or of gross energy (GE), digested on proportion of cubes was apparent when the lower energy cubes were used in Expt 1, indicating a similarity in digestible energy values between cubes and hay. The amount of protein digested was, however, the greater for cubes (Table 4). When the higher energy concentrate was used (Expts 2–4) coefficients for amount of protein digested were higher for cubes by both methods, but the amount of energy digested was significantly higher only as determined by Cr in Expts 3 and 4. Where there was a significant effect of feeding level on energy digested in Expts 3 and 4 (Table 5) coefficients are given for concentrate cubes and for hay at each level in Table 4.

Amounts digested by rhinoceroses

Slight diarrhoea was experienced within 4 days of the daily concentrate consumption rising to 13.1 kg and 11.6 kg in Expts 6 and 7 respectively in which the higher starch-containing concentrate was used. The condition abated within 2–3 days. In Expt 5 the concentrate containing the lower amount of starch was fed, and no diarrhoea was observed when the concentrate intake rose as high as 12.4 kg daily.

The overall mean amounts digested calculated from AIA data were higher than those calculated from Cr values in Expts 6 and 7, but they were similar in Expt 5 (Table 3). The coefficients of variation for AIA and Cr were similar in Expt 7, but the variation of the AIA data was greater in Expt 5 and that of Cr was slightly greater in Expt 6. The correlation amongst the diet mean responses, calculated by the two methods, was poor and significant only for amount of protein digested daily in Expt 5 ($P < 0.001$).

The experimental control in Expts 5 and 6 was less than that in Expt 7 and significant deviations of re-

Table 3. Mean apparent digestible dietary components (g/kg diet or MJ/kg diet) and their coefficients of variation as determined by two markers

Experiment	Marker	Overall means			Coeff. of var. (% of mean)		
		Dry matter	Crude protein	Gross energy	Dry matter	Crude protein	Gross energy
1	AIA	707	78	12.9	14.2	5.6	7.7
	Cr	441	61	8.2	14.3	11.1	9.8
2	AIA	—	84	12.2	—	21.4	28.8
	Cr	—	73	10.2	—	9.9	7.7
3	AIA	—	—	14.0	—	—	9.4
	Cr	—	—	9.4	—	—	5.7
4	AIA	—	63	10.2	—	17.6	14.4
	Cr	—	58	9.0	—	9.8	10.9
5	AIA	599	65	10.9	9.3	8.0	9.8
	Cr	604	65	11.0	5.3	6.8	5.0
6	AIA	674	96	12.1	9.8	8.2	10.9
	Cr	468	73	8.0	11.1	10.2	12.9
7	AIA	701	—	12.8	3.5	—	3.8
	Cr	636	—	11.5	3.6	—	4.0

sponse from linear regression on proportion of cubes in the diet occurred for all responses in Expt 6. The low energy cubes used in Expt 5 resulted in lower digestibility coefficients for them than for the hay (Table 4), whereas in Expt 7, in which the higher energy cubes were used, the coefficients for *DM* and *GE* digestibility of the cubes were 116% and 119%, respectively of the hay values.

The apparent digestibility of *MAD* fibre was determined in three of the experiments and the results are given in Table 6 (values were determined only for two of the diets in Expt 6). There was no consistent evidence that fibre digestibility declined as the proportion of cubes was increased as no trend occurred in Expts 1 and 6, although a decline was found in Expt 5 ($P < 0.05$) with an increasing proportion of cubes.

Faecal ciliate protozoa

On the occasions in Expts 2–4 when the proportions of concentrate cubes were increased the effect on the 4th day of each period was variable, but on average there was an increase in the number by 1.6×10^4 protozoa/g faeces. When there was a decrease in the proportion of cubes, the average effect on the 4th day was a decrease of 4.4×10^4 protozoa/g. When no change occurred in the proportion of cubes, but a change in the total amount of feed was made (Expts 3 and 4), there was on the 4th day an increase of 1.3×10^4 protozoa/g when the amount increased and an increase of only 0.4×10^4 protozoa/g when the amount decreased.

A somewhat similar response was still apparent by the 22nd day of each period. In Expt 2 increasing the proportion of cubes from 0.24 to 0.82 led to a linear increase in the number of ciliate protozoa by 5.6×10^4 ($P < 0.01$) (Table 7). In Expts 3 and 4 on the 22nd day there was no overall effect of increasing the proportion of cubes. In Expt 3 an increase in the

cube proportion from 0.24 to 0.67 caused no significant change in the numbers of protozoa. In Expt 4 there was an interaction ($P < 0.05$) in which the numbers were decreased only by increasing the amount of total feed containing the higher proportion of cubes.

DISCUSSION

Our data support evidence (Sutton *et al.*, 1977; Sauer *et al.*, 1979) that the use of *Cr* as a marker leads to lower digestibility values than those derived by *AIA*. As the values derived by *AIA* agree well with the results of total collection in horses (Sutton *et al.*, 1977) our *Cr* values may be underestimates.

Taking all experiments into account the coefficient of variation of one method was not consistently higher or lower than that of the other, so that the *AIA* method is to be preferred to the *Cr* method.

The evaluation in non-ruminant herbivores of the method proposed by Frape *et al.* (1976) for the determination of the digestible energy of feed ingredients was handicapped, not only by the relatively inflated errors inherent in measurements calculated by inert marker dilution, but also by the inadequate experimental control achieved in the first two rhinoceros experiments. However, the absence of significant deviations from linear regression in much of the data lends support to the use of this method for assessing the apparent amounts digested of hay and of cubes when they are fed together to non-ruminant herbivores.

The rhinoceroses received their experimental regimes for 240 days, during which no material change occurred in their clinical condition. In Expt 7 the average daily intake of dry matter was 14.2 kg per rhinoceros. The average daily intake of apparent *DE* per rhinoceros, as assessed by *AIA*, ranged from 190 MJ for the diet containing the lowest proportion

Table 4. Coefficients for apparent amount digested per kg cubes and hay (with their standard errors in brackets) using two markers in ponies, horses and rhinoceroses (g/kg, or MJ/kg)*

Experiment	Feed	Dry matter		Crude protein		Gross energy			
		AIA	Cr	AIA	Cr	AIA	Cr		
1	Cubes			102	86				
				(3.3)	(6.8)				
		707	441			12.9		8.2	
		(36)	(22)			(0.35)		(0.29)	
	Hay			44	28				
				(4.2)	(8.7)				
2	Cubes	—	—	122	93				
				(10.3)	(4.2)				
						12.2		10.2	
						(0.78)		(0.18)	
	Hay	—	—	41	50				
				(11.4)	(4.6)				
3+	Cubes	—	—	—	—			Low F	High F
								10.9	11.6
							(0.39)	(0.39)	
						14.0			
						(0.33)			
	Hay	—	—	—	—			7.6	8.3
								(0.34)	(0.34)
4†	Cubes	—	—	120	105	Low F	High F		
				(10.1)	(5.2)	11.2	9.3		
						(0.52)	(0.52)		11.2
									(0.90)
	Hay	—	—	25	27				7.5
				(7.1)	(3.7)				(0.63)
5	Cubes	399	228						
		(115)	(55)					5.6	2.5
							(2.01)	(0.97)	
					DEV				
	Hay	725	841					14.2	16.4
		(74)	(36)					(1.30)	(0.63)
6	Cubes								
	Hay		DEV		DEV				DEV
7	Cubes	753		—	—			13.9	
		(22)						(0.44)	
			DEV						DEV
	Hay	649		—	—			11.7	
		(22)						(0.44)	

* Where a common coefficient is given for cubes and hay the values did not differ significantly. F, level of feeding. Coefficients are given for each level of feeding where the interaction within diet was significant.

DEV, significant deviations from linearity.

† The use of only two proportions of cubes excludes a test for deviations from linearity.

Table 5. Mean apparent crude protein digested (g/kg diet) and mean apparent gross energy digested (MJ/kg diet) as influenced by level of total feed intake in Expts 3 and 4

Relative level of feed intake within experiment	Crude protein digested		Gross energy digested	
	AIA	Cr	AIA	Cr
			Expt 3	
100	—	—	14.3	9.1
133	—	—	13.7	9.8
SE of means			0.47	0.19
			Expt 4	
100	68	59	11.2	8.9
125	58	57	9.3	9.0
SE of means	3.9	2.0	0.52*	0.35

* Significant difference between treatment means ($P < 0.05$).

Table 6. Mean apparent digestibility (*D*) of modified-acid-detergent fibre in diet as determined by acid-insoluble ash

1 Proportion of cubes	Experiment				6 Proportion of cubes	<i>D</i>
	<i>D</i>	5 Proportion of cubes	<i>D</i>	<i>D</i>		
0.29	0.66	0.25	0.64	0.31	0.77	
0.43	0.60	0.41	0.60	0.47	0.77	
0.71	0.76	0.50	0.47			
0.86	0.58					
SE of means	0.039		0.044*		0.028	

* Significant linear decrease in digestibility with increasing cube proportion ($P < 0.05$).

of cubes to 220 MJ for the diet containing the highest proportion of cubes: the overall mean was 206 MJ. Each rhinoceros weighed approximately 1900 kg and on the assumption that their average daily energy consumption was at the maintenance level they required 109 kJ apparent *DE* per kg body weight daily (716 kJ/W^{0.75} daily). This value is 11% higher than the value calculated by Brody (1945) for mature animals of many species, and in comparison with species of the same natural Order 10% and 81% higher than the values calculated by the NRC (1978) for mature horses and by Kane *et al.* (1979) for mature Shetland ponies respectively. The lower value derived by the latter workers may have resulted from restricted activity in an equable environment and the estimation of the values by extrapolation of a regression line towards the origin.

Owen (1868) commented on the general resemblance of the abdominal anatomy of the horse to that of the rhinoceros (*R. indicus*). A comparison of the gross measurements of their gastro-intestinal tracts showed that the linear measurements of the small intestine, caecum and colon were almost identical. Energy requirements for maintenance increase only in proportion to W^{0.75} and the relative loss of intestinal surface area and absorptive efficiency per unit volume implied by the measurements in the rhinoceros are in

accordance with expectation. Energy demands for maintenance are directly proportional to surface area of the body so that no extension of the intestines is necessary for their surface area to remain directly proportional to those demands. This argument ignores any differences which may exist in folding of the intestinal mucosa.

The dry matter intakes of the equids and rhinoceroses were also in proportion to energy requirements rather than to gut volume which should therefore have induced a slower rate of passage of Cr in the rhinoceros. A close inspection of Fig. 1 indicates a retention of Cr by the rhinoceros for a longer period than occurred in the pony. The attainment of forage digestibility values by the rhinoceros which are at least as great as those found in the equids probably resulted, in part at least, from extended retention of ingesta in the hind gut facilitating microbial fermentation.

The results of Expt 2 imply that the rate of increase in the starch, soluble sugar, and acid content of the digesta reaching the large intestine following an abrupt increase in the starch content of the diet was moderated by digestion in the small intestine and so it stimulated the growth of the population of ciliate protozoa. The data from Expt 4, on the other hand, indicated that a large increase in starch dose, and prob-

Table 7. The effect of the proportion of concentrate cubes (C) in the diet and the level of feeding (low, L, high, H) on the mean number of ciliate protozoa (no. g faeces) $\times 10^{-4}$ in the faeces of horses and ponies on the 22nd day of each feeding period

C	Expt 2	Expt 3			Expt 4		
	Protozoa	C	L	H	C	L	H
0.24	5.2	0.24	6.9	6.3	0.23	5.0	6.7
0.38	8.8	0.67	5.9	5.9	0.57	14.0	3.3
0.52	7.5						
0.67	9.6						
0.82	10.8						
Coeff. of var.	7.1%*		154%			27%†	
of means							

* Highly significant linear increase in faecal protozoa with increasing cube proportion ($P < 0.01$).

† Significant interaction between feeding level and cube proportion ($P < 0.05$).

ably also in rate of passage, as would have occurred when the amount of feed and the proportion of cubes given were simultaneously increased, caused some suppression of protozoal growth. It is concluded that the conditions then were approaching those in which D(-)-lactic acid production is excessive and laminitis might be precipitated (Moore *et al.*, 1979), as Kern *et al.* (1973) detected no change in the total protozoal population per ml of caecal contents when oats were introduced to, or removed from, a feed of timothy hay, providing a constant total intake, although shifts in species distribution had occurred. Nevertheless changes in the population of faecal ciliates are interpreted with caution, as the evidence of Adam (1951) indicated the presence of two distinct populations inhabiting the large intestine of the horse, and other changes in the microbial populations, including those of methanogenic bacteria and of *Veillonella* species (Alexander, 1963), which will affect the decline in pH, have not been studied in any detail.

The rhinoceroses may have been more subject to changes in the pH of the large intestine, as dietary changes were introduced gradually, yet changes in the fluid content of faeces were apparent. This may have resulted from differences between species in bacterial and protozoal populations, or from differences in digestive enzyme secretion in the small intestine, or from both. Structural residues of hay 4-7 cm in length were of common occurrence in the rhinoceros faeces, indicating a poorer comminution of long hay than occurs in horses or ponies, although the data indicate no lower digestibility values for hay in rhinos. These data imply that in practice the starch content of the white rhinoceros diet should be low in accord with the diet normally given at Regents Park. The poor comminution of the hay on the other hand may indicate that giving it in a chopped form would be justified.

McDonald & Hamilton (1980) demonstrated that as the proportion of oats in the ration of steers, given oats and hay, was increased above 0.5, crude fibre digestibility fell from 56% at 0.50 to 44% at 0.77 oats. This effect could be attributed to a lower digestibility of oat fibre than of hay fibre, or to the suppression of fibre fermentation by the preferential fermentation of starch. The data in Table 6 suggest a similar effect did not occur consistently in our experiments, although the dietary proportions of concentrate attained, where MAD fibre digestibility was measured, were generally lower.

Nevertheless the data support the protozoal evidence and the conclusions that although the digesta entering the large intestine of horses and ponies may be of relatively uniform composition, some change in the fermentable portion can be initiated by diet and so play a role in N retention (Reitnour, 1979).

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