AN ASSESSMENT OF A "CONTROLLED RELEASE" CHROMIUM DEVICE FOR MEASUREMENT OF INTAKE IN CALVES

A Thesis presented in partial fulfilment of
the requirements for the degree
of Master of Agricultural Science
at Massey University, New Zealand.

AGGREY MBONEKO KASSANO

1988

ABSTRACT

Twelve Friesian male calves were used, in three blocks each with 4 calves, to assess the efficiency of a Controlled Release Device (CRD) designed to provide chromic oxide as a faecal marker.

Animals were weighed at the start and at the end of each replicate, after initial weight measurements calves were randomly allocated to one of 4 treatments (grass only, soya and grass, powdered milk and grass and liquid milk, carbohydrate concentrate and grass). Cut pasture was offered *ad libitum* while supplements were restricted at fixed levels and provided 11.28 MJME (metabolisable energy) and 175 g crude protein (CP) per calf daily.

After a 5-day preliminary period, two 5-day collection periods followed. The CRDs were administered by use of a 'balling gun' which introduced the device gently to the rumen. Collection of faecal and urine samples commenced 24h after administration of devices.

Faecal grab samples were collected from calves (*per rectum*) daily at 10.00 am, 01.00 pm and 04.00 pm while a 24 h representative faecal sample was taken every morning from the total day's collection. Daily faecal total collection was subsequently bulked for a 5-day sampling period.

A daily release of chromium (Cr) from CRDs was found to be 133 mg d^{-1} (obtained by measuring the amount of Cr 'loss' from three capsules which were regurgitated) which compares with the expected 130 mg d^{-1}.

There were no significant variations in Cr concentration between times within a day. Marked fluctuations in Cr concentration were noticed in Period 1 (first 5-days) of each block while concentration reached a more steady plateau in Period 2 of each block.

Similarly there were varying Cr release rates (RR) between days however, no statistical significance was shown because of the small number of calves within treatment

(n=3). The daily RR was found to be largely influenced by the amount of dry matter intake but not highly influenced by the type of ration.

The amount of Cr yield (recovery rate) was not statistically different from 100%. In real terms however, Cr recovery from calves which were on the liquid milk treatment was generally low. Such a recovery was associated with the fluid nature of the diet such that it could not intimately mix with the marker.

Two methods of digestion were compared for the Cr concentration assays. A significantly higher ($p < 0.001$) Cr concentration was obtained from faecal samples which were digested using a "high" bromate (HB) compared with "low" bromate (LB) digestion. Also within duplicate variations in Cr concentration were largely eliminated by using HB.

Supplementation considerably improved ration digestibility, Soya (0.77), powder milk (0.79) and liquid milk (0.83) as compared with grass only ration (0.74) there was also a favourable response in the nitrogen retention (NR) of the calves on the liquid milk diet which were superior, while the grass-fed calves performed relatively poorly. The response may be associated with high efficiency with which digestible energy (DE) from the milk supplement was utilized for liveweight gain.

ACKNOWLEDGEMENTS

It is my pleasure to acknowledge the guidance of my Supervisor, Dr G.F. Wilson.

I also wish to thank the following who gave very valuable assistance in various ways.

D. Hislop of No. 1 Dairy Farm (Massey University) for making calves available, B. Parlane of Animal Physiology Unit for facilitating conduct of the entire experiment, R. Watson and J. Rumball for their help during chemical analyses, R. MacKenzie of Chemistry Department for doing the tiresome spectrophotometry work and X. Wang for statistical assistance.

I am also quite grateful to my fellow post graduate students and other members of staff of the Animal Science Department who accepted to be involved at different stages of this work.

Appreciation is also due to S. Ryan for continuous encouragement and keeping an eye on language quality.

Many thanks to Trish Fleet for typing this thesis.

A lot of congratulations are accorded to my wife, Todo, for putting up with loneliness during the whole period of my study in New Zealand.

Lastly I am thankful to both the New Zealand and Tanzania Governments for the financial support offered towards this study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES (cont.)

Page/Facing Page

LIST OF FIGURES

LIST OF APPENDIX TABLES

Table

INTRODUCTION

Milk production has never met the demand for milk in less developed countries (LDCs). Thus milk used for calf rearing is also badly needed for human consumption, particularly in LDCs where whole milk is the major baby food.

The development of solid food intake (particularly pasture) in the calf is clearly an important topic for research, as the earlier this occurs the less the amount of whole milk is required.

Alternative techniques for the study of calf intake estimation are discussed as a part of the literature review. While accurate methods are available for the housed or zero-grazed calf, satisfactory techniques are presently not available for the grazing calf (Gleeson, 1971).

The recent commercial availability of a controlled release device (Captec Pty N.Z. Ltd, Auckland) which, when placed in the rumen of an animal, is said to release an indigestible marker (Cr_2O_3) at a constant rate, offers the possibility of accurately estimating faecal output in grazing animals. Food intake can then be calculated from faecal outputs provided an estimate of digestibility is also available.

This investigation was designed to study the usefulness of the CRDs for predicting intake in newly weaned calves. Specific objectives of the study undertaken with 12 calves fed indoors, include the following:

(1) To determine the rate of excretion of chromium from the CRDs and establish the main factors which affect the release rate. This information is required before CRDs are used in grazing calves. The RR associated with four feeding regimes were compared: Pasture only (G), Pasture and Liquid milk and carbohydrate (L), Pasture and Powdered milk (P) and Pasture and Soya beans (S).

(2) To determine whether the RR varies diurnally or over time (day 1 to day 10 after device administration). Such

information is needed to work out a faecal sampling regime for field studies.

(3) To study the recovery of administered Cr and factors affecting it, including the analytical method.

(4) In addition to the assessment of the CRDs the experiment provided information on the utilization (digestibility and nitrogen retentions) of autumn pasture, with and without a range of supplements, by newly weaned calves.

CHAPTER 1

LITERATURE REVIEW

1.1 ALTERNATIVE METHODS FOR MEASURING INTAKE OF PASTURE-FED CALVES

INTRODUCTION

A knowledge of pasture intake measurement in young calves and the assessment of whether this is enough to promote normal calf growth is of fundamental importance in animal production. However, there are great difficulties in measuring herbage intake by young ruminants and therefore various methods have been developed with varying degrees of success.

Intake of solid feed by calves can be measured by housing calves and feeding them cut pasture (Gleeson, 1971) and intake can be estimated in a number of different ways for grazing requirements (Meijs, 1981; Le Du and Penning, 1982). Field studies are necessary if there is a lack of indoor feeding equipment or in a situation where the normal "environmental conditions" could influence grazing behaviour, intake or performance.

Alternative ways in which intake of pasture-fed calves could be estimated are reviewed.

1.1.1 INDOOR METHOD

This is a method whereby pasture is cut from the field then weighed before being offered to stall-fed calves, commonly called 'cut and cart' method. The conditions for the success of the method are that all the refusals should be weighed so that the pasture intake can be determined by subtracting the refusal from the amount offered. Similar work to that done in the present study, involving

measurement of grass intake for housed calves, was done by Irish workers (Gleeson, 1971 and Keane and Harte, 1982). The method has some limitations in that it does not accurately replicate outdoor pasture and environmental effects where sward structure is likely to influence the grazing animal's behaviour (Hodgson, 1971). Also stall-fed calves are limited in selecting their diet as compared to their grazing mates.

1.1.2 OUTDOOR METHODS

Pasture intake of grazing calves can be estimated by measuring the disappearance of pasture from the grazed area over time.

Animal based techniques involve the estimation of faecal output (Le Du and Penning, 1982) together with an independent estimate of digestibility (Meijs, 1981) and both of these variables are used in the equation.

$$\text{Intake (I)} = \frac{\text{Faecal output (F) g d}^{-1}}{\text{1-digestibility (D)}} \qquad (1)$$

This method is discussed in detail by Le Du and Penning (1982).

1.1.2.1 SWARD BASED METHODS

The herbage mass (total mass of herbage per unit area of ground) is estimated at the beginning and at the end of the grazing period. The difference between the two gives an estimate of the apparent quantity of herbage consumed per unit area (Meijs *et al*, 1982). But since the herbage may also grow during the grazing period, a correction has to be made to allow for this, otherwise intake may be underestimated. This calculated consumption per unit area is

then converted to intake per animal/day by dividing the number of animal-days per unit area.

An advantage with the sward method is that the measurements also provide information on the herbage allowance (the weight of the herbage per unit of the animal liveweight) and the efficiency of grazing (herbage consumed expressed as a proportion of the herbage accumulated). The topic has been fully reviewed by a number of workers (Brown, 1954; Carter, 1962; 't Mannetje 1978; Frame, 1981 and Meijs, 1981).

1.1.2.1.1 Destructive and Non-destructive Methods

These methods are classified as either destructive (cutting) or non-destructive. Each of these has its merits and demerits.

(1) Herbage Cutting Methods

Cutting techniques, usually involve the harvesting of a measured proportion of the area of pasture allotted to the animal and weighing and sampling the cut herbage. The amount of residual herbage remaining after grazing, is similarly determined. This technique is fully discussed by the Grassland Research Institute, Hurley (1961) and Meijs *et al.* (1982).

The labour involved in cutting herbage samples by hand at ground level makes the method a difficult one, also herbage samples cut close to the ground, especially those cut by machine are frequently heavily contaminated by soil and may necessitate washing. For accurate assessment it has been suggested (Grassland Research Institute Hurley, 1961) to work on the basis of ash-free organic matter.

This method can only indicate how much herbage disappears in the course of grazing, this quantity is almost certainly greater than that actually ingested by the experimental animals because it includes herbage eaten by wild animals, in case of range lands (Cordova *et al,* 1978)

and herbage destroyed by trampling and that under dung pads (Holmes, 1980).

(2) Non-destructive Methods

These include eye estimation, height and density measurements and non-vegetative attributes. A review of different non-destructive techniques has been narrated by a number of workers (Brown, 1954; 't Mannetje, 1978 and Frame, 1981).

Eye estimation encompass estimation of herbage by visual appraisal (Pechaneck and Pickford, 1937 cited by 't Mannetje 1978). The sources of bias include (a) insufficient training - untrained observer tends to overestimate tall growing swards and underestimates dense swards; (b) idiosyncrasy of observers - some observers, although trained tend to persistently over- or underestimate herbage mass; (c) fatigue - after prolonged periods e.g. over 3 hours of intensive pasture examination, observers tend to over- or underestimate mass.

Height and density measurements may be used to estimate herbage mass after having first calibrated these relations by cutting and weighing. Height is normally defined as maximum or mean and is measured by a ruler (Meijs *et al*, 1982). Density is defined as the percentage ground cover and is estimated by point quadrant or visual appraisal (Bakhuis, 1960). Height and density measurements are integrated by the weighted disc grass meter (Earle and McGowan, 1979). The main source of bias in these variables include (a) sward structure; (b) lodged or trampled herbage; (c) botanical composition; (d) season and (e) grazing management.

Non-vegetative attributes. Herbage mass can be estimated from one of a number of non-vegetative plant attributes (e.g. capacitance, radioisotope attenuation, spectral analysis, after having first calibrated the respective parameter with actual herbage mass by cutting and weighing. Of these methods capacitance has been most intensively studied (Neal and Neal, 1973). Research

experience has shown that 'capacitance probe' has severe limitations in providing reliable herbage mass data due to the many extraneous factors that can influence meter readings (Angelone *et al.*, 1980a).

It has been concluded that (i) all non-destructive techniques are less precise than cutting methods on a per sample basis, they therefore require a greater intensity of sampling to achieve the equivalent level of precision; (ii) the method based on capacitance tend to be less precise than height measurement which in turn tend to be less than visual appraisal (Neal and Neal, 1973); (iii) relative precision is less for post grazed swards than for pre-grazed swards, and (iv) variability of pasture in terms of botanical composition, sward structure, morphology, sward density and moisture content decrease precision (Meijs *et al*, 1982).

1.1.2.2 ANIMAL-BASED METHODS

As indicated earlier, tthe indirect estimation of intake requires an estimate of faecal output (Le Du and Penning, 1982) and indigestibility (1-digestibility). These aspects are discussed separately.

1.1.2.2.1 Faecal output estimation

1.1.2.2.1.1 Total Collection can be achieved by fitting the animal with collection bags where faeces can be trapped. Measurement of total faecal output under grazing conditions has been carried out extensively (Kennedy *et al*, 1959; Holmes *et al*, 1961, Minson and Kemp, 1961 and Le Du and Penning, 1982). Dung bags and separators (faeces/urine) are fitted to the animal several days before the collection day to allow the animal to get used to the harness.

Since much time is spent in collecting faeces, this method is generally regarded as time consuming, expensive and impractical under some situations (Brisson, 1960). In a

somewhat subjective way, Cordova *et al* (1978) estimated that about 70 person-hours of field work were needed to obtain each individual faecal output measurement. This figures seems high, but is logical if all details of the method are considered and carried out to perfection. Besides constant changing, weighing and cleaning of faecal bags, other problems frequently arise, for example, supervision and rearranging of the harnesses to prevent faecal loss (Cordova *et al,* 1978).

A negative feature related to the faecal collection apparatus is its possible adverse effects on animal performance and behaviour (Corbett, 1960), for example reduction in liveweight gain, possibly due to lower intake and higher energy expenditure, was reported by Meijs (1981). However, Greenhalgh and Corbett (1960) bagged steers for at least 50 days and indicated that they showed no discomfort but later (Baker, 1974 cited by Meijs, 1981) found that distortion of hind legs in sheep was due to extra weight of faeces in the bags.

In view of objections raised against this method for estimating faecal output, and eventually intake, it may be concluded that a much less cumbersome procedure should be found for measuring faecal output particularly in situations where labour supply is critical.

1.1.2.2.1.2 Markers are as well termed indicators, tracers or reference materials which are used in the qualitative or quantitative indirect estimation of faecal output in animals.

When markers are used the labour requirements for the sampling of faeces may be lower than when total collection methods are used, although the preparation, administration and analysis of the marker could also involved a lot of work.

A known weight of a marker is fed daily to each animal and it is then assumed that this marker is quantitatively

excreted in the faeces (Kotb and Luckey, 1972) or that a constant proportion of the marker fed is excreted (Edin, 1918 cited by Kotb and Luckey, 1972). If a representative sample of the faecal excretion is obtained and analysed for its content of the marker, total faecal production can be estimated from the following equation:

Daily faeces produced (g) =

$$\frac{\text{weight of the marker given } (gd^{-1}) \times rr}{\text{mean concentration of marker in faeces } (gg^{-1}dm)} \quad (2)$$

where rr is the recovery rate of the marker and is:

$$\frac{\text{Total weight of the marker excreted in faeces (g)}}{\text{Total weight of the marker given (g)}}$$

Kotb and Luckey (1972) suggested that before a substance qualifies as an effective nutritional marker it should: (1) be inert with no toxic physiological or psychological effects (2) be quantitatively recovered in the faeces (i.e. neither absorbed nor metabolized within the gastro-intestinal (G1) tract (3) have no appreciable bulk (4) mix intimately with and remain uniformly distributed in the digesta (5) have no influence on G1 secretion, digestion, absorption or normal mortality nor on the microflora of G1 tract (6) have physical-chemical properties (i.e. easy to analyse) and cheap. Markers may either be internal or external. External markers are those which are added to the diet or administered orally. They include polymers (polyethylene glycol-PEG, plastics, glass and rubber); cells (Yeast and bacteria); charcoal; mineral salts (Barium sulphate) and metal oxides (Cr_2O_3, Fe_2O_3, TiO_2). These were fully discussed by Kotb and Luckey (1972) only a detailed discussion is specifically given to chromic oxide.

Chromic oxide (Cr_2O_3)

Chromic oxide or chromium sesquioxide is green in colour and practically insoluble in water, alcohol and acetone, but slightly soluble in acids and alkali. Chromic

oxide, among the external markers, is the most satisfactory for estimating faecal dry matter output and was first suggested by Edin (1918) cited by Kotb and Luckey (1972).

Factors Influence Accuracy of Using Chromic Oxide

The accuracy of the use of Cr_2O_3 in the estimation of faecal output depends largely on the fact that the marker is quantitatively recovered (the quantity of Cr_2O_3 excreted in the faeces is exactly the same as the quantity fed over a given period). Failure to attain a faecal Cr_2O_3 recovery 'close' to 100% might be due to lack of faecal representative sample for Cr_2O_3 analysis (Stevenson, 1962) and the errors from the type of analytical technique employed (Costigan and Ellis, 1987). Other factors which might result in lower or higher than 100% Cr_2O_3 recoveries are discussed in detail.

1. Period from Dosing to Start of Sampling

An incomplete recovery of Cr_2O_3 could be due to the period from dosing of capsule until start of faecal sample collection being too short for Cr_2O_3 release to attain equilibrium. When Cr_2O_3 was incorporated into the rations, a period between 2-5 days following the first ingestion of the index material was found to be necessary before faecal collection to establish a steady state of Cr_2O_3 in the gut and ensure a quantitative recovery of the marker (Crampton and Lloyd, 1951; Johnson, Dinusson and Bolin, 1964). Some workers have tried a pre-faecal collection period of up to 17 days (see Table 1.1) when Cr_2O_3 was administered in a number of different forms. In other trials (Border, Harris and Butcher, 1963) suggested that 6 to 7 days were required to reach an equilibrium while R.H. Laby (Pers. Comm.) recommended 5-7 days for CRDs to attain a steady release.

The time required for excretion of Cr_2O_3 to attain equilibrium appears to be influenced, among other factors, by level of intake, variation between animals and by characteristics of the feed itself; as the rate of excretion

Table 1.1 Mean Cr_2O_3 recovery and diurnal fluctuations of the marker in faeces.

First Author	Animal	Management	Food	Time of Feeding	Form of Cr_2O_3 Administration	Time of Dosing	Recovery Cr_2O_3 % Minimum	Maximum	Measured Total Collection	Estd. from Grab	Pre-dosing Period (days)	
Hardison (1953)	3 Steers	Grazing	-	-	10g capsule	07.30	12.00h 50	18.00h 180	100.2	99.9	(7)	11
	3 Steers	Indoors	Forage	06.00 1600	10g capsule	07.00	08.00h 80	16.00h 130	101.2	104	(7)	11
Smith (1955)	3 Cows	Grazing	Alfalfa	-	10g capsule	07.00	14.00h 65	24.00h 141	97.5	100.6	( )	7
Hardison (1956)	4 Cows	Indoors	Conc.	06.00 14.30	15g capsule	06.00	12.00h 91	16.00h 111				
	4 Cows	Indoors	Conc	06.00 14.30	7.5g capsule	06.00 14.30	97	103				
Davis (1958)	7 Cows	Indoors	Conc		10g capsule	09.00	02.08h 76	12.06h 119		102	( )	24h
	8 Cows	Indoors	Conc		5g capsule	09.00 21.00				93	( )	24h
Kane (1952)	3 Cows	Indoors	Conc	04.30 13.30	In diet	04.30 13.30	17.00h 94	09.00h 108			Data from Hardison (1953)	
Putnam (1958)	4 Cows	Indoors	Forage & Conc.		20g capsule	02.00 14.00	14.00h 85	124		99.6	(10)	2
McGuire (1966)	6 Steers	Indoors	Conc.	08.00 6 times/ day	0.5% in diet		04.00h 94	18.00h 106				
	6 Steers	Indoors	Conc.		0.5% in diet		14.00h 94	22.00h 105				
Cowlishaw (1963)	2 Steers	Grazing			20g oil	09.00				99.1	From sward (5)	10
	2 Steers	Grazing			20g sch paper	09.00				94.4	(5)	10

Table 1 (cont.)

First Author	Animal	Management	Food	Time of Feeding	Form of Cr_2O_3 Administration	Time of Dosing	Recovery Cr_2O_3 % Minimum	Maximum	Measured Total Collection	Estd. from Grab	Pre-dosing Period (days)	
Raymond (1955)	4 Wethers	Indoors	Alfalfa		1g capsule	10.00 17.00	18.00h 85	08.00h 120				
	5 Wethers	Grazing	-		1g capsule	10.00 17.00	14.00h 70	04.00h 130				
	5 Wethers	Indoors	Forage		1g capsule 1g Bentonite	10.00 17.00			99.8		(8)	12
	5 Wethers	Indoors	Forage		Drench	10.00 17.00			96.80		(8)	12
Pigden (1956)	Wethers	Grazing			10g capsule	once/day	45	180	87		(10)	4
	Wethers	Grazing			5g capsule	twice/day	65	135	95		(10)	4
	Wethers	Grazing			10/6g capsule	6 times/ day	98	104	101		(10)	4
	4 Wethers	Indoors			5g capsule	twice/day			97		(4)	10
Barnicoat (1945)	2 Lambs	Indoors	Ewes Milk		2g capsule	3 times/d			99.2		(4)	5
	2 Calves	Indoors	Separated cows milk		2g capsule	3 times/d			96.9		(5)	5
	1 Calf	Indoors	Separated cows milk + mixed meal		2g capsule	3 times/d			92.0		(5)	5
	2 Wethers	Indoors	Pasture silage		2g capsule	3 times/d			91.0		(8)	5
Lancaster (1953)	3 Cows	Grazing			7.5g capsule	06.00 16.00			101.3	101.1	(5)	40
Scaut (1961)	3 Wethers	Indoors	Forage		1g capsule	07.30 07.30			90.9		(5)	18
	3 Wethers	Indoors	Forage		1g capsule	16.30			98.5		(4)	18
	3 Wethers	Indoors	Forage		2g capsule	07.30 07.30			97.8		(4)	18
	3 Steers	Indoor	Forage		10g capsule	16.30 07.30			97.5		(4)	18
	8 Steers	Grazing	-		10g capsule	16.30			94.4		(8)	32
	8 Steers	Grazing	-		20g capsule	07.00			95.1		(6)	20

Table 1 (cont)

First Author	Animal	Management	Food	Time of Feeding	Form of Cr_2O_3 Administration	Time of Dosing	Recovery Cr_2O_3 % Minimum	Maximum	Measured Total Collection	Estd. from Grab	Pre-dosing Period (days)	
Hardison (1959)	8 Heifers	Indoors	Alfalfa forage		7.5g capsule	07.00 16.00			89.4	89.7	(4)	7
	6 Heifers	Indoors	Hay		1.5g capsule	07.00 16.00			97.5	96.4	(3)	6
	3 Cows	Indoors	Conc.		2.5g capsule	07.00 16.00			104.3	101.0	(3)	5
	3 Cows	Indoors	Conc.		7.5g capsule	07.00 16.00			82.9	87.4	(3)	5
	3 Cows	Indoors	Conc.		22.5g capsule	07.00 16.00			82.7	80.7	(3)	5
Troelsen (1965)	2 Wethers	Indoors	Long Hay	08.00 16.00	2g sch paper	09.00			96		Collection cont'd for several days after last administration	
	2 Wethers	Indoors	Pelleted Hay	08.00 16.00	2g sch paper	09.00			93-98			
Lambourne (1957b)	3 Wethers	Indoors	ad lib rye grass	08.30 17.30	1g capsule	08.30 17.30			115.3		(5)	10
	3 Wethers	Indoors	1/2 ad lib rye grass	08.30 17.30	1g capsule	08.30			104		(5)	10
	3 Wethers	Grazing	rye grass	-	1g Monst. Blue	07.00 16.00			102	99	(4)	14
Corbett	4 Wethers	Indoors	Chopped dried grass	07.00 11.30 19.00	1g oil	10.00			98.5		(15)	12
	4 Wethers	Indoors	"	"	1g rolled paper	10.00			99.5		(15)	12
	4 Wethers	Indoors	"	"	1g sch paper	10.00			98.6		(15)	12
Elliott (1960)	4 Wethers	Grazing	-	-	1g capsule	07.00			99.9		(5)	15

Table 1 (cont)

First Author	Animal	Management	Food	Time of Feeding	Form of Cr_2O_3 Administration	Time of Dosing	Recovery Cr_2O_3 % Minimum	Maximum	Measured Total Collection	Estd. from Grab	Pre-dosing Period (days)	
Kane (1953)	3 Cows	Indoors	Forage		15g in diet				98.1		(10)	4
Carneiro Viana (1959)	8 Wethers	Indoors	Chopped forage	08.00 16.00	2g capsule	08.00			99.1		(17)	10
	8 Wethers	Grazing	-	-	2g capsule	08.00			100.4		(17)	10
Crampton (1951)	2 Ewes	Indoors	Conc.	once/day	1% in diet				97		(6)	13
	2 Ewes	Indoors	Hay	once/day	1% in diet				86		(6)	13
Murdock (1957)	3 Heifers	Grazing	-	-	8g capsule	06.00 16.00			94.2		(10)	4
Curram (1957)	8 Pregnant cows	Indoors	Hay + Conc.	07.15 15.15	10g oil	07.15 15.15			89.8	90	(10)	6
	8 Pregnant cows	Indoors	Hay + Conc.	07.15 15.15	0.9% in diet	07.15 15.15			101	99.5	(15)	6
	8 Wethers	Grazing	-		1g oil in diet	15.30			88.8		(10)	8
	8 Wethers	Grazing	-		"	15.30			98.5		(10)	8
Christian (1965)	12 Wethers	Indoors	Dried Grass	08.00 13.00	2g cardboard capsule	08.00 13.00			92.8		(6)	66
Johnson (1964)	6 Wethers	Indoors	Alfalfa Pellets	07.00 19.00	0.25% paper in diet				101.8		(15)	5
	6 Wethers	Indoors	Alfalfa Pellets	07.00 19.00	0.24% powder in diet				93.3		(15)	5

* Numbers in brackets dosing period prior to faeces collection

After Valderrabano (1979)

of the marker is affected by the rate of passage of ingesta through the digestive tract (Lambourne 1955, 1957a; Cowlishaw and Alder, 1963 and Troelsen, 1965b).

2. Other sources of Errors

Low recoveries of Cr_2O_3 could be due to losses of Cr_2O_3 resulting from small amounts being converted to soluble chromium salts (Christian *et al*, 1965) or by a small amount of soluble chromate present as an impurity in the original dose being absorbed in the digestive tract of the animal (Moore, 1959). He further reported that 48 h after cessation of feeding, traces of Cr_2O_3 could still be found in the guts of wethers while, Deinum, Immink and Deijs (1962) cited by Valderrabano (1979) found traces of Cr_2O_3 in liver, kidneys and lymph glands of cows.

Losses of finely divided Cr_2O_3 particles may also arise during the process of grinding dried faeces (Stevenson, 1962).

Different methods for Cr_2O_3 determination in faeces were reported by Schurch *et al* (1950), Bolin *et al* (1952) and Christian and Coup (1954). Later the methods were further elaborated (Williams *et al*, 1962, and Fenton and Fenton, 1979) while Costigan and Ellis (1987) improved the procedure to be suitable for analysis of Cr_2O_3 derived from Controlled Release Devices.

Methods of Reducing Errors

A number of attempts were made to minimise the errors in the estimation of the mean marker concentration due to the variation of Cr_2O_3 excretion pattern.

1. Forms of Cr_2O_3 Administration. Different forms of Cr_2O_3 have been examined in an attempt to provide a more uniform flow of marker through the digestive tract and the latest ones are discussed.

Sustained Release pellets. These were made from a mixture of Cr_2O_3 and plaster of Paris at different proportions (50:50 and 20:80) and were reported by Pigden and Brisson (1957). These pellets reduced the diurnal fluctuations of Cr_2O_3 in faeces. Later it was found that they were easily regurgitated by animals both at pasture and indoors (Corbett *et al*, 1960).

Paper impregnated with Cr_2O_3. By impregnating Cr_2O_3 into a shredded paper, Corbett, Greenhalgh and McDonald (1958) found that when these were administered to cannulated sheep, the flow of the marker through the duodenum was more regular than when Cr_2O_3 was administered in gelatin capsules. Also problems in the gelatin capsules were lately reported by Hoogendoorn (1986). The irregular excretion of Cr_2O_3 when administered in capsules was probably due to the rapid passage of Cr_2O_3 to the omasum without being properly mixed with the food in the reticulo-omasum. The variation is further increased if the pattern of feeding is irregular (Langlands *et al*, 1963a). It had earlier been observed (Corbett *et al*, 1960), that the variability of Cr_2O_3 concentration was lowered when shredded paper was used than when a single sheet of rolled paper was used as a carrier. The major advantage of using paper as a carrier rather than an oil base or powder is, that any regurgitation of the dose can be readministered back to the animal without any loss of the marker.

Incorporation of marker into feed. This method is most suitable when animals are individually fed known quantities of feed (usually concentrates).

Foliar application of Cr_2O_3 was investigated by Harris *et al* (1967) by spraying a mixture of Cr_2O_3 and an adhesive material with a wetting agent over an ungrazed area of pasture. The results were not favourable in that there was an over estimation of the ground cover (pasture density) which affected marker distribution across the plots and therefore marker concentration on individual plants.

Controlled Release Devices (CRDs). This is the latest technological development (Laby, 1980 and Laby *et al*, 1984) in which Cr_2O_3 incorporated in the CRDs is continuously released from the carrier matrix into the digesta and was found to be more uniformly distributed in the faeces collected within the day. The structure of the device, content of the matrix, and the mechanism is discussed.

Device structure. The device construction was fully described by Ellis *et al*, 1981 and Harrison *et al*, 1981, 1982). It consists of a cylindrical barrel with a core of dissoluble matrix comprising of powdered biologically active agent and a carrier agent mixed and compressed into shape. The plastic barrel dimensions of the present device are 1.5 cm diameter x 9.0 cm long (Fig. 1.1). The core of matrix is held against a dissolution orifice at one end of the plastic cylinder by the action of a spring plunger compressed (25 cm long with 185 g force at 75% compression) at the opposite end.

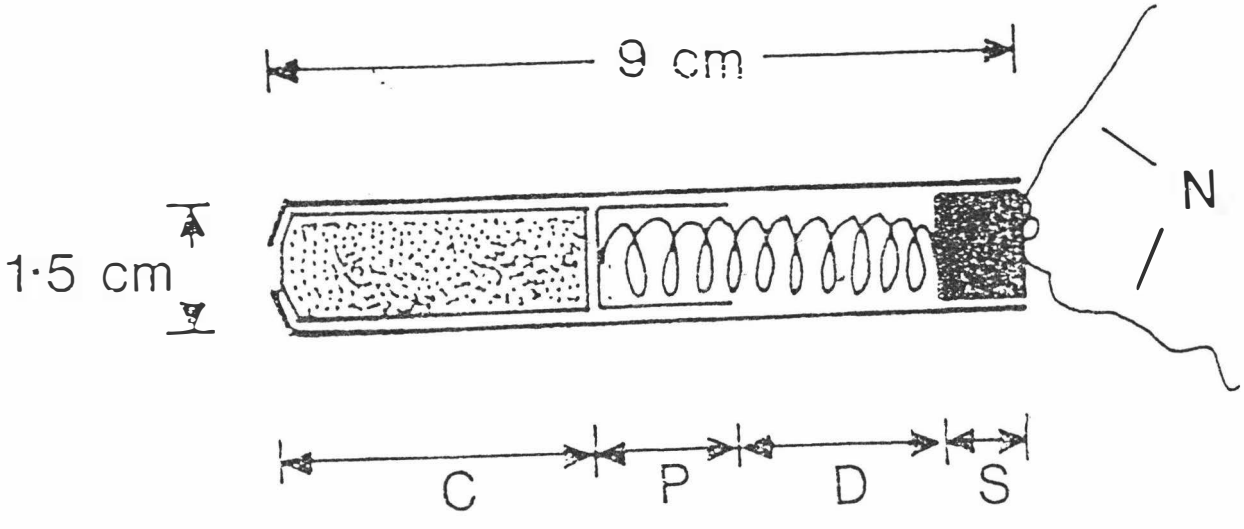

Fig. 1.1 Slow-release capsule of chromic oxide in sucrose mono-stearate (C = chromic oxide mixture, P = plastic plunger, D = daily measurement of plunger movement, S = sealed rubber plunger, N = nylon threads.

Content. According to Harrison *et al* (1981) chromic oxide (65% (w/w) is mixed with sucrose monostearate (50:50 w/w) and the mixture packed into a barrel of a 10 ml plastic syringe with a central nozzle of an orifice diameter of 9.53 mm. The release of the marker may be assessed by daily measurement of plunger movement and was fully described by Harrison *et al*, (1982).

Mechanism. The device has a constant release rate which is controlled by (a) spring strength (b) orifice diameter (c) matrix composition (Laby *et al*, 1984). In rumen fistulated sheep the device can be retained in the rumen using nylon filament anchored to the cannula (Harrison *et al*, 1981). In intact animals the filament is replaced by an elastic strip of plastic (8 x 100 mm) which is folded against the cylinder for oral dosing and which opens in to a "T" configuration for ruminal retention of the device.

2. Faeces Sampling Methods

Grab sampling

Another approach towards minimising errors involved in marker concentration fluctuations diurnally is the use of grab sampling method. The accuracy of using grab samples depend on the number of samples taken per day. The methods are fully discussed by Valderrabano (1979). It was suggested that grab samples should be taken at times when the concentration of Cr_2O_3 is similar to the mean daily value (see Table 1.1).

Frequency of Grabbing. Lambourne (1955) pointed out that a better estimation of the mean marker concentration could be established by grabbing twice daily compared with once per day. It was suggested (Pigden and Brisson, 1956; Hardison *et al* 1956; Balch *et al*, 1957 and Davis *et al*, 1958) that dosing and grabbing could take place in a single operation twice daily. They concluded that an unbiased estimate of the mean marker concentration would be obtained

by dosing at 9 and 15 h intervals and taking grab samples at the same time. Some workers (Pigden *et al*, 1956) have dosed Cr_2O_3 6 times/day (Table 1.1) in a move to try and equilibriate the mean Cr_2O_3 concentration within the day.

Ring Sampling

Another method of sampling is to collect from a number of defeacations voided inside a number of rings ($4m^2$) distributed at random on the sward (Raymond and Minson, 1955). Because of the more random nature of this technique than grab sampling at fixed times, the bias introduced in the estimation of faecal output is considerably reduced. Certainly the technique is relatively laborious and errors could result from differences in climate each time insects and wild animals dwelling in the area; these factors should be taken into account when the technique is put into use (Langlands, 1975). Coloured polystyrene granules (Minson *et al*, 1960) could be used for identifying individual defeacations of cattle.

Minimization of bias in sampling method.

Some bulking procedures should be employed in order to compensate for short term errors (Langlands *et al*, 1963a) introduced in estimating faecal output due exclusively to the sampling procedure. Also sample preparation process (grinding of the samples) for chemical analysis may result in some Cr_2O_3 losses which can be identified by adding known quantities of Cr_2O_3 to faeces and then check the quantitative recovery and therefore avoid bias (Stevenson, 1962).

1.1.2.2.2 Digestibility Estimation

Introduction

The estimation of grazing intakes from the equation (1) ($I = \frac{E}{1-D}$) involves determination of the indigestibility factors (I-D) which consequently requires the determination of the digestibility of the herbage consumed.

Direct measurements of digestibility of herbage harvested mechanically and hand-fed to confined animals can be accurate but they do not necessarily evaluate the 'actual' forage consumed by the animal grazing the same pasture. This is due to differences in selection of specific plant species or specific parts of the same plant, a habit which greatly influences herbage digestibility.

Before the use of oesophageal or ruminal fistulae procedures, the most commonly used methods for estimating digestibility of the diet selected by grazing animals were the marker-ratio techniques and faecal-index methods. These two will be reviewed first and then followed by techniques using fistulated animals and finally the *in-vitro/in vivo* methods.

(1) Marker-ratio techniques

In this method digestibility is calculated from two relative contents of a naturally indigestible component contained in the herbage grazed and excreted together with faeces (Kotb and Luckey, 1972 and Meijs, 1981). If the amount of indicators in the consumed herbage is completely indigestible, then the amount of the marker excreted must be equal to the amount of marker ingested:

$$\text{Herbage intake (I)} \times i_h = \text{Faeces output (F)} \times i_r$$

where, i_h and i_r are the % of indicator in feed and faeces respectively, and the ratio

$$F/I = i_h/i_f, \text{ hence the digestion coefficient } D = (1 - i_h/i_r)\ 100. \text{ (Meijs, 1981)}$$

Several plant components, such as lignin, silica, n-alkanes and chromogens, have been used as internal indicators and they are discussed in detail in the following sections.

Lignin This is a group of substances present in a plant cell wall which is insoluble in a solution of 72% sulphuric acid. This fraction of the plant was thought to be

completely indigestible by the ruminants (Crampton and Maynard, 1938).

The use of lignin as an internal indicator in animal nutrition studies was first suggested by Ellis *et al* (1946).

The recovery of lignin can be tested in experiments where the herbage is hand-fed to animals indoors in combination with total collection of faeces. With this technique an incomplete recovery of lignin varying from 87-96% was found by Kane *et al*, (1953); Ely *et al* (1953); Ellam and Davis (1961) and Ellam *et al* (1962). These low recoveries may be attributed to differences in the lignin content of dietary and faecal samples resulting from drying the samples with excessively high temperature (Van Soest, 1963). Also low lignin recoveries could result from loss through digestion. Barry and Manley (1984) found the apparent digestibility of lignin to vary between 6 and 18%.

Chromogen. The use of chromogen (plant pigment) as an internal indicator was first suggested by Reid *et al* (1950). They extracted chromogens from feed and faeces with 85% aqueous acetone and estimated their concentration by spectrophotometry.

Chromogen recovery can be tested indoors with total collection of faeces. An average of 100.5% of chromogen substance was recovered in 36 conventional digestion trials with sheep and cattle fed on forages and silage (Reid *et al*, 1950; Irvin *et al*, 1953 and Kemmink and Dijkistra, 1968 cited by Meijs, 1981). An incomplete recovery of 92% was reported by Meijs (1981). These differences in recoveries were said to be attributed to analytical errors particularly incomplete extraction of herbage pigments. Chromogen is said to be a pool of different materials and products subject to transformations in the digestive tract, its indigestibility is therefore depending on animal as well as type of diet due to the possible transformation of pigment in the forage with growth stage or even during the feed making process (in case of hay or silage).

n-alkanes These are carbon chains present in herbage species mainly in the cuticular wax, and they have been suggested (Mayes and Lamb, 1984) as internal markers because they are indigestible, and relatively easy to analyse. Those contained in herbage species are predominantly odd-chain in the range of C_{27}-C_{35} which include nonacosane (C_{29}) hentriacontane (C_{31}) and tritriacontane (C_{33}) being the most abundant while pentatriacontane (C_{35}) is relatively less abundant (about 12 mg kg^{-1}dm) which may limit the accuracy of digestibility estimation. C_{29}, C_{31} and C_{33} have the concentration of about 5-9 times higher than C_{35}.

To minimise errors in digestibility estimation due to between-animal variation in alkane recovery in faeces, n-alkanes of similar chain length were dosed to the animal and then compared with the naturally (odd-chain alkanes) occurring ones in the herbage.

Intake calculation is done from a pair of a natural dietary alkane (i) and a dosed alkane (j) as follows:

$$\text{herbage intake (kg dmd}^{-1}) = \frac{\frac{Fi}{Fj}(Dj + 1c.Cj) - 1c.Ci}{Hi - \frac{Fi}{Fj}.Hj}$$

Where Hi, Ci and Fi are the respective concentrations ($mgkg^{-1}$dm) of the natural odd-chain alkane in herbage, concentrate and faeces; Hj, Cj and Fj are the respective concentrations ($mgkg^{-1}$dm), of the even-chain alkane in herbage, concentrate and faeces; 1c is the intake of concentrate ($kgdmd^{-1}$) and Dj is the amount of alkane j, dosed by pellet (mgd^{-1}). Table 1.2 shows the results of the work of Mayes *et al* (1986) regarding intake estimated by using n-alkanes.

Table 1.2 Estimates of daily herbage DM intake (g) using n-alkanes

Lamb	Treatment	Actual herbage intake	Herbage intake estimates*				C_{35}-faeces output
			C_{27}-C_{28}	C_{29}-C_{28}	C_{31}-C_{32}	C_{33}-C_{32}	
1	G LA	504	502	505	495	515	460
2	G LB	485	443	465	472	484	459
3	G MA	676	624	632	629	665	654
4	G MB	657	594	630	634	664	565
5	G HA	896	813	826	830	863	835
6	G HB	909	842	866	862	902	853
7	GCLA	354	328	352	341	355	340
8	GCLB	351	298	340	336	353	350
9	GCMA	488	505	492	477	490	421
10	GCMB	483	420	451	439	466	460
11	GCHA	515	488	530	507	539	478
12	GCHB	631	573	621	618	656	582
Discrepancy (actual intake - calculated intake):							
	(discrepancy)*		32012	11374	12324	2788	27705
	Mean discrepancy		44	20	26	0	41

* First four columns represent intake estimates using alkane pairs: natural alkane-dosed alkane. Fifth column represents intake estimate using C_{35} as an indigestible internal marker.

Little is known of their fate in the ruminant digestive system. It is not known if dietary alkanes remain attached to particular material or enter the liquid phase of the digesta.

The use of dosed alkane and an adjacent shorter odd-chain herbage alkane would be expected to give a slight underestimation of herbage intake, whereas the use of longer odd-chain adjacent alkane should slightly over-estimate intake. Thus a mean of two estimates should give an estimate of herbage intake close to the true value.

The concurrent dosing with C_{29} or C_{32} alkanes and Cr_2O_3 enables both herbage intake and diet digestibility to be estimated in the same animal. However, hexatriacontane (C_{36})

is an alkane not present in the herbage. If it were to behave according to the pattern of recovery of other herbage alkanes, it should be completely recovered and so can substitute for Cr_2O_3 and, if dosed together with C_{28} or C_{32}, would allow measurements of intake and digestibility.

Silica. Wildt (1874) cited by Kotb and Luckey (1972) suggested silica in plants as an internal marker for nutrition studies. For a pelleted ration, Jones and Handreck (1965) suggested that reliable estimates of digestibility could be obtained with silica if precautions are taken to prevent contamination. It was shown that urinary silica was less than 1.8% of the ingested silica even though the silica content of the diet was abnormally high. The recovery of silica in these faeces was close to 100%.

In another development, Van Dyne and Meyer (1964a) found that there was a temporary accumulation of silica in the digestion tract of grazing animals which contributed to variable recoveries apart from variation arising from inadequate method of analysis which is based on determining silica as an acid insoluble ash (AIA).

Errors in estimation of digestibility by means of the silica ratio technique can arise from dust (Gallup, Hobbs and Briggs, 1945), soil contamination (Van Dyne and Meyer 1964a; Kellaway, 1969) or losses of silica in urine. The excretion of silica in urine is not necessarily proportional to that ingested (Forman and Sauer, 1962).

Limitations of the indigestible Marker Ratio Method

Although internal indicators have been widely used, a number of workers have questioned their adequacy because of uneven distribution of the internal indicator throughout the plant (Elliot and Fokkema, 1960a). Workers noted a variable chemical composition with stages of growth and even time of harvesting (Kivimae, 1960) which had a marked influence on the amount of internal indicator. Analytical procedures for determining indicator content in both feed and faeces are laborious and subject to bias.

For these reasons the indicator methods are infrequently used, and also because of the difficulty involved in accurately sampling the herbage consumed, which is necessarily subjective and thus potentially biased (Raymond, 1954).

(2) The Faecal Index Technique

This technique involves the prediction of digestibility from the composition of faeces. A series of conventional digestion trials are conducted in which forage of varying digestibilities are fed to animals indoors. After measuring the content of the internal marker in the faeces (eg. N) an equation was developed which shows the best relationship between the content of the marker in the faeces and the digestibility. A simple equation of faecal index proposed by Holmes (1980) is as follows:

$$OMD - 0.4 + 0.1 \text{ (g N kg}^{-1} \text{ faecal DM)}$$

where

OMD = organic matter digestibility
N = nitrogen
DM = dry matter
Kg^{-1} = per kilogram

Blaxter and Mitchell (1948) showed that the faecal nitrogen excretion was related to DM consumption. Later Lancaster (1954) developed the faecal nitrogen index (FN1 = 1/(1-OM) digestibility) which describes the dependence of faecal nitrogen concentration on food OM digestibility.

However, the use of nitrogen as an indicator of digestibility has been criticised since a large proportion of faecal nitrogen is of microbial origin (Van Soest, 1982). If a faecal index is used to estimate digestibility, it should be based on control animals which do not receive any supplement but graze on the same pasture. This method does not account for possible associative effects of the supplement on the digestibility of the pasture.

To minimise errors in the estimation of the digestibility of grazed pasture, the N regression must be derived with material similar to that selected by the animal when grazing (Langlands, 1967b) since general relationships have been shown to be imprecise as a result of effects of season and fertiliser treatment on the relationship between faecal N and digestibility.

The technique is therefore of little use under continuous grazing management where opportunity exists for widespread selection between plants or parts of the plant. It is at its best under strip grazing management in which herbage grazed is equivalent to that harvested by machine for indoor digestibility trials.

(3) Techniques using Fistulated Animals

Animals may be fitted with either an oesophageal or a rumen fistula. These techniques provide a sample of the feed actually selected and ingested by the animal to be collected. This is then used in *in vitro* determinations of digestibility.

Oesophageal fistula The establishment of oesophageal fistulas in ruminants was first described by Torell (1954). An oesophageal fistula basically consists of a longitudinal surgical opening into the oesophagus of the animal which allows samples of masticated herbage mixed with saliva to be collected. This mixture is commonly termed 'extrusa'. The accuracy of the technique for obtaining estimates of the digestibility of the herbage selected depends on the similarity of the extrusa sample and the herbage eaten.

The optimum size of the fistula appears to be between 4-6 cm^2 in order to get an extrusa sample weighing between 100 and 200 g. Large fistulas usually cause swallowing problems and difficult to keep the 'plug' in position.

Rumen fistula A rumen fistula essentially consists of an orifice of the appropriate dimensions made on the rumen wall. A variety of closure devices (Harris *et al*, 1967) have

been developed which can be opened when sampling of rumen contents are required.

In a method described by Cook (1964) all rumen contents are removed before sampling and the rumen flushed with water, then the animal is allowed to graze freely. After a certain period of time, the animal is handled again to remove the rumen content.

(4) The *In-vitro* technique

The two-stage *in vitro* digestion procedure for prediction of herbage digestibility was developed and fully discussed by Tilley and Terry (1963). In this technique samples of dried herbage were exposed to 48h incubation with a buffered rumen liquor containing rumen microorganisms and a further 48h incubation with acidified pepsin (the protein digesting enzyme from the true stomach). The technique contains the essential two-stages necessary to solubilise all the cell contents and simulate biodegradability of cell wall fractions.

The *in-vitro* technique has the advantages of speed, cheapness and precision (Shelton and Reid, 1960) as well as being applicable to forages in all stages of maturity. Its inherent disadvantage lies in its dependence on a supply of rumen inoculum, requiring access to animals fitted with rumen cannulae and fed under controlled conditions to minimise variations in the composition of the rumen liquor. These disadvantages in the two-stage *in-vitro* technique led to the development of an 'enzyme' digestion technique which has an obvious advantage of circumventing the variability of rumen inoculum.

A 'cellulase' Digestion Technique

The use of commercially available cellulase enzyme from fungus (*Trichoderma viride*) has been thoroughly discussed by Johnes and Hayward (1973).

The samples of herbage of known *in-vivo* dry matter digestibility are weighed and the enzyme solution containing

the appropriate amount of cellulase added. Then the containers are incubated for 48h at 40°C and shaken twice daily. After 48h the residues are filtered off on to tared sintered glass filters washed well with water and finally acetone. After drying overnight at 100°C, the residues are weighed and the amount digested calculated as a percentage of the original 200 mg sample.

In-vivo digestibility may, if required, be predicted from the cellulase values from the equation:

$$y = 0.72x + 32.95$$

where, y = % *in vivo* dry matter digestibility
x = cellulase dry matter digestibility

From these calculations, Johnes and Hayward (1973) found that the amount digested with cellulase is, numerically, some 25 units lower than the *in vivo* value for samples of low digestibility and some 15 units lower for samples of high digestibility.

1.1.2.3 Other Techniques

(1) Grazing Behaviour Methods

Feed intake (I) by grazing animals is a function of:

- time spent eating (T)
- the number of bites/unit of time (R)
- average size of each bite (S) (Hodgson, 1982).

Therefore, $I = T \times R \times S$

Each of these components has to be measured in conjunction with the other because grazing activities (grazing, ruminating, lying and standing or walking) sometimes may take place simultaneously (Hoogendoorn, 1986). Recently methods of measuring number of bites and bite sizes have been developed (Stobbs and Cowper, 1972 and Stobbs, 1973a).

Estimates of the time spent grazing may be derived from the continuous monitoring of activity or by using an

internal sampling technique (Hodgson, 1982). The former is likely to be more accurate, but is difficult to carry out unless automatic equipment is available.

The mean rate of biting over 24h or a full grazing period may be calculated from a total grazing time and the total number of bites taken. Rate of biting is normally estimated over a short period of time.

Bite size can be measured with oesophageally fistulated animals when the material eaten is quantitatively recovered at the fistula. With an open oesophagus the recovery can be low and variable. However, when the lower oesophagus of cattle was blocked with a foam rubber plug the mean recovery of organic matter was 95% (Stobbs, 1973a).

Hodgson (1982) added two variables to the above equation. They are total number of grazing bites/day(the product of T and R) and the rate of herbage intake (the product of R and IB, intake/bite). These five variables conveniently describe the ingestive behaviour. Estimation of herbage consumption by grazing cattle using eating behaviour has considerable merits because it is reasonably precise (10.1% cv) could be applicable to a wide range of pasture conditions (Chacon *et al*, 1976), measurements are easily taken and laboratory analyses are minimized.

(2) Liveweight Methods

The technique of weighing animals, before and after feeding to get liveweight change as to estimate intake was suggested by Erizian (1932) cited by Le du and Penning (1982) and used by Allden (1969). It depends on observations made over short periods of time.

The formula below was used:

$$I = (W_{t2} - W_{t1}) - L + F + U + R$$

where, W_{t2} and W_{t1} = Liveweight after and before grazing respectively

L = weight of water drunk

F = weight of faeces produced during period of grazing

U = weight of urine produced during period of grazing

R = loss of weight by respiration and transpiration (CO_2, CH_4 and H_2O) "insensible loss of weight"

The method requires the measurement of voided faeces and urine by harnessing the animals with dung bags and urine collection containers (Allden, 1969). Animals are weighed and then turned out to graze and any water consumption is measured. With very frequent weighing intervals it is not necessary to measure urine or faeces weights separately but these figures can be derived from the changes in animal weight.

There are inaccuracy problems in measuring changes in animal weight due to evaporation (insensible loss of weight) in the field. Therefore (Allden, 1969) made observations on the similar fully harnessed animals without allowing them to graze during the same period. Due to the fact that these animals were subjected to less intensive movement and therefore lower metabolism they were likely to be less active than the grazing ones and this could lead to underestimation of "insensible loss of weight". Allden and Young (1959) had earlier used a different method but Horn (1978) cited by Le Du and Penning (1981) fitted load cells to the feet of cattle by so doing he could continuously monitor the weight change of the animals, and therefore estimate intake.

This technique provided intake data in the form of fresh herbage of unknown dry matter content which is

ambiguous in experiments like pasture productivity or animal nutrition where information on a dry matter or organic matter basis is needed.

It is important therefore, that a representative sample of selected herbage be collected for dry matter or organic matter determination of the diet selection.

The method is useful for short period intake estimation and may be suitable for bite size and rate of biting calculation.

(3) Animal Production Methods

This method offers an alternative to the more demanding techniques based upon pasture measurements or faecal output/digestibility relationships. It involves weighing the animal product and keeping records.

Herbage Intake (HI) is estimated indirectly from the energy requirements for maintenance and production of the animals involved (Em + p) and the total requirement equated with herbage of a given energy concentration (E_n).

$$\text{Thus, } HI = \frac{Em + p}{E_n}$$

Baker (1982)

Maintenance

It is generally accepted that maintenance requirements are proportional to some power (0.75) of liveweight and that they are dependent also on the extent of the animals' activity. The maintenance requirements of grazing animals is higher than that of stall-fed animals. For calculation of dry matter or organic matter intake the energy content of the consumed herbage is necessary, requiring fistulated animals or sward-cutting methods to provide estimates of the selection effect of grazing animals.

The extent to which activity allowances should be increased for grazing animals cannot be stated with certainty. ARC (1980) concluded that estimates made of

energy lost of grazing do not warrant the inclusion of any additional allowance of work of grazing but they did acknowledge the increased needs arising from extra walking, climbing or standing.

Milk

The net energy requirement for milk production is the energy value (EV_L, MJ kg^{-1}) of the milk secreted. This may be estimated for cows from the fat (BF) protein (P) and lactose (L) content (g kg^{-1}) or from fat and solids not fat (SNF) contents (Tyrell and Reid, 1965):

$$EV_L = 0.03840\ BF + 0.02226\ P + 0.01992L - 0.1081$$

or

$$EV_L = 0.0386\ BF + 0.0205\ SNF - 0.236$$

Liveweight Gain or Loss

The quantity of feed eaten reflects the amount available on pasture, and the time it was eaten in relation to the time of weighing, have major effects on fill and observed liveweight. Therefore errors and possible bias in estimates of gains can readily arise from fluctuations in the quantities of digesta in the alimentary tract (Corbett, 1978).

There are several ways of standardizing weighing procedures so that errors and biases are minimized (Grassland Research Institute, Hurley 1961 and Campbell, 1969).

Animals, particularly lactating ones may go through periods of liveweight loss, and adjustments are required to estimate requirements. The average value of body tissue energy for cows has been taken as 20 MJ (MAFF, 1975) 21 MJ (Van Es, 1978) or 25 MJ per kg liveweight (NRC, 1978 and Vermorel, 1978.

1.2 METHOD FOR FEED AND FAECAL CHROMIUM ANALYSIS

INTRODUCTION

It is important that an analytical method that is simple, specific, accurate, rapid and one which requires common laboratory equipment be developed.

Techniques developed for Cr analysis hitherto include atomic absorption spectroscopy (AAS), inductively-coupled plasma emission spectroscopy (ICPES) and the titration method.

Attention will be paid to the commonly used technique (AAS) and a less detailed review will be made regarding the other two in an effort to make a comparison of these methods.

1.2.1 Atomic Absorption Spectroscopy

The method was first introduced by Walsh (1955) cited by Williams *et al* (1962) and later improved by Czarnocki *et al* (1960). Basically Cr concentration in a solution is measured by use of a flaming technique (nitrous or acetylene) as described by Goguel (1970).

1.2.1.1 Procedures In AAS procedures, sample handling is done in one container and this eliminates the need for repeated dilution and relies upon a sulphuric-phosphoric acid digestion and bromide oxidation.

Sample dissolution. Three general procedures used for dissolving samples into solution ready for Cr analysis included Sodium peroxide (Na_2O_2) fusion, perchloric acid ($HClO_4$) digestion (Day, 1954) and oxidation with bromate and phosphoric acid solution (Williams *et al*, 1962)

During the fusion process, Na_2O_2 oxidises Cr_2O_3 to CrO_4^{-1} ions. The best ratio of Na_2O_2 to specimen was found

to be 8:1. However, Mellor and Thompson (1938) cited by Fisher *et al* (1972) pointed out that excess peroxide still remained at a stage of acidification (the conversion by H_2SO_4 of chromate to dichromate) causing reduction of chromate to chromic salts. These workers suggested that a further boiling for 15 minutes decomposed any remaining excess peroxides and that this could be accomplished in 8 minutes in the presence of a catalyst such as ferric ions. Reactions involved in the fusion process are as follows:

$$Cr_2O_3 + 3\ O_2^{2-} \longrightarrow 2\ Cr\ O_4^{2-} + O^{2-}$$

$$2CrO_4^{2-} + 2\ H^+ \longrightarrow Cr_2O_7^{2-} + H_2O$$

(Fisher *et al*, 1972)

Perchloric acid digestion is not widely used because saturated perchlorate usually spray unsatisfactorily (Williams *et al*, 1962), also crystallization of this salt in the atomizer could easily lead to errors and delays in analysis.

Bromate-phosphoric acid oxidation has been preferred as compared to the other two dissolution methods because of its greater simplicity. During the process chromium (III) is oxidized to chromium (VI).

Cr (III) ⟶ Cr (VI)

green insoluble — soluble orange dichromate form

It is a procedure based on that of Christian and Coup (1954) involving digestion of the ashed sample with phosphoric acid-manganese sulphate solution and potassium ($KBrO_3$) later the procedure was well elaborated by Williams *et al* (1962) and slightly modified by Costigan and Ellis (1987). Wet digestion method of Coup and Lancaster (1952)

was also modified on similar lines by Christian and Coup (1954) largely because of its low daily output. This was due to long boiling periods necessary because Cr_2O_3 was not readily attacked by the oxidizing agent at a temperature of the diluted acid mixture. Some workers (Williams *et al*, 1962) carried out digestion with half quantity of the acid mixture (H_2SO_4 + orthophosphoric acid and manganese sulphate) together with one fifth of bromate (aqueous solution), and by boiling until water had evaporated and the H_2SO_4 started to fume until Cr_2O_3 was brought into solution but not all the dichromate. A further boiling with a small amount of $KBrO_3$ in a dilute solution was sufficient to oxidise all the chromic compounds to dichromate. The presence of H_2SO_4 in the acid mixture kept the temperature below the critical point where dichromate was reduced to insoluble chromic compounds. Thus the modification reduced the long boiling time to 10-15 minutes.

1.2.1.2 Factors Controlling Rate of Dissolution

Acidity. Bolin, *et al* (1952) found that temperature, digestion time and acidity were critical factors during the digestion with the digest mixture of perchloric-sulphuric acid. A search of literature (Smith, 1935 cited by Day, 1954) showed that hot, concentrated perchloric acid (70 to 85% $HClO_4$, by weight) had reducing as well as oxidizing properties and that the reducing reaction increased directly with the concentration of the acid and the temperature. These reducing properties are assumed to result from the formation of hydrogen peroxide as a decomposition product.

Previously it was demonstrated that addition of H_2SO_4 to Cr_2O_3 digests depressed their optical densities. This was supported by Compton (1952) cited by Czarnocki *et al* (1960) when he designed a study and found a decrease in optical density associated with increasing H_2SO_4 concentration. A

summary of the optical density data is presented in Table 1.3.

The difference in optical densities (OD) resulting from the range of acid molarities encountered in different digests of feed and faeces are not necessarily of sufficient magnitude to introduce major errors in the Cr_2O_3 assay (Czarnocki *et al*, 1960).

Table 1.3 The Influence of Sulphuric Acid on the Optical Densities of Potassium Dichromate Solutions[1]

Molarity[2]	O.D. day of preparation	O.D. 2 days later
0.00	0.422	0.422
0.05	0.403	0.398
0.10	0.388	0.381
0.15	0.378	0.373
0.20	0.368	0.368
0.25	0.360	0.358
0.30	0.358	0.347
1.00	0.299	0.290

1 Wavelength 350 nm
2 Expressed in terms of sulphuric acid

It was noted that some of the solutions showed an appreciable decrease in OD after storage for 2 days (Table 1.3), the decrease being greatest at the highest molarities.

Time of digestion. It was advocated that the digestion of the samples be terminated when they change in colour from green to yellow, orange or red. Day (1954) found that time of digestion was a critical factor when sulphuric:perchloric acid mixtures were employed, whereas Kimura and Miller (1957) reported that time of digestion did not affect Cr_2O_3 recoveries when perchloric acid was the only acid used.

Further results of the work of Czarnocki *et al* (1960) showed that the OD of the solutions removed immediately following the colour change where lower than those of the solutions allowed to digest for a longer period. The mean results of their experiment are presented in Table 1.4.

Table 1.4 The effect of Length of Time of Digestion on the Concentration of Chromium in the diluted digest as measured by Optical Densities at 350 mμ[1]

	Times in minutes[2]						
	0	5	10	15	20	25	30
Feed	0.313	0.425	0.440	0.434	0.427	0.418	0.419
Feed C-3-60	0.331	0.411	0.418	0.414	0.407	0.414	0.398
Feed C-2-60	0.342	0.390	0.402	0.392	0.392	0.395	0.389
Excreta T-3-60	0.319	0.337	0.338	0.331	0.335	0.341	0.340
Excreta C-2-60	0.452	0.477	0.479	0.477	0.471	0.471	0.404
Mean	0.351	0.408	0.415	0.410	0.406	0.408	0.404

[1] Each value represents the mean of three determinations
[2] Time of heating after colour change from green to orange

It is apparent that the OD of solutions removed immediately following the colour change were lower than those of solutions allowed to digest for a longer period. Solutions digested for 5 to 30 minutes following the colour change had comparable OD. Analysis of variance confirmed that interpretation. In view of these findings, digestion for 15 minutes following the colour change was recommended.

Temperature of digestion. Day (1954) stressed that the temperature of digestion could influence the OD of Cr_2O_3 digests while Czarnocki *et al* (1960) employed microburners to heat the digestion flasks and made no comments concerning temperatures.

More studies carried out by Czarnocki *et al* (1960) with a rheostat setting of 0 (lowest heat), 3, 5 and 8 showed mean OD of 0.440, 0.433, 0.422 and 0.434 respectively. Analysis of variance failed to reveal a significant different in OD associated with rheostat settings. It would therefore appear that although temperature variations may influence the Cr_2O_3 assay, such variation would have to be very large to introduce significant error.

Recently Costigan and Ellis (1987) found that rate of heating during the digestion process had some effect on the subsequent results. Too fast a rate would result in sample loss due to "bumping" while a very slow temperature use was time-wasting. A time span of 90 minutes proved to be an adequate compromise using the available equipment.

Figure 1.2 shows the effect of digestion temperature on observed concentrations of Cr_2O_3 determined on faecal samples. There was a temperature (170^{o}C) below which there was incomplete solubilization of the chromium, while at the other extreme (above 240^{o}C), there was an apparent loss of chromium, probably a result of formation of insoluble complexes (Czarnocki *et al*, 1960). Day (1954) had earlier critisized the use of mixtures of two acids because of her observation that time of heating with this mixture needed very careful control in order to avoid errors due to overheating. However, she advised that in case mixtures are to be used, H_2SO_4 should be a part of the mixture because it slows down and moderates the (temperature/time) digestion reactions.

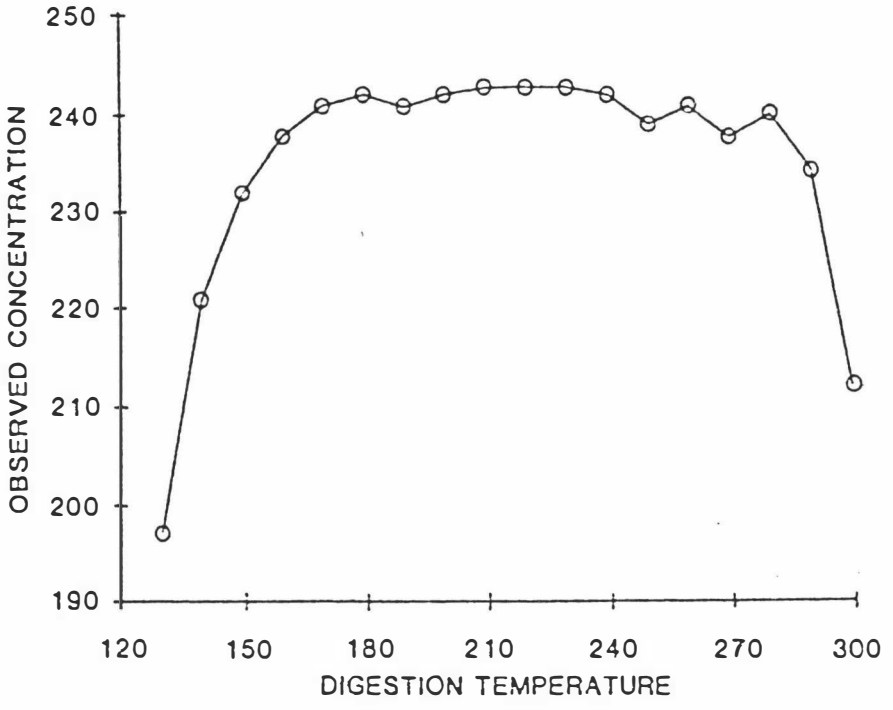

Fig. 1.2 Effect of digestion temperature on observed concentration of Cr_2O_3 determined on a standard faecal sample containing 243 μg Cr_2O_3/g.

1.2.1.3 Recovery Tests on Chromium (III) oxide

As a check on the possible accuracy of any chosen method, recovery time of chromic oxide added to faeces should be made. The mean percentage recoveries from replicate analyses of samples spiked by the addition of known amounts of chromium (III) oxide suspension are indicated in Table 1.5 after the work of Costigan and Ellis (1987). Repeated measurements of blank and low concentration solutions showed the detection limit in solution to be about 0.5 $\mu g g^{-1}$dm of faeces.

In each instance recoveries from the base samples were not practically different from each other within the limits of detection. However, the total recovery (x = 98.6%) was significantly less than 100% possibly because of impurities or some interference (Williams *et al*, 1962).

Table 1.5 Mean percentage recoveries (SD, N=10) of various amounts of Cr_2O_3 added to digestion tubes containing (A) no faecal material, (B) 0.2 g of faeces with no Cr content, and (C) 0.2 g of faeces analysed as containing 243 µg Cr_2O_3/g

Base sample	µg Cr_2O_3 added				
	40	80	120	160	200
A	98.7 (1.1)	98.8 (0.7)	98.3 (0.6)	98.9 (0.5)	98.7 (0.6)
B	98.9 (1.1)	99.4 (0.7)	99.0 (0.9)	98.3 (0.9)	98.0 (0.8)
C	98.3 (1.1)	98.5 (0.7)	98.3 (0.6)	98.5 (0.3)	- -

1.2.1.4 Inhibitors of Cr(III)oxide absorption:

(1) K_2Cr_2O. Although dichromate added to faeces post-ashing could be totally recovered, there is usually a substantial loss of Cr(III) oxide when the spike is added prior to ashing (Costigan and Ellis, 1987).

Table 1.6 A comparison of the use of Cr_2O_3 and $K_2Cr_2O_7$ as analytical spikes added to faeces prior to ashing (spike solution or suspension both containing the equivalent of 40 µg of Cr_2O_3/ml).

Composition of Spike		Recovery (%)	
Vol. of Cr_2O_3 suspension (l)	Vol. of $K_2Cr_2O_7$ solution (l)	Mean (n=4)	SD
1000	0	98.7	1.0
750	250	97.0	0.5
500	500	94.6	1.7
250	750	91.3	0.5
0	1000	90.6	1.9

The effect is demonstrated by the data in Table 1.6. Clearly, the greater the amount of dichromate added prior to ashing, the less was the percentage of total chromium recovered.

(2) Extraneous ions: The influence of extraneous ions likely to be present in plant or faecal material obscure Cr(III) absorption. Previous studies (Yanagisawa *et al*, 1970 and Kitagawa *et al*, 1980) categorised interelement effects in flames as either enhancing (Cu, Al, Mg and Ca); depressive (Na, K, Zn and Si) or selective (Fe).

Further examination of the calcium and magnesium interferences showed that over the range 100-1000 ppm of Ca, chromium absorption showed little change and that in the presence of 500 ppm of Ca, magnesium did not affect the absorption. Supporting results are indicated in Table 1.7 (Williams *et al*, 1962).

Table 1.7 The effect of additional ions on chromium absorption in phosphoric acid-manganese sulphate-potassium bromate solution * in the presence and absence of 500 ppm of calcium.

Ion Added	Concentration (ppm)	Absorption by solution containing: No added calcium		500ppm of added calcium	
		20ppm Cr%	50ppm Cr%	20ppm Cr%	50ppm Cr%
Nil	1	19.5	38.0	23.0	45.0
K^+	200	19.5	38.0	23.0	45.0
Na^+	200	19.5	38.0	23.0	45.0
Ca^{2+}	200	23.0	45.0	23.0	45.0
Mg^{2+}	200	22.5	44.0	23.0	45.0
Al^{3+}	100	21.0	40.5	22.5	44.5
Fe^{3+}	100	19.5	38.5	23.0	44.5
SO_4''	100	20.0	38.5	23.0	45.0
PO_4'''	200	20.0	39.0	23.0	45.0
SiO_3''	100	6.0	14.0	22.5	44.0
Mixture	-	22.5	44.5	23.0	44.5

* 3 ml of phosphoric acid-manganese sulphate solution (30 ml of 10% $^{w}/_{w}$, $MnSO_4.4H_2O$ in 1 l of 85% H_3PO_4) and 4 ml of 4.5% $^{w}/_{v}$ of potassium bromate per 200 ml.

K+, Na+, Ca2+, Mg2+, PO4''' each 100 ppm, Al2+, SiO4'', SO2'' each 50 ppm.

It is thus possible to correct for both Ca and Mg interferences by the addition of sufficient Ca to both 'standards' and 'test solution' to ensure that at least 100 ppm of Ca was present.

Since silicate and aluminium both interfere seriously in flame methods (emission and absorption) for determination of Ca (David, 1959) due to the formation of refractory compounds in the flame, it seemed possible that Ca may prove a satisfactory suppressant for these two interfering ions in Cr(III) oxide absorption.

1.2.2 Comparison of Different Methods

Methods to be compared include the AAS, ICPES and to a lesser extent Titration method.

Instrument sensitivity. Practically all methods seemed to give good precision within batches according to the work of Lee *et al* (1986).

The most suitable range of Cr concentration for analysis was found to be 0.80-70.00 ppm in solution (Williams *et al*, 1962). The upper limit could certainly be increased according to the choice of 'chromium line'. Further results given by Williams *et al* (1962) indicated that by use of AAS, Cr concentration could be detected down to a level of 0.15 ppm. The variability in the AAS measurements arise from a combination of a number of factors: the presence of potential interferences as described by Williams *et al* (1962), Fisher *et al* (1982), Roofayel *et al* (1984) and Lee *et al* (1986); change in oxidation state (Kitagawa *et al*, 1980 and Aggette *et al*, 1981) and inability to reproduce optimised instrumental operating conditions exactly (Lee *et al*, 1986 and Costigan and Ellis, 1987). Of the two AA methods (Nitrous oxide-acetylene flame/air acetylene flame) the nitrous oxide-acteylene flame gives a more consistent calibration curve and it is free from interference.

The ICPES differs from classical flame, spark and arc discharges in the means by which free atoms are 'excited'. Emission spectra from atoms and ions are observed when the sample aerosol is introduced into an inductively coupled radio frequency argon 'plasma' torch. A plasma is a gas ionized to a degree sufficient to have a significant effect on its properties (Lee, 1981). The resulting interaction produces temperatures between 6,000-10,000^{o}K. These temperatures are much hotter than conventional AAS flames, so that much of the sample is vaporized and excited and little if any is subject to chemical interaction. Therefore reasons for excellent sensitivity in ICPES are associated

with high temperature, low background, stable discharge (Greenfield *et al*, 1975) and a minimum sample preparation often encountered in AAS.

The titration method has a relatively high demand for equipment and is usually not straight forward due to large numbers of chemical changes that take place. However, for both AAS and titration methods, chromium in solution must be oxidized to Cr(VI) which is not a prerequisite for the ICPES determination.

Generally AAS has been more popular than other methods because AAS instruments are far more available, the procedure is rapid and do not require continuous observation.

CHAPTER 2

MATERIALS AND METHODS

2.1 EXPERIMENTAL DESIGN

Calves of similar weight and age were brought in doors in groups of four and were arranged in a complete randomized block design with three replicates.

The Controlled Release Devices, containing Cr_2O_3, were administered and animals within blocks allocated at random to one of the following feeding regimes:

1. Pasture only (G)
2. "Soya bean concentrate supplement" and pasture *ad lib* (S)
3. "Powdered milk supplement" and pasture *ad lib* (P)
4. "Liquid milk supplement" and pasture *ad lib* (L)

Within each block an adjustment (preliminary) period of at least 5 days was allowed and then this was followed by 2 collection periods of 5 days each during which intakes were measured and faecal and urine outputs collected.

2.2 EXPERIMENTAL FEEDS

2.2.1 Pasture

Fresh pasture, predominantly ryegrass (*L. perenne*) and white clover (*T. repens*) was cut from paddock No. 34 of No. 3 dairy farm (Massey University) every morning between 07.00 and 08.00 am and afternoon between 01.00 and 02.00 pm. Two separate harvests were made so that only fresh pasture was fed to calves.

The harvesting was done by use of a small reciprocating lawn mower (John Allen & Sons, Ltd, Cowley, Oxford, England). At the beginning of Block II this mower was replaced with a rotary selfloading mower (Masport, Auckland). The latter mower could easily be adjusted to cut the grass regrowth

close to ground level. The harvested grass was at a vegetative stage of growth at all times of harvesting to ensure high digestibility.

2.2.2 Milk

Milk used in the experiment was reconstituted from a fat-fortified milk powder (Ancaf).

Milk substitute powder was mixed with warm water (about 38°C) before feeding (0.52 kg of powder in 3.48 l of lukewarm water) and was fed half in the morning at 08.00 am and another half at 02.00 pm in the afternoon making sure that each half was reconstituted just before feeding.

2.2.3 Concentrate supplements

Three concentrate supplements were formulated as shown in Table 2.1 and fed as follows: soybean mix (1 kg d^{-1}) and milk powder mix (milk powder + carbohydrate) (0.75 kg d^{-1}) and were designed to provide 11.28 MJME and 175g CP for each calf daily basing the calculations of nutritive value on that provided by Holmes and Wilson (1984). The carbohydrate (CHO) mix (0.23 kg d^{-1}) together with liquid milk (4 l of 0.52 kg powder/day) also provided a similar level of nutrients.

Table 2.1 Ingredients used in Supplement Composition

Ingredients	Concentrate Supplements		
	Soybean mix (%)	Milk Powder mix (%)	Carbohydrate mix (%)
Soybean meal (50% CP)	25.0	-	-
Maize meal (8% CP)	20.0	8.4	27.0
Barley meal (11% CP)	50.0	21.1	68.0
Milk Powder (30% CP)	-	69.0	-
Molasses	5.0	1.5	5.0
	100.0	100.0	100.0

The milk powder mix was fed in a powder form while soybean mix and carbohydrate mix were fed in pelleted form. Fresh supplements were provided every morning at 08.00 am and refusals were measured at the same time.

2.2.4 Water

Water was provided *ad lib* in ten litre plastic buckets throughout the day. Calves were taught to drink water from individual buckets placed in each metabolism cage. Water temperature was always close to the room temperature (about 15^{o}C).

2.3 EXPERIMENTAL ANIMALS

Twelve six week old Friesian male calves were weighed using an electronic scale, accurate to $\pm$ 0.5 kg, at the beginning and at the end of each block period. All measurements were done at 08.00 am before feeding in order to minimise the possible errors due to difference in gut fill.

After weighing, the calves were randomly placed in metabolism cages (1.83 x 1.12m) constructed of weld mesh and a metal floor. The room in the Animal Physiology Unit was well ventilated and not temperature controlled.

The relative humidity varied because of the warm water used to wash the floor at different times of the day, however, it did not drop below 65%. Lights in the room were on for 24 h.

Any calves which showed signs of scours were treated immediately by administering "scourban" (Vetc. Products Auckland) drench. No regular drenching with antibiotics was done.

2.4 EXPERIMENTAL PROCEDURES

2.4.1 Chemical analysis

(1) Food

The diets fed were sampled daily. Duplicates of 200 g pasture samples were taken at each time of feeding for both oven and freeze drying (for determination of dry matter and components easily destroyed by high temperature, respectively). The dried pasture and a 50 g concentrate sample were ground through a 1 mm mesh in a laboratory mill (Christy and Norris Ltd. Chelmsford Essex) and stored in airtight plastic containers. Chemical analyses carried out are as indicated in Table 2.2, also pasture samples were incubated *in vitro* to obtain digestibility coefficients.

Table 2.2 Chemical analyses of food

Component	Equipment/Method	Manufacturer	Reference
Dry matter (DM) content	Force draught oven for 12h	Birmingham & Blackburn Constr. Co. Ltd.	AOAC (1984)
Ash content	Muffle furnace 550°C for 12h	W.D. McGregor Ltd	AOAC (1984)
N-content	Kjeltec Auto 1030 Analyser	Tecator AB HÖganäs Sweden	AOAC (1984)
Gross Energy (GE) content	Adiabatic bomb calorimeter	Gallenkamp Towers Ltd	AOAC (1984)
In-vitro digestibility	Cellulase method		Johnes & Hayward (1973)
Chromic oxide	Instrumentation laboratory 457 AA/AE spectrophotometer		ILAA/AE(1981)

(2) Faeces

Daily representative faecal samples Each cage had a flat metal tray placed on the rear half of the cage floor to trap the faeces. At 08.00 am the daily total faecal output for the past 24 h was collected and weighed. Before the faeces were put in the refrigeration room, fresh representative (duplicate) samples (100-150g/sample) were taken, one for oven drying and another for freeze drying. Representative faecal output from each calf were bulked for two-5 day periods within each block.

Grab samples Faecal grab samples were collected either directly from the rectum or the top part of the already defecated dung pat. The latter was necessary when no faecal sample was obtained by stimulating the rectum. Sampling was done once/day (10.00 am) during Block I, three times/day (10.00 am, 01.00 pm, 04.00 pm) during Blocks II and III. After collection, the grab samples were processed as the daily representative samples and grinding was done like the procedure used in feed processing. All faecal samples were analysed for DM content, ash content, and Cr_2O_3 content.

(3) Urine

Urine was collected, via a large metal funnel underlying the wire mesh floor of the cage, into a 5 l plastic bucket containing 40 ml of 5NHCl per 3 l of urine (to minimise loss of nitrogen by ammonia gas). Collection of a 24 h urine sample was done every morning at 08.00 am, a sample (10 ml or 1% of total quantity) was extracted and bulked with previous days sample as to make a composite sample for each calf for a 5-day collection period which was stored in a freezing room ($-10^{o}C$) until when it was analysed for N and GE content.

(4) Chromium(III)oxide

Introduction The standard method for chromic oxide analysis used in this laboratory in the past was essentially that of Costigan and Ellis (1987) and is described as Method I below. Unfortunately the results obtained contained some

unusually large differences in Cr_2O_3 concentration between duplicates and it was suspected that there was limited oxidation and therefore less chromic(III)oxide was actually converted to chromium (VI) a soluble component (see details under Literature Review). Therefore, the analyses were repeated using Method II in which a higher amount of bromate (oxidizing agent) was used, and comparisons were made between the two methods with regard to the repeatabilities between faecal sample duplicates for Cr_2O_3 concentration.

Method I - Low Bromate (LB)

Reagents: All reagents were analytical grade and solutions were in all cases made up in de-ionised and distilled water.

1. Acid digestion mix: 250 ml orthophosphoric acid (85%) $^{w}/_{w}$
   50 ml aqueous $MnSO_4$ $4H_2O$ (10%) $^{w}/_{v}$

Final mixture was: 97.3 ($^{v}/_{v}$)
85% phosphoric acid: 10% $^{w}/_{v}$ $MnSO_4.4H_2O$

2. Potassium bromate: 4.5% $^{w}/_{v}$ $KBrO_3$
   (aqueous solution)

Procedure Two 1.00 g samples of dried faecal material were weighed into tared 25 ml pyrex beakers which were numbered. The beakers and contents were placed into an oven at 105°C to dry for 24 h. After drying the beakers were weighed to determine the dry matter weight and then samples were ashed at 550°C in a furnace for 12 h overnight.

Digestion: The beakers were reweighed to determine the ash weight and then digestion of the ash followed.

- 6 ml of acid digesta were added to the ash in beakers. The heating block was heated to 140°C and beakers were placed in the block while covered with glass to prevent evaporation of the mixture. Boiling was allowed to continue for 20 min.
- The beakers were later removed from the block and allowed to cool to below 100°C, after cooling, 3 ml 4.5% potassium bromate was added to each beaker, and placed

in reheated block with a final temperature of 210°C (at centre). Glass was normally placed over beakers.

- After 45 min the beakers were removed from the block, allowed to cool and the contents transferred into 50 ml flat bottomed conical flasks. Beakers were thoroughly rinsed with distilled water to remove any remaining amount of digestion mixture, then the residue added to flasks and vigorously shaken to dissolve the digestion mixture.
- Flasks were topped with distilled water, by use of a dispenser, resulting to a final volume of 50 ml.
- The flasks were then thoroughly shaken (agitated) and allowed to settle for 24 h.
- About 10-15 ml aliquot was 'poured off' into numbered plastic potteles. By only taking small amounts from the top, a clear sample with minimal suspended material was obtained for AA spectrophotometry.

Method II - High Bromate (HB)

Reagents: Sample preparation and digestion procedures were exactly the same as in Method I, except in Method II 8 ml of potassium bromate (high bromate, $KBrO_3$) were used for oxidation instead of the conventional 3 ml.

Preparation of Standard Solution for AAS calibration

Objective: The use of blank solution on the AAS reading was to compensate for any physical and/or chemical interference arising from the sample digestion or background chromium.

Procedure: Blanks (or chromium free) faecal samples were collected from 4 calves on different treatments (as in previous part of Materials and Methods) just before CRDs were administered. The samples were dried, ashed and digested by using same procedure as for the treatment samples. Then the following amounts of 100 ppm $K_2Cr_2O_7$ solution was added.

Table 2.3 Proportions used in preparation of standards

Standard (Cr^{2+} Conc. ppm)	$K_2Cr_2O_7$ (ml added)
0	0
2.5	1.25
5.0	2.50
10.0	5.00
20.0	10.00

The mixture was then made up to 50 ml volume by the addition of distilled water and thoroughly shaken and allowed to stand overnight. The following morning 15-20 ml were poured off into labelled plastic potteles for AAS reading.

Spectrophotometric Procedures

Background: The 1L 457 AA/AE spectrophotometer was used for determining the concentration of Cr_2O_3 in solutions prepared from faecal samples. The instrument uses an air supply (20% oxygen) and nitrous oxide-acetylene flame (3000°C). Lamp current, slit width and wavelength are as indicated by the Instrumentation Laboratory Inc. (1981).

Standards: The instrument was first calibrated by flaming the standard solutions (as prepared in the previous section) and gauging them against Chemistry Laboratory Standards also fed into the spectrophotometer, through a built in microcomputer, their absorbence was automatically displayed on the keyboard of the spectrophotometer according to the respective standards dilution series (0, 2.5, 5, 10 and 20).

Flaming: After calibration of the instrument the concentration of chromium in each sample contained in the numbered plastic potteles was read by first dipping the capillary into distilled water for a second and then dipping the capillary into the sample solution, long enough (5 sec) to give a steady reading on the keyboard. Sometimes capillary

was removed from the solution, as a precaution, to determine whether the acetylene flow was adequate. In case the flame became fuel-lean by showing signs of going out, an increase of acetylene was made until the flame was maintained and could give a steady reading. Readings of Cr_2O_3 concentration were in microgram Cr_2O_3 per gram dry matter of the sample (μg Cr g^{-1} dm).

Interferences: Cleaning of the burner and recalibration of the instrument was done after each 60-sample run to minimise interferences from other elements which could clog the nebulizer during the flaming process.

2.5 STATISTICAL ANALYSIS

Analysis of variance was done for Cr concentration (High and Low Bromate), Cr Release Rate, Cr Recovery Rates and Cr Concentration (within day sampling times).

Standard errors (SE) for means of Cr recovery rate, DM intakes, DE intakes and Faecal and Urinary energy outputs were calculated.

Regression equations were developed to relate: Nitrogen Retention (NR) and Digestible Energy Intake (DEI); Digestible Energy Concentration and Supplement proportion in the ration.

Statistical Analysis was carried out by using the SPSSX and SAS programme.

The following symbols have been used in this thesis to determine the level of significance of difference between means.

* differences significant at the 5% level of probability
** differences significant at a 1% level of probability
*** differences significant at 0.1% level of probability
NS No significant difference

CHAPTER 3

RESULTS

3.1 ANIMALS AND CRD ADMINISTRATION

The liveweights of the 6-8 week old calves were taken prior to entering the metabolism crates and at the conclusion of the experiment and are shown in Table 3.1. Dates for CRDs dosing in calves in Block I, II and III were 5th May, 9th June and 29th June respectively, and for commencement of collection in the respective Blocks were 19th May, 10th June and 30th June. The majority of calves remained healthy but as indicated in Table 3.1 a total of four calves suffered from scours, all during the first 5-day periods in Blocks I and II. In each case 30 ml d^{-1} of "scourban" (Vetc Products, Auckland) was administered and this quickly controlled the problem.

Earlier, calf No. 6 had regurgitated the capsule while on the field and this was reinserted on 14 May prior to the start of Block I. Two more calves, the heaviest ones in the experiment (Table 3.1) regurgitated the CRDs during the first collection period in Block II and they were reinserted within 6 h.

3.2 CHEMICAL DESCRIPTION OF FEEDS

3.2.1 Herbage

The nutritive value of pasture was assessed by chemical analysis of the pasture samples (procedures of analysis are discussed under Methods) and the results are shown in Table 3.2.

Table 3.1 Experimental details of animals and treatments.

Block	Treatment	Calf No	Age (days at start of Period I)	Liveweight (KG) In	Liveweight (KG) Out	10-day liveweight gain (kg)	Capsule Irregularity	Illnesses or infections
I	S	2	46	67	74	7	-	NIL
	P	7	49	69	73	4	-	Scours from 19-23/5
	L	1	43	71	76	5	-	NIL
	G	6	50	68	70	2	CRD reinserted 14 May in the field	NIL
Means (SE)				68.75 (±0.53)	73.25 (±1.25)	4.50 (±1.04)		
II	S	24	45	65	69.0	4.0	-	NIL
	P	25	43	73	79.2	6.2	-	NIL
	L	26	43	65	74.7	9.7	-	NIL
	G	23	39	56	61.7	5.7	-	NIL
Means (SE)				64.75 (±3.47)	71.15 (±2.89)	6.4 (±1.20)		
III	S	27	53	76	78	2	CRD reinserted 6 July	Swollen knee 8 July Scours 1-3 July
	P	28	57	76	84.6	8.6	CRD reinserted 4 July	
	L	30	54	73	75.5	2.5	-	Scours 1-4 July
	G	29	48	69	73.1	4.1	-	Scours 2-3 July
Means (SE)				73.5 (±1.66)	77.8 (±2.48)	4.3 (±1.50)		

Table 3.2 Chemical Composition of pasture and the *in vitro* (Dry matter) digestibility.

		(SE)	Max. Value	Min. Value
Crude Protein	(%) (Nx6.25)	25.00(0.43)	26.36	23.86
Ash	(%)	10.95(0.36)	12.12	9.87
Gross Energy	(MJ/kg DM)	19.16(0.24)	19.65	18.68
In vitro DDM	(%)	75.42(0.02)	76.99	74.53
**In vivo* DDM	(%)	74.11(1.36)	79.50	70.39

* Measured from "grass only" treatment (G) (n=3).

3.2.2 Supplements

A summary of some characteristics of the supplement including the concentration of dry matter (DM), crude protein (CP), organic matter (OM), ash and gross energy (GE) fed to calves during the trial is given in Table 3.3.

Table 3.3 The mean composition of supplements fed to calves.

Supplement	DM (%)	CP (%DM)	OM (%DM)	Ash (%DM)	GE (MJ kg^{-1})
S	89.32 $\pm$ 0.70	22.29	95.73	4.27	18.74
P	93.15 $\pm$ 0.02	27.00	93.97	6.03	21.80
L	95.97 $\pm$ 0.05	31.01	93.07	6.93	22.60
*CHO	88.18 $\pm$ 0.001	12.19	96.87	3.13	18.35

* Carbohydrate mix

Supplements Offered

The carbohydrate concentrate (CHO) was fed along with the suckled milk (L treatment) but was mixed with the powdered milk for the P treatment. The amounts of supplements fed and the quantities of nutrients offered are shown in Table 3.4

Table 3.4 The quantities of nutrients offered to individual calves in form of supplements per day.

Supplement	As fed (g)	DM (g)	CP (g)	OM (g)	GE ($MJkg^{-1}$)
S	1000	893.20	199.30	855.07	16.73
P	750	698.59	188.59	656.50	15.23
L	750	701.85	179.45	635.30	14.51

The supplements were fed for 5 days (10 days for Block I) prior to the commencement of the two 5-day collection periods.

3.3 FEED DRY MATTER INTAKES

Supplements were offered together with an *ad lib* supply of herbage (G) to each calf.

Table 3.5 shows the average amounts of supplements and herbage actually eaten by calves per day during consecutive 5-day periods (Periods 1 and 2) within each of the three Blocks I, II, III). Herbage intakes were much lower in supplemented treatments than for those fed grass only. Also dry matter intakes were generally higher

Table 3.5 Averages of the actual amounts (dmd^{-1}) of supplements and herbage eaten by calves during the experiment (Means ± SE of 5 days)

Block	Period	Diet	TREATMENTS S	P	L	G
I	1	Grass (g/calf/d)	519.94(± 42.84)	333.83(± 33.30)	439.69(± 31.16)	1134.95(± 71.27)
		Suppl.(g/calf/d)	816.03(± 30.14)	664.80(± 19.11)	678.19(± 0)	-
		Total dm (g/calf/d)	1333.97(± 65.98)	998.63(± 37.73)	1117.88(± 31.16)	1134.95(± 71.27)
		Suppl.proportion (%)	61.17	66.57	60.67	
	2	Grass (g/calf/d)	524.23(± 7.77)	277.58(± 65.75)	219.74(± 71.73)	1308.22(± 86.40)
		Suppl.(g/calf/d)	902.30(± 5.20)	696.99(± 0.00)	678.00(± 0.00)	-
		Total dm (g/calf/d)	1426.49(± 47.74)	973.58(± 65.75)	897.93(± 71.43)	1308.22(± 86.40)
		Suppl.proportion (%)	63.25	71.49	75.51	
II	1	Grass (g/calf/d)	288.84(± 41.06)	508.44(±115.43)	326.28(± 61.83)	713.71(± 40.55)
		Suppl.(g/calf/d)	367.91(± 69.08)	137.43(± 25.91)	625.69(± 26.20)	-
		Total dm (g/calf/d)	656.75(± 77.78)	645.87(± 93.37)	951.95(± 62.21)	713.71(± 40.55)
		Suppl.proportion (%)	56.02	21.28	65.73	
	2	Grass (g/calf/d)	807.72(± 22.20)	951.99(± 23.72)	662.78(± 21.97)	1120.64(± 53.54)
		Suppl.(g/calf/d)	459.73(± 99.21)	245.80(± 54.14)	659.50(± 4.19)	-
		Total dm (g/calf/d)	1267.64(±106.06)	1197.79(± 53.82)	1322.08(± 23.39)	1120.64(± 53.54)
		Suppl.proportion (%)	36.27	20.52	49.88	
III	1	Grass (g/calf/d)	876.22(± 42.87)	922.49(± 58.75)	910.96(± 88.93)	1435.22(±105.14)
		Suppl.(g/calf/d)	747.18(± 36.09)	586.18(± 11.77)	678.22(± 0.002)	-
		Total dm (g/calf/d)	1623.40(± 32.33)	1508.57(± 70.07)	1589.18(± 88.91)	1435.22(±105.14)
		Suppl.proportion (%)	46.03	38.85	42.68	
	2	Grass (g/calf/d)	975.97(± 43.53)	1006.39(± 49.46)	1072.70(± 60.11)	1804.92(± 97.33)
		Suppl.(g/calf/d)	802.70(± 25.56)	665.10(± 9.13)	678.10(± 0.00)	-
		Total dm (g/calf/d)	1778.67(± 51.42)	1671.49(± 46.07)	1750.80(± 60.11)	1804.92(± 97.33)
		Suppl.proportion (%)	45.13	39.79	38.73	
		Treatment means (SE)				
		Grass	665.49(±107.09)	666.79(±135.34)	605.35(±137.74)	1252.94(±148.68)
		Suppl.	682.64(± 88.20)	499.18(± 99.39)	666.28(± 8.67)	-
		Total	1347.82(±158.32)	1165.99(±153.78)	1271.64(±141.24)	1252.94(±148.68)

during the second 5-day collection period than in the first period.

On average the supplements contributed 51(S), 43(P) and 45%(L) of total dry matter intake. The lower values for the P treatment were largely due to a very low intake of dry matter by the calf in Block II.

3.4 ASSESSMENT OF CRD PERFORMANCE

The physical specifications of the CRD provided by Captec Pty Ltd (Manufacturer of Captec Chrome) state that the capsules contained 65% (by weight) of chromium sesquioxide in the matrix.

The weight of the matrix lost from capsules in calves No. 27, 28 and 30 during the trial in a 10-day collection period, was measured by difference in weight. This provided an estimate of a release rate (RR) of 133 mg chromium per day (mg Crd^{-1}). This compares with the expected range provided by the manufacturer of 117-143 mg Crd^{-1}.

Assessment of the performance of the CRDs depend on the establishment of satisfactory methods for Cr determination, the measurement of time required for the CRD to deliver a constant amount of Cr and the measurement of total faecal dry matter output in an indoor experiment. Results pertaining to each of these aspects and calculations of Cr recovery percentages are provided in the following sections.

3.4.1. Digestion Methods

Initial results obtained using the standard Cr analysis (see Methods) for faecal Cr concentration as shown in Table 3.6 (designated as "Low Bromate") varied significantly ($P<0.001$) between consecutive days and appeared to be very low when converted to total excretion per day values. Poor oxidation was suspected.

Table 3.6 Daily Cr Concentration ($\mu g g^{-1}$ dm) in faeces in which Cr was determined by using both High (HB) and Low (LB) bromate during the second 5-day measurement period of Block II

	TREATMENT							
	S		P		L		G	
Date	HB	LB	HB	LB	HB	LB	HB	LB
24/5	11.17±1.060	4.10±0.020	9.76±0.000	3.28±0.000	10.36±0.000	6.99±0.000	10.29±0.000	1.66±0.000
25/5	10.33±0.070	6.87±0.002	8.05±0.000	2.29±0.010	11.21±0.000	7.68±0.010	10.61±0.000	2.73±0.003
26/5	10.76±1.040	8.09±0.000	8.63±0.006	3.15±0.003	13.13±0.100	8.94±0.001	7.47±0.000	1.30±0.001
27/5	11.50±0.000	8.76±0.000	7.06±0.000	3.00±0.000	15.24±0.000	11.68±0.000	7.68±0.000	3.67±0.490
28/5	11.29±0.000	6.88±3.580	7.20±0.000	2.64±0.010	16.28±0.000	12.66±0.000	8.24±0.000	1.42±0.000
Means (SE)	11.01±0.002	6.94±0.400	8.14±0.060	2.87±0.001	13.24±1.130	9.50±1.110	8.86±0.150	2.16±0.040
Significance of Difference (HB & LB)	***		***		***		***	

Faecal samples were therefore re-analysed using a higher amount of bromate in order to ensure complete oxidation and a comparison between the values is presented in Table 3.6 for the faecal samples obtained during Period 2 of Block II, as this was a "scourfree" period.

The difference due to chemical methods of analysis was highly significant ($P<0.001$).

The influence of two levels of bromate is further illustrated in Fig. 3.1. There were consistent differences in all treatments as a result of using 8 ml of bromate (HB) compared with 3 ml(LB). The between duplicate variability was also diminished by using HB levels.

3.4.2 Day to Day Variations in Release Rate

The daily excretion of Cr during Blocks II and III are shown in Fig. 3.2. The data obtained from Block I were not comparable as the CRDs were inserted at an earlier date relative to the collection period (See Table 3.1).

According to the results indicated in Fig. 3.2 there are large variations of Cr RR between days. These results further indicated that RR was below expected levels (133 mg Cr d^{-1}) throughout most of Period 1 within Block II and III whereas gradual equilibration started during Period 2.

The data from the second period of all blocks are therefore of most interest with respect to establishing "normal" day to day variations in Cr concentration which is an important consideration when devising 'optimum' sampling regimes in field studies.

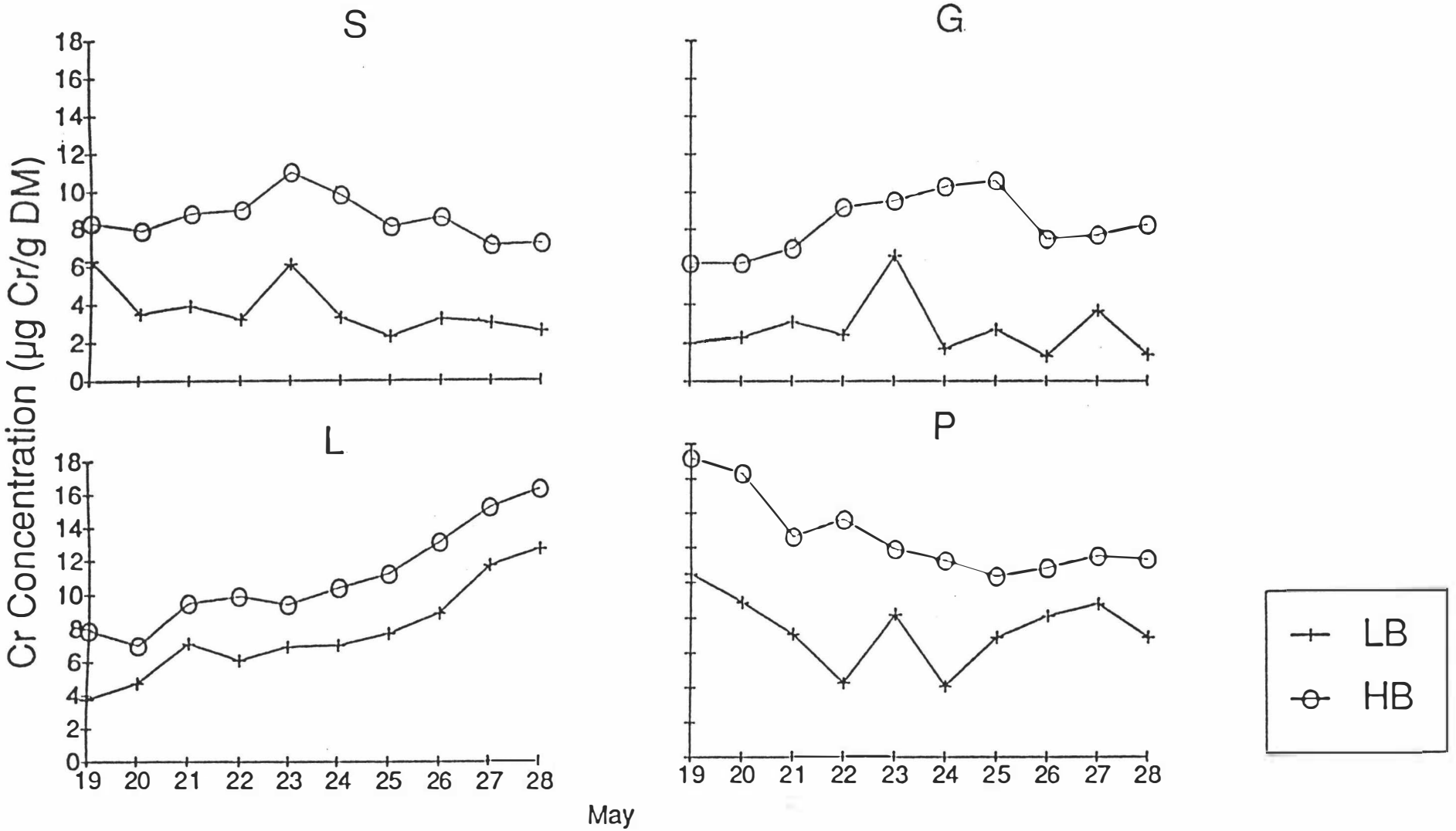

Fig. 3.1 Daily Chromium concentration in faeces when Cr was determined using Low (LB) and High (HB) bromate concentration

(Values are shown for individual calves within Block I)

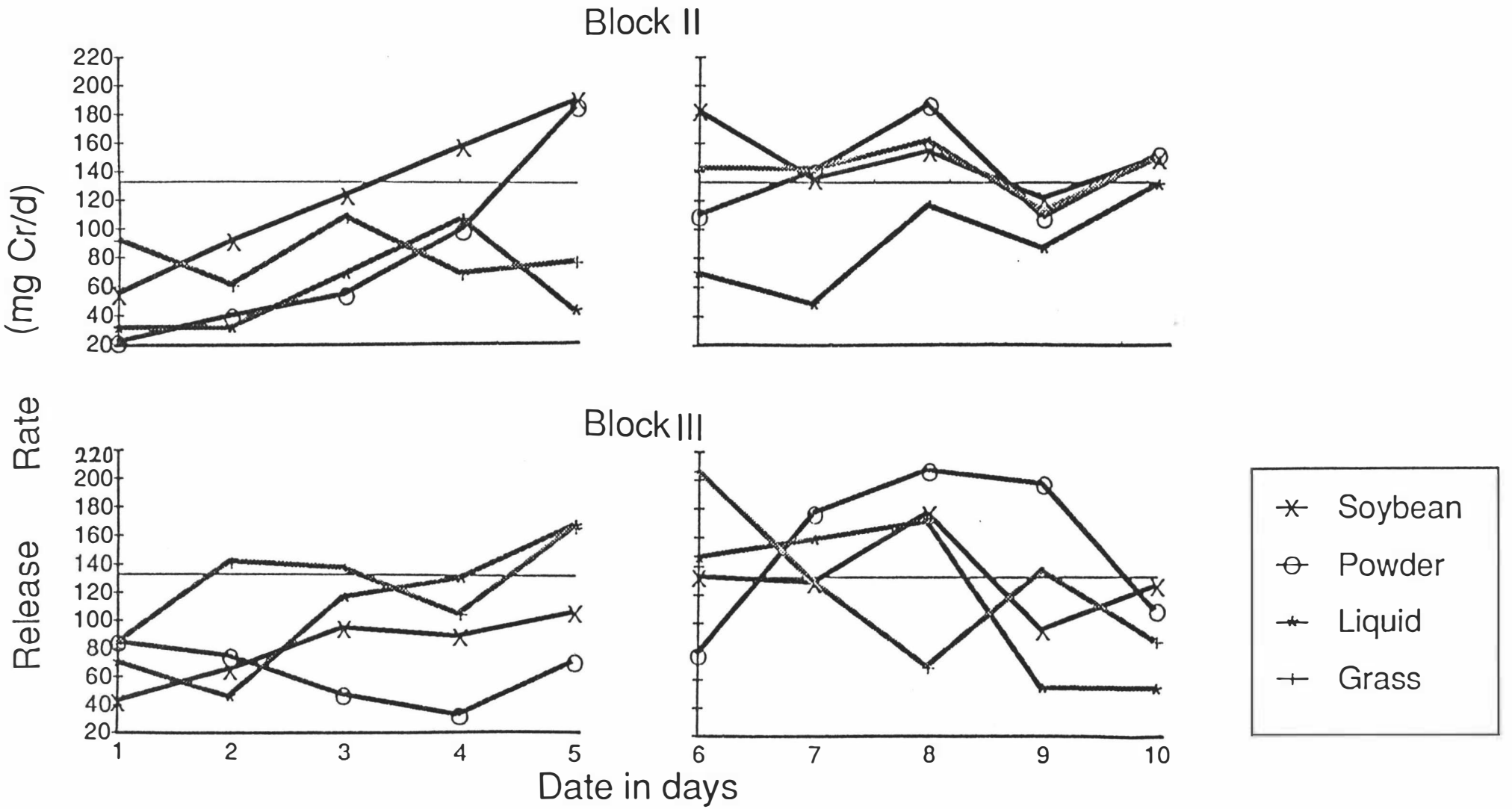

Fig. 3.2 Chromium excreted (mg/d) by individual calves (2 X 5 day period) during Block II & III over a 10-day collection period

Table 3.7 Mean release rate (RR) of chromium (mg Crd^{-1}) from individual calves during the second period of each of the three Blocks (Means ± SE for 5 days)

	TREATMENTS				
Block	S	P	L	G	Significance
I	123.14 ± 27.87	98.74 ± 14.03	95.82 ± 12.74	116.79 ± 15.08	
II	148.09 ± 23.03	138.83 ± 32.50	90.04 ± 15.03	141.19 ± 17.79	
III	131.37 ± 12.98	152.96 ± 25.60	120.35 ± 23.62	125.46 ± 23.76	
Means (SE)	134.20 ± 12.72	130.18 ± 15.45	103.16 ± 29.76	127.82 ± 12.37	NS

3.4.3 Ration Effect on Chromium Release Rate

The influence of possible ration effects on release rate are shown in Table 3.7.

The results obtained from the analysis of data indicated that there were no significant differences caused by the rations in the excretion of Cr (see Anova in Appendix Table 2). However, the means of most treatments and especially that for the L treatment was below the expected RR of 133 mg Cr d^{-1}.

3.4.4 Day to Day and Diurnal Variations in Chromium Concentration

The Cr concentration in grab samples taken from all calves at three different times of the day on individual days during Block II are shown in Fig. 3.3.

Day to day variation was clearly much larger during the first compared with the second period. The day to day effects appeared more important than within day variations (see Appendix Table 4). The main effects of time of sampling during the day are given in Table 3.8.

These results confirm that there is very little variation associated with time of sampling and hence time of feeding.

3.4.5 Chromium Recovery

The concentration of Cr in the faecal material as a percentage of the (assumed 133 mg) total amount of Cr fed to the calves via CRDs was estimated during the second 5-day collection period of each block. A relatively steady RR was expected after a period of more than five days (see Fig. 3.2). The results from daily representative faecal samples of individual calves are shown in Table 3.9.

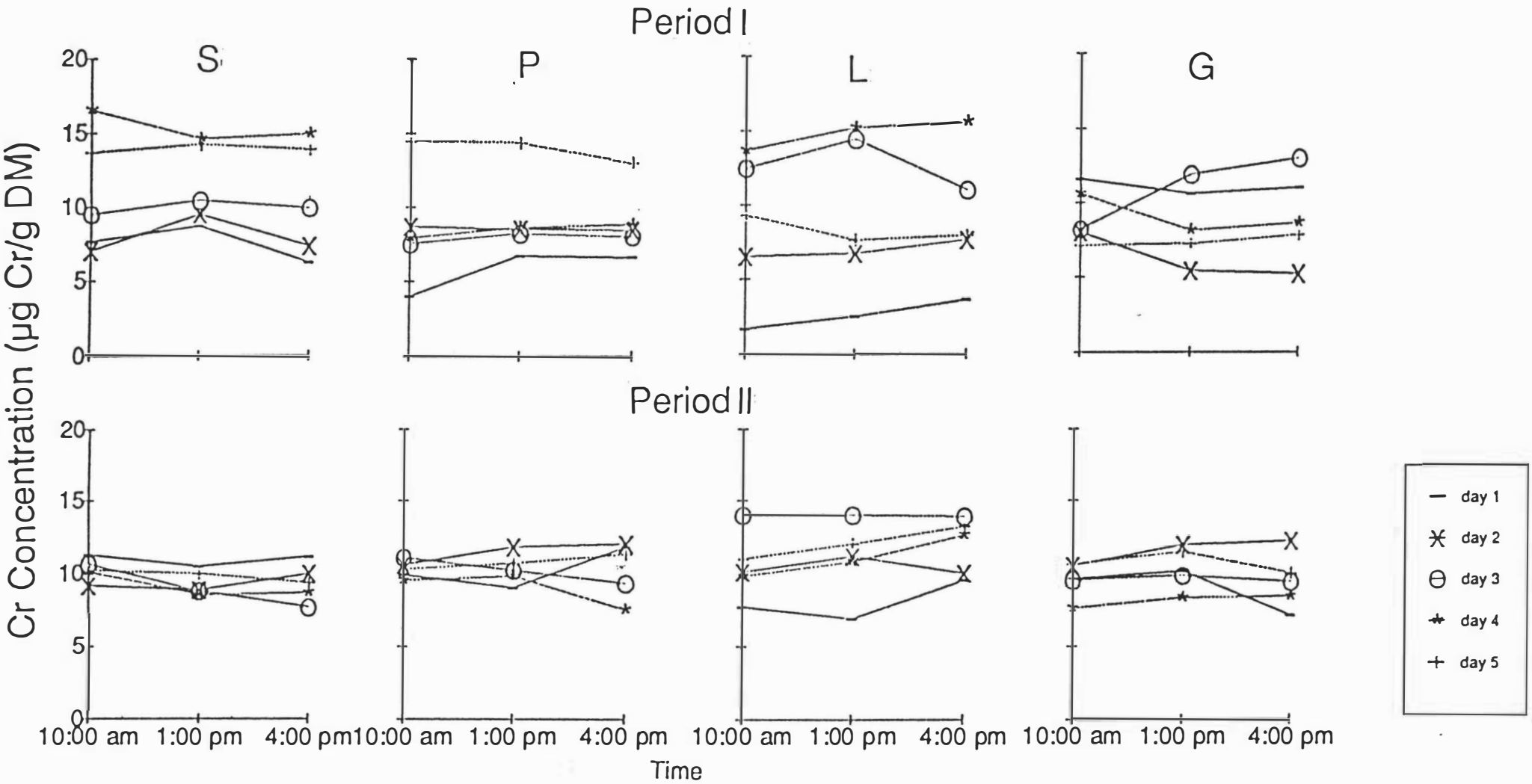

Fig. 3.3 Diurnal variation in Cr concentration (µg/g) in faeces DM taken 3 times/day from individual calves during Block II

Table 3.8 The effects of time of sampling during the day on chromium concentration ($\mu g g^{-1}$ dm) in faeces during period 2 of Block II (Means ± SE of 5 days)

Treatment	TIME WITHIN DAY			Means (SE)	Day's Representative Sample	Significance
	10.00 am	1.00 pm	4.00 pm			
S	10.32 ± 0.10	9.34 ± 0.03	9.39 ± 0.11	9.68 ± 0.02	8.87 ± 0.11	NS
P	10.30 ± 0.00	10.13 ± 0.14	10.38 ± 0.58	10.22 ± 0.00	9.86 ± 0.03	NS
L	10.54 ± 1.04	10.98 ± 1.15	11.50 ± 0.55	11.01 ± 0.00	9.80 ± 0.17	NS
G	9.54 ± 0.09	10.30 ± 0.18	9.11 ± 0.49	10.08 ± 0.01	9.32 ± 0.03	NS
Means (SE)	10.18 ± 0.00	10.19 ± 0.01	10.10 ± 0.09	10.17 ± 0.09	9.46 ± 0.01	NS

Table 3.9 Chromium Recoveries (%) from daily representative faecal samples taken from individual calves during the second periods of Blocks I, II and III

	TREATMENTS				
Block	S	P	L	G	LSD
I	92.59	72.05	67.63	87.80	
II	111.35	104.38	67.70	106.16	
III	98.77	115.00	90.49	94.33	
Means (±SE)	100.90 ± 5.5	97.14 ± 12.93	75.27 ± 7.62	96.10 ± 5.34	26.71 (NS)

Analysis of variance indicated no significant difference between recovery means. However, treatment L looked suspiciously low although insignificant (LSD 26.71). Such a result was due to a small number of calves used within treatments.

Mean recoveries were low in Block I presumably due to exhaustion of Cr in the CRDs. According to the manufacturer, the prescribed life time of the device is 25 days whereas Block I calves had had the devices for 25 days by the end of collection period.

3.5 UTILIZATION OF RATIONS FOR CALF GROWTH

3.5.1 Energy Utilization

The amount of energy (MJME d^{-1}) consumed by calves was found by calculating the difference between the energy eaten by the calf and that lost by way of faeces and urine output and also an assumed value for methane losses (12% of digestible energy, for above maintenance level of feeding; McDonald *et al* 1981).

These values together with estimates of digestible energy (DE) coefficients and the metabolisable energy (ME) concentration (MJME kg^{-1}dm) of rations are provided in Appendix Table 6. The effects of time (age and weight) on the utilization of rations is also indicated. A summary of the most important of these variables is indicated in Table 4.1.

The DE intake and concentrations appeared higher for the L treatment than the other 3 treatments however, this was based on very few animals per treatment.

The DE concentration of the four rations during each of the 5-day periods for individual calves in relation to the proportion of the total intakes as supplements are shown in Fig. 3.4. Generally grass DE concentration declined over time. It is also indicated that L treatment

Table 4.1 Energy Utilised by Calves fed different rations (Means ± SE of 4 periods)

	TREATMENT				
	S	P	L	G	Significance for treatment difference
*Means of 5 days (± SE)					
Apparent DEI (1) (MJ/calf/d)	17.17 ± 8.00 (22.44 ± 4.36)	16.51 ± 7.70 (22.69 ± 4.10)	21.88 ± 4.73 (27.24 ± 2.82)	15.18 ± 4.72 (21.01 ± 4.85)	NS
Proportion of UEO (2) to DEI (%)	9.00 ± 3.00 (7.00 ± 6.14)	9.50 ± 2.50 (7.50 ± 0.06)	5.50 ± 0.06 (4.50 ± 0.06)	12.50 ± 1.50 (7.00 ± 0.00)	NS
DE Concentration (%)	77.50 ± 3.50) (76.00 ± 2.00)	74.50 ± 4.50 (78.0 ± 0.00)	84.00 ± 2.00 (85.51 ± 2.50)	74.50 ± 2.50 (73.50 ± 0.06)	*
ME Concentration (MJME/kg DM)	11.69 ± 0.76 (11.89 ± 0.03)	11.63 ± 1.28 (12.65 ± 0.003)	14.31 ± 0.05 (14.89 ± 0.15)	10.80 ± 0.00 (11.66 ± 0.01)	NS

* For Blocks II and III

1 Digestible Energy Intake

2 Urinary Energy Output as % of DEI

Unbracketed numbers = Period 1

Bracketed numbers = Period 2

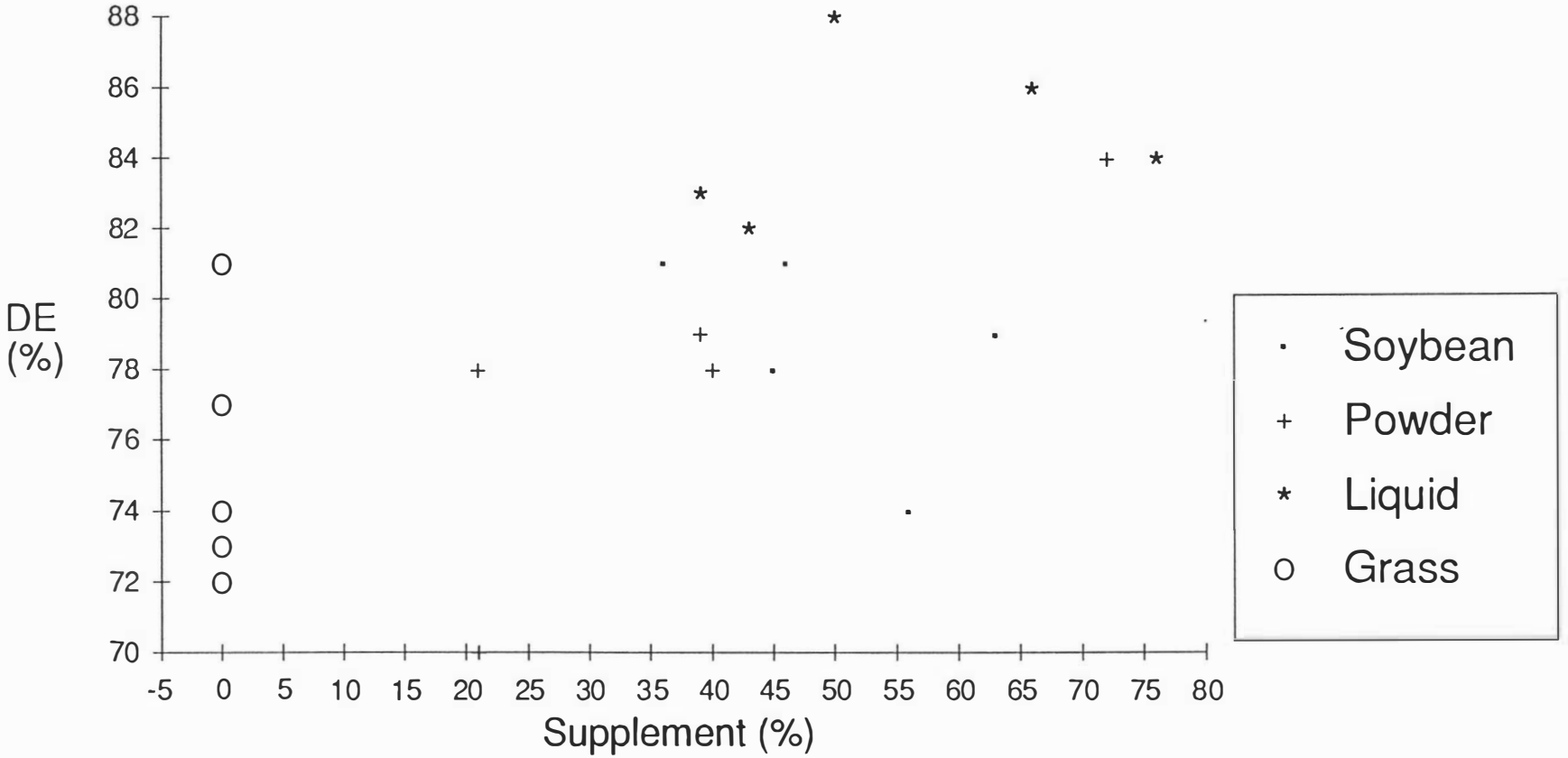

Fig. 3.4 Relation between DE concentration and supplement proportion in the ration

had significantly higher ($P<0.05$) amount of DE concentration in the ration.

The proportion of DE lost in urine also appeared to be relatively low in the L treatment calves compared with the remaining treatments (Table 4.1) and this proportion also consistently decreased over time (Period 2 vs Period 1). The mean ME intake for the 3 calves on each of the treatments during period 2 were 16.10, 15.20, 18.40 and 14.73 for S, P, L and G treatments respectively. These apparent differences were associated with differences in DE intakes as well as faecal and urinary losses.

The ME concentrations were 11.23, 11.79, 12.14 and 14.60 MJME kg^{-1}dm for G, S, P and L rations respectively.

The mean liveweight (LW) of calves and their weight gains during the duration of the experiment are shown in Table 4.2. They ranked P>L>S>G but were based on only three values per treatment.

Table 4.2 The means of liveweights (initial and final) and daily liveweight gain (LWG) for calves on different rations

Treatments	LW (kg) (Initial)	LW (kg) (Final)	LWG (kg d^{-1})	CV (%)
S	69.30±3.71	73.67±2.56	0.44	3.06
P	72.67±1.97	78.93±3.39	0.63	4.13
L	69.67±2.36	74.50±0.02	0.57	3.45
G	64.33±4.20	68.27±3.58	0.39	3.03

3.5.2 Nitrogen Balance

The amount of nitrogen retained (NR) was found by subtracting the sum of the N losses via faeces and the

urine from the total N intake. The results for Blocks I, II and III are shown in Table 4.4.

During Block II Period 1, all calves except L were in a negative N-balance. This was associated with low amounts of dry matter intake (See Table 3.5). In all Blockss, calvess on L treatment had the highest NR as a proportion of N-intake.

The relationship between NR and digestible energy intake is further elaborated in Fig. 3.5. Analysis showed a strong overall positive correlation (r = 0.80) between nitrogen retained and the quantity of digestible energy consumed. DE intake had a significant ($P<0.01$) effect on NR. The pooled regression was as follows:

$$NR = -18.79\ (\pm\ 6.52) + 1.24\ (\pm\ 0.30)\ DEI,\ R^2 = 0.63$$

Relationships between NR and DE intake of individual rations were further computed and are presented in Table 4.3

Table 4.3 Relationship between NR and DE intake for individual rations.

Treatment	EQUATION				
	a	b	r	R^2	Significance
S	$Y = -16.17(\pm 7.13) + 1.19(\pm 0.31)x;$		0.97	0.94	NS
P	$Y = -28.21(\pm 12.92) + 1.60(\pm 0.64)x;$		0.93	0.86	NS
L	$Y = -4.43(\pm 14.63) + 0.46(\pm 0.63)x;$		0.59	0.35	NS
G	$Y = -13.20(\pm 0.80) + 0.75(\pm 0.04)x;$		1.00	0.99	*

Footnote:

Y = NR (g d^{-1})
X = DE intake (MJ/calf/day)
a = Y intercept
b = regression coefficient
r = correlation coefficient
R^2 = coefficient of determination

Table 4.4 Means N R (gd^{-1}) and as a proportion of N-intake (%) for individual calves in the three Blocks

		TREATMENTS			
Block	Period	S	P	L	G
I	2	13.28 (25)	-0.13 (-0.30)	10.63 (25)	5.24 (10)
II	1	-7.81 (-31)	-7.33 (-27)	13.11 (29)	-8.20 (-28)
	2	7.68 (16)	-1.50 (- 3)	21.41 (35)	1.73 (4)
III	1	17.78 (28)	14.54 (23)	23.35 (33)	2.02 (3)
	2	16.24 (23)	15.28 (21)	10.63 (25)	5.24 (10)

Numbers out of brackets = Means NR for 5-day period
Numbers in brackets = NR as a proportion of N-intake (%)

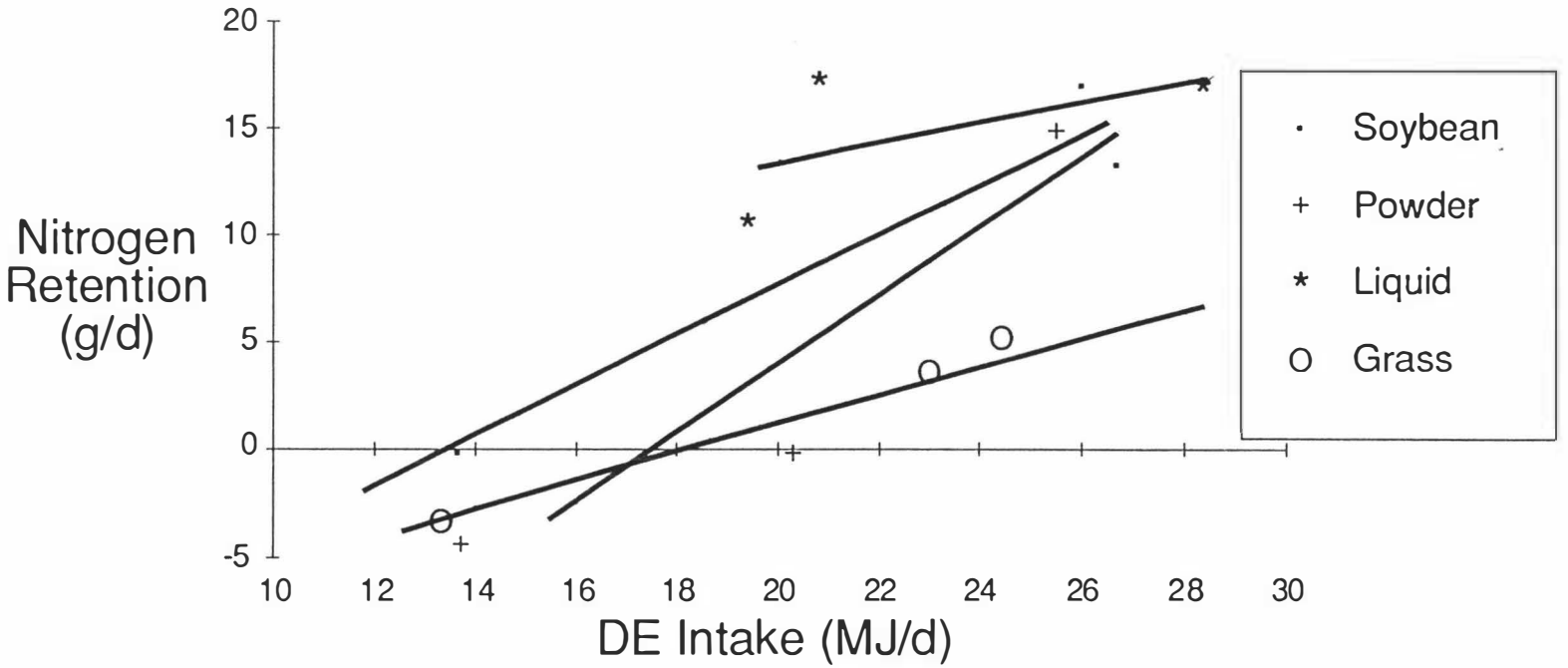

Fig. 3.5 Relation between NR (g/d) and DE Intake (MJ/d) of calves fed different supplements

The regression for the L treatment calves was significantly different ($P<0.05$) from that for the G calves.

3.5.3 Accuracy of *in-vitro (cellulase) method* of Determining Digestibility

In field studies CRDs may be used to estimate the amount of faecal output which is required for indigestibility estimation. This poses a question as to whether *in-vitro* digestibilities are sufficiently accurate for intake assessment. This aspect was assessed by undertaking *in vitro* digestion of the herbage used to feed the calves indoors as shown in Table 4.5.

Table 4.5 The *in vivo* and *in vitro* digestibility of herbage.

Period/ Block	*In vivo* digestibility (% DMD)	*In vitro* digestibility (% DMD)	*In vivo* digestibility (% OMD)
P_1B_1 +	79.60[a]*	74.64[b]	78.74
P_2B_1	74.11[b]	74.76[b]	79.02
P_1B_2	76.00[b]	76.99[b]	81.51
P_2B_2	73.00[b]	74.53[b]	79.15
P_1B_3	70.39[b]	74.95[b]	79.24
P_2B_3	71.56[b]	76.53[b]	81.03
Means (SE)	74.11±1.36	75.41±0.002	79.78±0.08

* Values followed by a common letter are not significantly different (using LSD).
\+ P and B indicate Period and Block respectively.

The mean *in vivo* herbage digestibility result (74.11 ± 1.36) was not significantly different from the mean *in vitro* figure (75.41 ± 0.002) but some individual values differed considerably.

There was a gradual decline of the *in vivo* digestibility values over time but no such trend was apparent in the *in vitro* values.

CHAPTER 4

DISCUSSION

4.1 ANALYTICAL METHOD

The results indicate a significant difference ($P<0.001$) between the use of low bromate, 3 mls used by Costigan *et al* (1987) and the high bromate, 8 ml used in the present experiment. Previously, a procedure based on that of Christian and Coup (1954) involving digestion of the ashed faecal sample with phosphoric acid-manganese sulphate solution and potassium bromate was investigated by Williams *et al* (1962) and later widely adopted. In this experiment it was found that lack of Cr solubilization was mainly due to inadequate oxidation of chromic (III) oxide which eventually gets converted to soluble chromate which releases Cr (VI) in solution (Fisher and Lee 1982 and Lee *et al*, 1986).

These results confirm earlier observations (Curran *et al*, 1967, Krohn and Konggaard, 1976) that digestion procedures might be one of the causes for low Cr recoveries and therefore a source of 'long-term' errors (Langlands *et al*, 1963 and Langlands, 1987).

The HB, apart from accelerating the rate of oxidation decreased the between duplicate variability in Cr concentration, this is indicated by the broad range of SE (0.00 to 3.31) duplicate means for LB samples and by a narrow range of SE (0.00 - 0.45) for HB sample means for Blocks I-III.

A parallel relationship resulted (Fig. 3.1) when data for HB were plotted against LB chromium concentration during Block I. Such a relationship certainly demonstrated the even dissolution of Cr(III) at a higher bromate level than 3 mls. To a certain extent the behaviour of these curves are affected by the type of

ration, and the amount of dry matter intake (see Appendix Table 6).

While the addition of extra bromate was most successful in reducing variability between duplicate samples and increasing the Cr concentration, more work is needed to investigate the 'optimum' amount of bromate to be used during digestion as to have maximum oxidation of Cr(III) into soluble chromate.

4.2 VARIABILITY OF MARKER EXCRETION BETWEEN DAYS

Results in Fig. 3.2 indicated large variations in marker RR between days. According to Langlands *et al* (1963a), when referring to gelatin capsules and Cr impregnated paper suggested causes of such variation could be either 'long term' or 'short term' errors. They advocated that long term errors could result from an inadequate preliminary period of incomplete recovery of the marker during analysis whereas short term errors typically arise because of diurnal variation in marker excretion as a result of inadequate mixing of feed and marker in the rumeno-reticulum. At least some of these reasons for variation between days may therefore apply to CRDs as to other forms of Cr administration.

In both Blocks II and III a more even state of Cr release was generally noticed during the second period (Fig. 3.2) after an average of 7 days following insertion of the CRDs. However, according to the manufacturers prescription for this particular type of device the release rate is expected to be constant and steady between days 5 and 25 of the capsule administration. Le Du and Penning (1982) recommended a preliminary dosing period of 7 days (with animals being dosed with Cr twice daily) and they also recommended a 5-day sampling period similar to what was used in the present study. These results were further confirmed by Valderrabano (1979) who

administered Cr to lambs for a period of 7, 12, 14, 19, 28 and 33 days prior to starting faecal collection, and showed that after 7 days an equilibrium between marker fed and excreted was reached.

It can therefore be concluded, that a preliminary period of not less than 7 days is to enable the CRD to give an equilibrated Cr release before faecal collection starts.

Ration effects The results indicated that the type of diet did not have a significant influence on release rate, in agreement with results of Laby *et al* (1984). However, the possibility remains that the L treatment may lead to lower RR of Cr (see Table 4.6) as the data in this experiment was based on few calves within treatment (n = 3).

The effect of the supplement in each ration upon Cr release rate is summarised in Table 4.6.

Table 4.6 The proportion of supplement (%) in each ration fed to calves and the mean RR of Cr.

TRT	*RATION COMPONENTS		RR (mgd^{-1})	% Deviation from expected RR (133 mg d^{-1})
	Grass	Supplement		
S	49.38	50.65	$134.20^{a} \pm 12.72$	+0.90
P	57.19	42.81	$130.18^{a} \pm 15.45$	-2.12
L	47.60	52.40	$103.16^{a} \pm 12.37$	-18.68
G	100.00	-	$127.82^{a} \pm 12.37$	- 3.89

* Data from Table 3.4

Means with same letter don't differ significantly

Calves on all rations except S gave a RR below the expected. This trend was previously documented by other workers (Langlands *et al*, 1963a); Hopper *et al*, 1978, and Moran *et al*, 1987), that many factors affecting RR have a depressive rather than enhancing influence on Cr release (Lee *et al*, 1986).

The results also indicated that the lowest RR was from L calves which also had the least proportion of grass (47.60%) total intake and highest proportion of supplement intake (52.40%) but in form of liquid. The fluid nature of the diet might be implicated with causing low RR although this was not statistically different from the other rations. Langlands (1975) used gelatin capsules in sheep and he was of the view that incomplete mixing of the marker and the food (intended to be marked) in the digestive tract of the animal could partly lead to low RR.

4.3 DAY TO DAY AND DIURNAL VARIATION IN CR CONCENTRATION

4.3.1 Diurnal Effects

Chromium concentration in grab samples taken at equidistant times of the daylight did not differ significantly. However, the concentration was at its maximum at 01.00 pm (10.19 ± 0.01 µg Crg^{-1} dm). More marked differences were earlier documented by a number of workers (Raymond and Minson, 1955, Smith and Reid, 1955 and Brisson *et al*, 1957) while using gelatin capsules in grazing cows. They found higher Cr concentrations in the morning than afternoon samples. High concentration of Cr in morning faecal samples was attributed to more active feeding and rumination which the calf starts early in the morning, therefore by the time sampling started there was already a thorough mixing of feed and the marker. Also Nicoll and Sherington (1984) found a significant cyclic

pattern in mean Cr concentration when they used shredded Cr_2O_3 paper and a frequency of twice daily sampling.

The data in Table 3.8 backed-up by Fig. 3.3 indicated some variations in diurnal Cr concentrations although they were not statistically significant. This probably reflected normal fluctuations in either dry matter intake or digestibility or both, attributes which were not measured at sampling times but measured on a 24 h basis. However, there is a lot of evidence (Hodgson, 1968) that a curvilinear relationship exists between digestibility of the diet and the amount eaten which eventually influences the rate of food passage in the reticulo-rumen and is therefore reflected in the concentration of Cr in faecal output. The influence of diet digestibility was also highlighted by Hopper *et al* (1978) who found variations in Cr concentration in faecal material from sheep fed on forage with digestibility ranging from 71 to 81% compared with 70-79% in the present study.

The extent of diurnal fluctuations may depend on the way in which supplements are fed. In the present experiment, supplements were available continuously (with exception of liquid milk proportion of L treatment) rather than at discrete meals. This is due to the fact that concentrates, whose energy is readily available for metabolic purposes, remain in the anterior portion of the rumen and thus secure a rapid entry to the reticulum and omasum. While roughages, whose energy is not readily available and therefore requires predigestion treatment by the rumen microorganisms, are forced to the rear of the rumen, from which point they must make a slow movement until regurgitation and remastication (Van Soest 1982). To be able to isolate the influence of herbage and 'dry' supplement when fed together, some workers (Uden *et al*, 1980) used polyethylene glycol (PEG). In the present study PEG was not used because it was found to be

associated with a number of serious limitations like lack of a specific, sensitive and accurate method of analysis which explains partly its occasional failure to achieve complete recovery or reproducible results. These limitations were earlier sounded by MacRae (1974).

The results from the present study also showed that there was no significant difference between Cr concentration from the samples taken at equidistant times of the day (10.17 $\pm$ 0.09 μgCr g^{-1}dm) and the day's representative faecal sample concentration (9.46 $\pm$ 0.01 μg Crg^{-1} dm), it is therefore concluded that sampling more than one time within a day is unimportant because fluctuations in Cr concentrations were more noticeable between days than within day samples. The best time for faecal sampling should be synchronized with feeding schedule and if calves do not defecate naturally they should be made to do so by rectal stimulation (rectal grab sampling) as demonstrated by Hardison and Reid (1953).

In this experiment the mean of samples taken at 04.00 pm was found to be similar to those composited for a 24 h period. This time is also convenient in New Zealand where routine milking is also at 04.00 pm, a time when faecal samples can be taken from lactating cows.

It was concluded from the results of this study that fluctuations in Cr concentration is not strictly diurnal, since the time of feed consumption and the amount of feed consumed at a given time do not appear to influence the Cr concentration-time relationship.

4.3.2 Day to Day Effects

Variation between days in faecal Cr concentration were more important than diurnal variations in the present study; and this was probably associated with erratic dry matter intakes which caused inverse variations in Cr concentration.

These variations were noticeably larger in Period 1 than Period 2 of each measured block (see Fig. 3.2). Lack of consistency in Period 1 was possible due to the fact that the plunger of the CRD required time to function in a linear manner but time is also required before an equilibrium can be reached in the digesta.

It can therefore be concluded that diet digestibility and quantity of dry matter intake are largely responsible for most of the observed day to day variations once the CRDs have become equilibrated. It then follows that faecal samples must be taken on as many different days as possible in order to get a 'truly representative' composite sample prior to Cr analysis, if accurate faecal outputs are to be estimated.

4.4 CHROMIUM RECOVERY

Results from this experiment indicated that the mean Cr recoveries from calves on treatments S, P and G (100.90, 97.14 and 96.10% respectively) were close to 100% whereas that of L treatment (75.27%) was much lower, although not statistically different. Recoveries from the S, P and G calves were similar to that estimated by Le Du and Penning (1982) whose percentage was 96.50 (SD $\pm$ 0.056); a mean value from 55 experiments covering both cattle and sheep. The low recovery found from L calves could also be due to inadequate mixing of marker and food in the gastro-intestinal tract.

In all rations except S the Cr recoveries were slightly below 100%. Langlands (1975) noted losses through absorption which were unlikely in this study because of the young age of the calves, he also attributed low recoveries to losses of faeces and regurgitation of capsules. When this study was in progress, three calves lost devices (see Table 3.1) and in each case regurgitation of the device was from the

heaviest calf in the group. It is speculated that the size of the calf is associated with regurgitation because a heavy calf is expected to have a larger oesophagus which could regurgitate the device more easily.

However, there was a negligible influence of regurgitation on Cr recovery rate because, in the first place, recovery estimates were based on a 5-day mean and secondly it was observed that the devices had not been out of the calves for very long (<6 h) before they were reinserted. Similar problems were earlier observed by Wittayanuparpyuenyong (1987) when carrying out an outdoor trial.

Recovery variations due to differences between individual CRDs was considered to be negligible in this study because all capsules used were of a single size (CV=5%) suitable for calves and other species like goats and sheep (Ellis *et al*, 1985). To minimise problems of regurgitation it can be recommended to vary wing length, barrel width/length and wing flexibility when manufacturing CRDs for specific size and species of animals. Reasons for heavier animals to easily regurgitate the capsules than the lighter ones still remains ambiguous.

In this study loss of Cr through the urine was considered to be insignificant. Uden *et al* (1980) confirmed that there were negligible losses through urine and by way of absorption. Rose (1962) cited by Kotb and Luckey (1972) had earlier suggested such inevitable losses were to be corrected by a 7% factor while Valderrabano (1979) found a mean of 1.84% of Cr_2O_3 administered was not recovered and was attributed to the absorption of the marker in the digestive tract. Some workers thought of the possibility of a systematic retention of Cr in the digestive tract of ruminants because of its length and complexity.

4.5 ENERGY UTILIZATION

The amount of energy that a young calf retains for growth purposes from the digestion of food determines the period to total weaning and indeed has a considerable bearing on the economics of calf rearing.

Results from this experiment indicated that calves which were on L treatment (liquid milk and grass) consumed a significantly larger amount ($P<0.01$) of DE as compared with G (grass only) treatment or even S (soya and grass) and P (powered milk and grass) treatments (see Table 4.7). The inferiority of S, P and G treatments compared with L treatment was mainly associated with the proportion of DE lost via faeces as it was shown that L calves had the least gross energy intake.

Further speculation could not be made at this stage because the data was limited by small numbers of animals ($n = 3$ per treatment) and the experiment was not designed especially for measurement of ration utilization; as such work was previously done by Whittayanuparpyuenyong (1987).

Leaver *et al* (1969) and Aston and Taylor (1980) had previously shown that fibre and cellulose digestion was generally reduced when concentrates were used to supplement roughages. This is not usually the case with liquid supplements (treatment L in this study) which normally by-pass the rumen through the oesophageal groove (Kaiser, 1976).

The present results show that the response to liquid milk supplementation relative to other concentrates was always higher during the first than the second experimental period. This trend was similarly observed by an Irish worker (Keane, 1982) when working with grass-fed calves. Depending on the prevailing 'cost effective strategy' for supplementing grass-fed calves it might be worthwhile feeding milk at 'low' level with *ad lib*

Table 4.7 Gross Energy Intake, Faecal and Urinary energy losses as a percentage of DE intake (MJ/calf/day) (Means ± SE of 3 Blocks n = 12)

	TREATMENTS				
	S	P	L	G	Significance
Gross Energy Intake	b 48.48	b 49.03	b 43.17	a 54.40	*
Apparent DE Intake	b 19.81 ± 2.59	b 19.61 ± 3.06	a 24.56 ± 2.68	b 18.11 ± 2.89	*
Faecal EO+ (%)	b 28.67 ± 3.89	b 29.42 ± 3.39	c 18.61 ± 2.21	a 36.29 ± 0.19	**
Urinary EO (%)	b 7.07 ± 1.55	b 8.40 ± 1.48	a 5.01 ± 0.82	b 9.06 ± 3.48	*

+ Energy output

Values having common letters are not significantly different

quantities of good quality autumn pasture during the early rearing period of the calf.

The observed differences between the L and P treatments were due to the form in which the rations were presented to calves. Keane and Harte (1982) also noticed inconsistence between L and P treatment and suggested that powdered milk entered the reticulo rumen while the liquid milk bypassed the rumen hence P was likely to have been poorly fermented.

It was not possible to measure (directly) the digestibility of the supplements alone however, "text book" values would suggest that the two milk supplements would be expected to have a value of 90-95% and the soya supplement about 85%, all substantially above that measured for the pasture.

The digestibility of the three supplemented rations (S, P, L) would therefore be expected to be greater than that for G and the extent of difference depends on the proportion of supplement: grass consumed. The relation actually observed is shown in Fig. 3.4. While the digestibility of L was clearly higher than G, the values for P and S were not, which suggests that the supplement was reducing the digestibility of the grass (or vice versa). The mean comparison between the expected and the actual (measured) digestibilities are summarized in Table 4.8.

The elevation in digestibility by 5% in P (grass and milk powder ration) compared to G (grass only) provided an additional 1.70 MJ/calf/day intake in terms of DE.

Table 4.8 Actual and expected dry matter digestibilities of herbage, supplement and herbage and supplement ration.

Ration	TREATMENTS S Exp.	S Actual	P Exp.	P Actual	L Exp.	L Actual
Herbage	*0.74	-	0.74	-	0.74	-
Supplement	0.85	-	0.92	-	0.92	-
Herbage & Supplement	0.795	0.770	0.830	0.790	0.830	0.830

Exp. Expected

* Actual mean digestibility (n=6) for G treatment

Similarly by feeding L (grass and liquid milk) and S (grass and soya) there was a respective increase in digestibility by 9 and 3% as compared with G (grass only) ration. This increase in digestibility as a result of supplementation gave an additional 6.15 and 1.9 MJ/calf/day DE for calves on L and S treatments respectively. Previous work (Thomas and Hinks 1983) gave results similar to the ones in the present study which showed intake to increase to a certain level when concentrates were combined with roughages and digestibility of basal ration were promoted.

The results in Tables 4.1 and 4.7 also show that the addition of supplements to a grass ration decreased the proportion of DE which is lost in the urine and hence ME intake was much higher for the L treatment, followed by S, P and G.

It can be concluded that supplements partly contribute to higher amounts of DE intake when fed together with herbage. The resultant high digestibility

characteristic of supplement increased the amount of DE that was actually eaten as compared with unsupplemented herbage (G) ration.

4.5.1 Calf Performance

From the limited amount of data on this aspect it was found that calves varied greatly in the rate of daily LW gain. There was a positive correlation ($r = 0.59$, $P<0.05$) between the LW gain and the concentration of ME in different rations. Calves on G treatment (unsupplemented) performed poorly. The rates of gain were 390, 440, 570 and 630 g/calf/day for calves on G, S, L and P rations (see Table 4.2). The general response was found to be in agreement with that of Whittayanurparpyurenyong (1987) who worked with outdoor calves and Byford (1974) who worked with indoor-fed calves. The results from Byford's work indicated daily weight gains of 580 and 310 g/calf/day for the supplemented and unsupplemented calves respectively. The observed slight difference may be due to variation in ingredients used for supplement composition but herbage digestibilities, 76 and 74% used in Byford's and present experiment respectively, were not significantly different.

The relatively low daily LW gain for the grass-fed calves in the present experiment was associated with the efficiency with which GE was utilized for LW gain .

In the present experiment, ME concentration for rations G, S, P and L were 11.23, 11.79, 12.14 and 14.60 MJME kg^{-1}dm respectively. This order of increasing energy concentration was positively associated with weight gains for respective calves. ARC (1980) associated the efficiency with which energy was utilized for different productive function (K) to be partly governed by the energy concentration. On the contrary, Kay *et al* (1970) by feeding diets of 13.0, 11.7 and 10.0 MJME kg^{-1}dm to

early weaned calves (6 weeks of age) found higher weight gains on lower ME concentration diet. In their experiment it is documented that high ME concentration was achieved by inclusion of tallow, which might have decreased intake through fats influence on rumen digestion.

4.6 NITROGEN UTILIZATION AND DE INTAKE

The relationship between NR and DE intake (Fig. 3.5) attracts a lot of attention because it shows clear differences between the efficiency of use of DE between treatments. This suggests differences in urinary energy losses or in K-values if it is assumed that the N content of the rations was not limiting growth rate (and hence N-retention).

4.6.1. Nitrogen Balance

The results of the experiment showed that large amounts of N were lost through urine. This is in conformity with the results of Blaxter *et al* (1966). The amount of urinary N loss varied significantly ($P<0.01$) according to rations on which the calves were fed. The losses were calculated as a percentage of N intake which were 55.51, 69.06, 50.91 and 70.80% from calves on rations S, P, L and G respectively. There was a poor relationship between the amount of N intake and the urinary N losses. Faecal N losses were 13.64, 11.50, 10.28 and 13.92 g N d^{-1} which accounted for an average of almost 25% of the total N intake in all rations and were low ($P<0.001$) compared with the urinary N losses.

In both faecal and urinary losses, grass-fed calves recorded the highest N excretion (26.70 and 70.80% respectively). From these results it can be concluded that CP in grass-fed calves was inefficiently utilized unless supplements were fed. From results of Reid (1986) it seems unlikely that N was limiting in the herbage fed

to the calves. Beever *et al* (1978) indicated that N content of autumn pasture was higher than spring pasture. At this stage energy cannot be disregarded as a limiting factor.

Influence of Supplements A summary of NR results and the relation between NR and DE intake is in Table 4.3 was plotted in the graph (Fig. 3.5). It was found that L ration was outstandingly superior ($P<0.01$) compared with the other supplements. The NR for L ration was in the range of 25-35% of total N intake compared with -28 to 10% for G.

The superiority of L treatment was associated with a high digestibility and a higher ratio of ME to CP intake. This view is in good agreement with results of Davey (1974) who found milk and milk substitutes fed to calves to contain surplus protein compared with other supplements where energy rather than protein is usually limiting. Preston *et al* (1965) found that when calves were fed on a diet with 21.75% CP, energy rather than protein limited NR.

It can be concluded that liquid milk supplement has a positive NR influence as compared with other supplements which have a relatively low ME:CP ratio and therefore L can result in relatively large amounts of NR when fed with herbage which is rather poor in energy.

The P calves were in a negative N balance during Blocks I and II but recovered to an average of 14.91% (NR as a proportion of N intake) during Block II (see Appendix Table 5). There was a strong relationship ($r = 0.93$) between N intake and the amount of N retained. It is true that low dry matter intakes during Block I (986 gd^{-1}) and Block II (921.83 gd^{-1}) resulted in negative N balance while in Block III (1590.03 gd^{-1}) gave positive N balance. This was associated with age of the calves. In Blocks I and II the calves on P treatment were 41 and 49 days old respectively while in Block III the calf on

similar treatment was 57 days old (Table 3.1) which had a relatively well developed digestive system to digest the powdered milk. These results are supported by those of the Irish workers (Keane and Harte 1982). It can therefore be concluded that milk powder would not be easily digested by a pre-ruminant calf but will give higher NR when supplemented to grass than feeding grass alone.

The results of S treatment calves were relatively poorer than calves on L treatments but on average better than G treatment in terms of NR. An average of 10.05 g N d^{-1} were retained by calves fed on S treatment compared with 3.45, 14.96 and 1.88 for the P, L and G treatments respectively.

The soya fed calves probably gave slightly poorer results than liquid milk fed calves because soya bean protein had a lower digestibility and/or biological value (BV) when compared with protein of liquid milk origin. This contention was also supported by Gorril and Nicholson (1969) by replacing a liquid milk supplement with a soya protein concentrate, a low yield of N and GE digestibility was evident. It is suggested that some improvements can be made on soya bean protein availability by using heating techniques. Nitsan *et al* (1971) enhanced soya protein digestibility from 73 to 89% by moist heating. However, the improvement on soya bean can be done as long as it is economically feasible as compared with feeding high quality autumn pasture to young calves.

4.7 CALF HEALTH

Four calves scoured during the entire experimental period. The incidence was mainly concentrated in Block III and not associated with any one particular treatment

group. There was no evidence to suggest that Cr yield and calf performance were affected.

CHAPTER 5

FINAL DISCUSSION AND CONCLUSIONS

The results of the present study indicated that the use of CRDs minimized although not completely removed diurnal fluctuations in Cr-release. It is now confirmed that within day variations in Cr concentration are of little practical significance in situations where the CRDs are used.

There were more marked variations in Cr concentration between days. Results of the present study associated such fluctuations largely with time of CRDs administration/faecal sampling periodicity and erratic dry matter intake, digestibility or both. The expected period between CRDs insertion and the recommended time for sampling is at least 5 days (R.H. Laby, pers. comm.) however, results from the present study showed faecal Cr concentration equilibration to start not earlier than 7 days.

Daily Cr release rate was found to be markedly influenced by the amount of dry matter intake (Table 3.5) and had an inverse relationship with faecal output. It can therefore be concluded that day to day changes in quantities of dry matter intake are partly responsible for the observed fluctuations in Cr release rate.

Although the data in this study were based on too few calves to be able to measure valid statistical effects caused by rations on RR, it was found that there was no significant effect of the treatment on the rate of Cr release from the CRDs. Calves on liquid milk had the least proportion of grass intake and highest proportion of supplement intake which may have resulted in lower RR

because of the fluid nature of the L ration compared to S, P and G rations.

In this study, the CRDs resulted in Cr yields (recovery rates) which were not statistically different from 100% (see Appendix Table 3) although those of L calves were much lower than those of S, P and G calves. With the exception of the L treatment, the Cr yield from the CRDs was found not to be influenced by the type of the ration. The possible effects of a liquid diet on Cr release rate need further study. The observed difference from 100% would be due to within capsule differences although the manufacturer had earlier indicated that devices of the type used in this study had a CV of 5%.

Although regurgitation of the devices remain a potential problem when used in field studies, their loss in the present experiment had an unnoticeable influence on Cr yield because reinsertion of the devices was done within a short period of time. Such Cr losses were obscured by the procedure of the technique which used 5-day collection period means. A method of measuring empty CRDs at the end of field studies would obviously be a desirable development. It can be recommended that a modification of CRDs, by the manufacturer, in terms of wing length, barrel width, length and a control of factors which lead to wing flexibility, could probably minimize the incidences of regurgitation.

Results of the present study confirmed that the conventional 3 ml of bromate ($KBrO_3$) was inadequate to bring about complete oxidation during the conversion of insoluble Cr(III) to soluble Cr(VI). Increasing bromate to 8 ml significantly elevated Cr concentration in the acqueous solution during sample digestion, also the adjustment reduced the within duplicate variation. However, the 'optimum' amount of bromate required to cause maximum oxidation of Cr(III) is hitherto unknown. Further work is needed to explore this aspect.

The supplements studied in this experiment had resulted in significantly different LW gains in field studies (Wittayanuparpyuenyong, 1987), and the results from the present study suggest that the reasons for the differences may be due to different intakes of DE (and ME) and also the efficiency with which ME was utilized. The efficiency of use of protein fraction of autumn pastures was shown to be particularly important.

REFERENCES

Aggette, J. and O'Brien, G. (1981). Formation of chromium atoms in air-acetylene flames. Part 1. *Analyst* 106: 497.

Ibid. (1981). Formation of chromium atoms in air-acetylene flames. Part 2. *Analyst* 106: 506.

Alexander, G.I. (ed) (1973). Manual of techniques for field investigations with beef cattle. Canberra, Australia: Commonwealth Scientific and Industrial Research Organisation (CSIRO).

Allden, W.G. and Young, R.S. (1959). The summer nutrition of weaner sheep: Herbage intake following periods of differential nutrition. *Aust. J. Agric. Res.* 15: 989.

Allden, W.G. (1969). The summer nutrition of weaner sheep: the voluntary feed intake, body weight change and wool production of sheep grazing mature herbage of sown pasture in relation to the intake of dietary energy under a supplementary feeding regime. *Aust. J. Agric. Res.* 20: 499.

Angelone, A.; Toledo, J.M. and Burns, J.C. (1980a). Herbage measurement *in situ* by electronics. 1. The multiple proetype capacitance metre. A brief review. *Grass and Forage Science* 35: 25.

AOAC (1984). Official methods of analysis. Association of Official Analystical Chemists, Washington D.C.

ARC (1980). Agricultural Research Council: The Nutrient Requirements of Ruminant Livestock, Commonwealth Agricultural Bureaux.

Aston, K. and Taylor, J.G. (1980). Effects of supplementing maize and grass silage with urea or ammonia on the intake and performance of fattening bulls. *Anim. Prod.* 31: 243.

Baker, R.D. (1982). Estimating herbage intake from animal performance in "Herbage Intake Handbook" Edited by J.D. Leaver. British Grassland Society, pp. 77-94.

Bakhuis, J.A. (1960). Estimating pasture production by use of grass length and sward density. *Neth. J. Agric. Sc.* 8: 211.

Balch, C.C., Reid, J.T. and Strause, J.W. (1957). Factors influencing the rate of excretion of administered chromium sesquioxide by steers. *Brit. J. Nutr.* 11: 184.

Barry, T.N. and Manley, T.R. (1984). The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 2. Quantitative digestion of carbohydrates and proteins. *Br. J. Nutr.* 51: 493.

Beever, D.E.; Terry, R.A.; Commell, S.W. and Wallace, A.S. (1978). The digestion of spring and autumn harvested perennial ryegrass by sheep. *J. Agric. Sci. (Camb).* 90: 463.

Blaxter, K.L. and Mitchell, H.H. (1948). The factorization of protein requirements of ruminants and of the protein value of feeds with particular reference to the significance of the metabolic faecal nitrogen. *J. Anim. Sci.* 7: 351.

Blaxter, K.L. and Wood, W.A. (1951). The nutrition of the young Ayrshire calf. Some factors affecting the biological value of protein determined by nitrogen balance method. *Br. J. Nutr.* 5: 55.

Blaxter, K.L.; Clapperton, J.L. and Wainman, F.W. (1966). Utilization of the energy and protein of the same diet by cattle of different ages. *J. Agric. Sci. (Camb).* 67: 67.

Bolin, D.W.; King, R.P. and Klosterman, E.W. (1952). A simplified method for determination of chromic oxide (Cr_2O_3) when used as an index substance. *Science* 116: 634.

Border, J.R.; Harris, L.E. and Butcher, J.E. (1963). Study of the quantitative faecal recovery of chromic oxide when administered to sheep as a component of paper. *J. Anim. Sci.* 22: 1117 (abstr.)

Brisson, G.J.; Pigden, W.J. and Sylvester, P.E. (1957). Effect of frequency of administration of chromic oxide on its faecal excretion pattern by grazing cattle. *Can. J. Anim. Sci.* 37: 90.

Brisson, G.J. (1960) Indicator methods for estimating amounts of forage consumed by grazing animals. *Proc. Int. Grassld Congr.* 8: 435.

Brown, D. (1954). Methods of surveying and measuring vegetation. Commonw. Bur. Pastures and Field Crops, Hurley, Bull. 42.

Byford, M.J. (1974). The comparison of pasture and concentrates as early-weaning foods for calves. M. Agric. Sci. Thesis presented at Massey University.

Campbell, J.B. (1969). Experimental methods for evaluating herbage. *Can. Dept. Agric.* Publ. 1315.

Carter, J.F., Bolin, D.W. and Erickson, N.D. (1960). Evaluation of forages by the agronomic "difference" method and the chromogen-chromic oxide "indicator" technique. *N. Dakota Agric. Exp. Sta., Bull. 426.*

Carter, J.F. (1962). Herbage sampling for yield:tame pasture. In: "Pasture and Range Research Techniques". By joint committee of Am. Soc. Agron.; Am. Dairy Sci. Assoc. Comstock Publishing Association pp. 90-101.

Chacon, E. and Stobbs, T.H. (1976). Influence of progressive defoliation of a grass sward on the eating behaviour of cattle. *Aust. J. Agric. Res.* 27: 709

Chambers, D.T. (1961). The effect of two meal supplements on the progress of calves receiving autumn grass. *An. Prod.* 3: 147.

Christian, K.R. and Coup, M.R. (1954). Measurements of feed intake by grazing cattle and sheep. VI. The determination of chromic oxide in faeces. *N.Z. J. Sci. Tech.* A36: 328.

Christian, K.R.; Williams, V.J. and Carter, A.H. (1965). The estimation of faeces output in stall-fed sheep using chromic oxide marker. *N.Z. J. Agric. Res.* 8: 530.

Coblentz, E.J.; Morrill, L.; Parrish, D.B. and Dayton, A.D. (1976). Nutritive value of thermo-alkali processed soya materials for young calves and rats. *J. Dairy Sci.* 59: 481.

Cook, C.W. (1964). Symposium on nutrition of forage and pastures: collecting forage samples representative of ingested materials of grazing animals for nutiritonal studies. *J. Anim. Sci.* 23: 265.

Colluci, P.E.; Chase, L.F. and Van Soest, P.J. (1982). Feed intake apparent diet digestibility and rate of particulate passage in dairy cattle. *J. Dairy Sci.* 65: 1445.

Corbett, J.L.; Greenhalgh, J.F.D. and McDonald, I. (1958). Paper as carrier of chromium sesquioxide. *Nature, London* 182: 1014.

Corbett, J.L.; Greenhalgh, J.F.D. and McDonald, I. (1960). Excretion of chromium sesquioxide administered as a component of paper to sheep. *Br. J. Nutr.* 14: 289.

Corbett, J.L. (1960). Faecal-Index techniques for estimating herbage consumption by grazing animals. *Proc. Int. Grassld. Congr.* 81: 438.

Corbett, J.L.; Langlands, J.P.; McDonald, I. and Pullar, J.D. (1966). Comparison of direct animal calorimetry of the net energy values of an early and late season growth of herbage. *Anim. Prod.* 8: 13.

Corbett, J.L. (1978). Measuring animal performance. In: "Measurement of Grassland Vegetation and Animal Production". Edited by L.'t. Mannetje. *Bull. Bur. Past: Hurley No. 52* (Commonwealth Agricultural Bureaux; Farnham Royal).

Cordova, F.J.; Wallace, J.D. and Piepper, R.D. (1978). Forage Intake by Grazing Livestock: A Review. *J. Range Mgt 31(6):* 430.

Costigan, P. and Ellis, K.J. (1987). Analysis of faecal chromium derived from controlled release marker devices. *N.Z. J. Tech.* 3: 89.

Coup, M.R. and Lancaster, R.J. (1952). The measurement of feed intake by grazing cattle and sheep. II. The determination of chromic oxide and monastral blue in cow faeces. *N.Z. J. Sci. Tech.* A34: 347.

Cowlishaw, S.J. and Alder, F.E. (1963). A comparative study of paper and oil as carriers of chromium sesquioxide administered to grazing steers to determine their faecal output. *J. Brit. Grassld. Soc.* 18: 328.

Crampton, E.W. and Maynard, L.A. (1938). The relation of cellulose and lignin content to the nutritive value of animal feeds. *J. Nutr.* 15: 383.

Crampton, E.,W. and Lloyd, L.E. (1951). Studies with sheep on the use of chromic oxide as an index of digestibility of ruminant rations. *J. Nutr.* 45: 319.

Curran, M.K.; Leaver, J.D. and Weston, E.W. (1967). A note on the use of chromic oxide incorporated in a feed to estimate faecal output in ruminants. *Anim. Prod.* 9: 561.

Czarnocki, J.; Sibbald, I.R. and Evans, E.V. (1960). The determination of chromic oxide in samples of feed and excreta by acid digestion and spectrophotometry. *Can. J. Anim. Sci.* 41: 167.

Davey, A.W.F. (1974). Nutrition of pre-ruminant calf. *Proc. N.Z. Soc. Anim. Prod.* 34: 133.

David, D.J. (1959). Determination of calcium in plant material by atomic-absorption spectrophotometry. *Analyst* 84: 536.

Davis, C.L.; Byers, J.H. and Luber, L.E. (1958). An evaluation of chromic oxide method for determining digestibility. *J. Dairy Sci.* 41: 152.

Day, K.M. (1954). Source of error in determination of chromic oxide using perchloric-sulphuric acid digestion method. *Science* 120: 717.

Dyne, G.M. van and Lofgreen, G.P. (1964). Comparative digestion of dry annual range forage by cattle and sheep. *J. Agric. Sci.* 23: 823.

Earle, D.F. and McGowan, A.A. (1979). Evaluation and calibration of an automated rising plate for estimating dry matter yield of pasture. *Aust. J. Exp. Agric. Anim. Husb* 19: 337.

Ellam, C.J. and Davis, R.E. (1961). Lignin excretion by cattle fed a mixed ration. *J. Anim. Sci*. 20: 484.

Ellam, C.J.; Reynolds, P.J.; Davis, R.E. and Everson, D.O., (1962). Digestibility studies by means of chromic oxide, lignin and total collection techniques with sheep. *J. Anim. Sci.* 2: 189.

Elliot, R.C. and Fokkema, K. (1960a). The use of chromic oxide for the estimation of faecal output. *Rhodesia Agric. J.* 57: 43.

Ellis, G.H.; Matrone, G. and Maynard, L.A. (1946). A 72 percent H_2SO_4 method for the determination of lignin and its use in animal nutrition studies. *J. Anim. Sci.* 5: 285.

Ellis, K.J.; Laby, R.H. and Burns, R.G. (1981). Continuous controlled-release administration of chromic oxide to sheep. *Proc. Nutr. Soc. (Aust.)* 6: 145.

Ellis, K.J.; Laby, R.H.; Costigan, P.; Zirkler, K. and Choice, P.G. (1982). Continuous controlled release administration of chromic oxide to cattle. *Proc. Nutr. Soc. (Aust.)* 7: 177.

Ellis, K.J.; Costigan, P. and Schlink, A.C. (1985). An intra-ruminal device for controlled infusion into the fore-stomach of free ranging animals. Seminar on "research and development of controlled-release technology for agrochemicals using isotopes": International Atomic Energy Agency in corporation with the Food and Agricultural Organization of the United Nations, (IAEA/FAO), Vienna, Austria.

Ely, R.E.; Kane, E.A.: Jacobson, W.C. and Moore, L.A. (1953). Studies on the composition of lignin isolated from orchard grass hay cut at four stages of maturity and from the corresponding faeces. *J. Dairy Sci.* 36: 346.

Fenton, T.W. and Fenton, M. (1979). An improved procedure for the determination of chromic oxide in feed and faeces. *Can. J. Anim. Sci.* 59: 631.

Fisher, M.T.; Atkin, P.R. and Joplin, G.F. (1972). A method for measuring faecal chromium and its use as a marker in human metabolic balances. *Clin. Chim. Acta* 41: 109.

Fisher, M.T. and Lee, J. (1982). Multi-element analysis by inductively-coupled plasma emission spectrometry in animal diets and faeces containing chromium marker. *Anal. Chim. Acta,* 139: 333.

Forman, S.A. and Sauer, F. (1962). Some changes in the urine of sheep fed hay high in silica. *Can. J. Anim. Sci.* 42: 9.

Frame, J. (1981). Herbage mass. In: "Sward measurement handbook". Edited by J. Hodgson, R.D. Baker, A. Davies, A.S. Laidlaw and J.D. Leaver. British Grassland Society pp. 39-69.

Gallup, W.D.; Hobbs, C.S. and Briggs, H.M. (1945). The use of silica as a reference substance in digestion trials with ruminants. *J. Anim. Sci.* 4 68.

Gleeson, P.A. (1971). Grass as feed for calves. *Ir. J. Agric. Res.* 10: 151.

Goguel, R. (1970). An improved nitrous oxide burner for atomic absorption spectroscopy. *N.Z. J. Sci.* 13: 603.

Gorrill, A.D.L. and Nicholson, J.W.G. (1969). Growth, digestibility and nitrogen retention by calves fed milk replacers containing milk and soybean proteins supplemented with methionine. *Can. J. Anim. Sci.* *49*: 315.

Grassland Reserach Institute, Hurley (1961). Research techniques in use at the Grassland Research Institute, Hurley. *Commonw. Bur. Pasture Field Crops, Hurley, Berkshire, Bull. 45*.

Greenhalgh, J.F.D. and Corbett, J.L. (1960). The direct estimation of the digestibility of pasture herbage. 1. Nitrogen and chromogen as faecal-index substances. *J. Agric. Sci.* 55: 371.

Greenfields, S.; McGeachin, H. McD. and Smith, P.B. (1975). Plasma emission sources in analytical spectroscopy. 1. *Talanta* 22: 1.

Grosskopf, J.F.W. (1959). Some factors affecting the secretion of abomasal juice in young dairy calves. *Onderstepoort J. Vet. Res.* 28: 133.

Hardison, W.A. and Reid, J.T. (1953). Use of indicators in the measurement of dry matter intake of grazing animals. *J. Nutr.* 53: 35.

Hardison, W.A.; Engel, R.W.; Linkous, W.N.; Sweeney, H.C. and Graf, G.C. (1956). Faecal chromic oxide concentration in 12 dairy cows as related to time and frequency of administration and to feeding schedule. *J. Nutr.* 58: 11.

Harris, L.E.; Lofgreen, G.P.; Kercher, C.J.; Raleigh, R.J. and Bohman, V.R. (1967). Techniques of research in ranch livestock nutrition. *Utah Agric. Exp. St. Bull. No. 471*.

Harrison, F.A.; Laby, R.H. and Mangan, J.L. (1981). A slow release marker capsule for studies of digestion in sheep. *J. Physiol.* 319: 1P.

Harrison, F.A.; Laby, R.H. and Mangan, J.L. (1982). The release and movement of chromic oxide from a capsule in the rumen of sheep. *Proc. Nutr. Soc.* 41: 55A.

Hodgson, J. (1968). The relationship between the digestibility of a sward and the herbage consumption of grazing calves. *J. Agric. Sci. (Camb.)* 70: 47.

Hodgson, J. (1971). The development of solid food intake in calves. 5. The relationship between liquid and solid food intake. *Anim. Prod.* 13: 593.

Hodgson, J. (1982). Ingestive behaviour. In: "Herbage Intake Handbook" Edited by J.D. Leaver. British Grassland Society pp. 113-135.

Holmes, C.W. and Wilson, G.F. (1984). In: "Milk production from pasture" Butterworths, Wellington.

Holmes, W.; Jones, J.G.W. and Drake-Brockman, R.M. (1961). The feed intake of grazing cattle. II. The influence of size of animal on feed intake. *Anim. Prod.* 3: 251.

Holmes, W. (ed.) (1980). Grazing management. In: "Grass its production and utilization". British Grassland Society, p. 129.

Hopper, J.T.; Hollaway, J.W. and Butts, W.Jr. (1978). Animal variations on chromium sesquioxide excretion. *J. Anim. Sci.* 46: No. 4: 1096.

Hoogendoorn, C.B.J. (1986). Studies on the effects of grazing regime on sward and dairy cow performance. Ph.D. Thesis, Massey University.

Instrumentation Laboratory Inc. (1981) Instrumentation Laboratory Atomic Absorption/Atomic Emission (ILAA/AE). Operators manual. Jonspin Road, Wilmington, Maryland, USA.

Irvin, H.M.; Wiseman, H.G.; Shaw, J.C. and Moore, L.A. (1953). The rate of plant pigements in digestion trial studies. *J. Anim. Sci.* 12: 541.

Johnes, D.I.H. and Hayward, M.V. (1973). A cellulase digestion technique for predicting the dry matter digestibility of grasses. *J. Sci. Food Agric.* 24: 1419.

Johnson, D.E.; Dinusson, W.E. and Bolin, D.W. (1964). Rate of passage of chromic oxide and composition of digesta along the alimentary tract of wethers. *J. Anim. Sci.* 23: 499.

Jones, L.H.P. and Handreck, K.A. (1965). The relation between the silica content of the diet and the exccetion of silica by sheep. *J. Agric. Sci. (Camb.)* 65: 129.

Kaiser, A.G. (1976). The effects of milk feeding on the pre- and post-weaning growth of calves, and on stomach development at weaning. *J. Agric. Sci. (Camb.)* 87: 357.

Kane, E.A.; Ely, E.R., Jacobson, W.C. and Moore, L.A. (1953). A comparison of various digestion trial techniques with dairy cattle. *J. Dairy Sci.* 36: 325.

Kay, M.; MacLeod, N.A. and McLaren, M. (1970). Nutrition of the early weaned calf XI. Intake of diets differing in energy concentration. *Anim. Prod.* 12: 413.

Keane, M.G. and Harte, F.J. (1982). Supplementation of grass-fed calves. 1. Effects of milk feeding method and concentrates with and without protected protein on intake and performance. *Ir. J. Agric. Res.* 21: 105.

Keane, M.G. (1982). Supplementation of grass-fed calves. 2. Effects of different levels of milk and concentrates on intake and performance. *Ir. J. Agric. Res.* 21: 117.

Kellaway, R.C. (1969). The estimation of digestible energy intake from forages by ruminants. *Aust. J. Exp. Agric. Anim. Husb.* 9: 578.

Kennedy, W.K.; Carter, A.H. and Lancaster, R.J. (1959). Comparison of faecal pigments and faecal nitrogen on digestibility indicators in grazing cattle studies. *N.Z. J. Agric. Res.* 2: 627.

Kimura, F.T. and Miller, V.L. (1957). Improved determination of chromic oxide in cow feed and faeces. *J. Agric. Food Chem.* 5: 216.

Kitagawa, K. and Yanagisawa, M. (1980). Spectroscopic study of atomization process and inter-element effects on the flame emission of chromium and iron in air-acetylene flame. *Anal. Chim. Acta* 115: 121.

Kivimae, A. (1960). Estimation of digestibility of grassland crops from their chemical composition. *Proc. 8th Int. Grassld. Congr.* pp. 466.

Kotb, A.R. and Luckey, T.D. (1972). Markers in nutrition. *Nutr. Abstr.* 42: 813.

Krohn, C. and Konggaard, S.P. (1976). The use of chromic oxide (Cr_2O_3) for determining the individual feed intake in group-fed dairy cows. *Acta Agriculture Scandinavia* 26: 251.

Laby, R.H. (1980). Controlled release in animal production; modern technology developments. *Proc. Aust. An. Prod.* 13: 6.

Laby, R.H.; Graham, C.A.; Edwards, S.R. and Kautzner, B. (1984). A controlled release intra ruminal device for the administration of faecal dry matter markers to the grazing ruminants. *Can. J. Anim. Sci.* 64(Suppl): 337.

Lambourne, L.J. (1955). Recent development in the field study of sheep nutiriton. *Proc. N.Z. Soc. Anim. Prod.* 15: 36.

Lambourne, L.J. (1957a). Measurement of feed intake of grazing sheep. I. Rate of passage of inert reference substance material through the ruminant digestive tract. *J. Agric. Sci. (Camb.)* 48: 273.

Lancaster, R.J. (1954). Measurement of feed intake of grazing cattle and sheep. V. Estimation of feed-to-faeces ratio from the nitrogen content of the faeces derived from the pasture. *N.Z. J. Sci. Tech.* 36A: 15.

Langlands, J.P.; Corbett, J.L.; McDonald, I. and Reid, C.W. (1963a). Estimation of faeces output of the grazing animals from the concentration of chromium sesquioxide in a sample of faeces. 1. Comparison of estimates from samples taken at fixed times of the day with faeces output measured directly. *Br. J. Nutr.* 17: 211.

Langlands, J.P. (1967b). Studies on the nutritive value of the diet selected by grazing sheep. III. A comparison of oesophageal fistula and faecal index techniques for the indirect estimation of digestibility. *Anim. Prod.* 9: 325.

Langlands, J.P. (1975). Techniques for estimating nutrient intake and its utilization by grazing ruminants. In: "Digestion and metabolism in ruminants". Edited by A.C.I. Warner, University of New England Publication, Armidale, Australia pp. 320-332.

Langlands, J.P. (1987). Assessing the nutrient status of the herbivores. In: "The nutrition of the herbivores". Edited by J.B. Hacker and J.H. Ternouth. Academic Press, Australia pp. 363-390.

Leaver, J.D.; Campling, R.C. and Holmes, W. (1969). The effect of level of feeding on the digestibility of diets for sheep and cattle. *Anim. Prod.* 11: 11.

Le Du, Y.L.P. and Penning, P.D. (1982). Animal based techniques for estimating herbage intake. In: "Herbage Intake Handbook". Edited by J.D. Leaver. The British Grassland Society pp. 37-76.

Lee, J. (1981). Evaluation of the inductively-coupled argon plasma emission spectrometer during 'bedding-in' and its performance on the analysis of biological materials. A.B.D. Tech. Rep. No. 3, DSIR, New Zealand pp. 1-40.

Lee, J.; Fisher, M.T. and Marè, B. (1986). Comparison of Techniques for Chromium Sesquioxide Analysis in Marker Studies. *J. Sci. Food Agric.* 37: 366.

Le Neindre, P. (1981). Influence of milk and concentrates on intake, digestibility and performance of beef calves. Proc. IVth European Grazing Workshop, Theix, France.

MacRae, J.C. (1974). The use of intestinal markers to measure digestive function in ruminants. *Proc. Nutr. Soc.* 33: 147.

Mannetje, L.'t (ed) (1978). Measuring quantity of grassland vegetation. In: "Measuring of grassland vegetation and animal production". Commonwealth Agricultural Bureaux, Hurley, Berkshire, England No. 52 pp. 63-95.

Mayes, R.W. and Lamb, C.S. (1984). The possible use of n-alkanes in herbage as indigestible faecal markers. *Proc. Nutr. Soc.* 43: 39A.

Mayes, R.W.; Lamb, C.S. and Colgrove, P.M. (1986). The use of dosed and herbage n-alkanes as markers for the determination of herbage intake. *J. Agric. Sci. (Camb.)* 107: 161.

McDonald, P.; Edwards, R.A. and Greenhalgh, J.F.D. (1981). In: "Animal Nutrition" (3rd Ed.) Longman, London p. 204.

Meijs, J.A.C. (1981). Herbage intake by grazing dairy cows. Agricultural Research Report 909. Centre for Agricultural publication and documentation, Wageningen, The Netherlands.

Meijs, J.A.C.; Walters, R.J.K. and Keen, A. (1982). Sward methods. In: Herbage Intake Handbook. Edited by J.D. Leaver. The British Grassland Society, pp. 11-36.

Ministry of Agriculture, Fisheries and Food (1975). Energy allowances and feeding systems for ruminants. Technical Bulletin 33, HMSO, London.

Minson, D.J.; Taylor, J.C.; Alder, F.E.; Raymond, W.E.; Rudman, J.E.; Line, C. and Head, M.J. (1960). A method for identifying the faeces produced by individual cattle or groups of cattle grazing together. *J. Brit. Grassld. Soc.* 15: 86.

Minson, D.J. and Kemp, C.D. (1961). Studies in the digestibility of herbage. IX. Herbage and faecal nitrogen as indicators of herbage organic matter digestibility. *J. Brit. Grassld. Soc.* 16: 76.

Moore, J.H. (1959). The use of indicators in the digestibility studies. *Agric. Prog.* 34: 48.

Moran, J.B., Lemerle, C. and Trigg, T.E. (1987). Excretion patterns of chromium sesquioxide in dairy cows and sheep. *J. Aust. Inst. Agric. Sci.* 53:(4) 290.

Moule, G.R. (ed.) (1965). Field investigations with sheep. In: "A manual of techniques". Commonwealth Scientific and Industrial Research Organization publication.

NRC (1978). National Research Council. The Nutrient Requirements of Dairy Cattle (5th Ed). National Academy of Science, Washington, D.C.

Neal, D.L. and Neal, J.L. (1973). Uses and capabilities of electronic capacitance instruments for estimating standing herbage. *J. Brit. Grassld. Soc.* 28: 81.

Nicoll, G.B. and Sherington, J. (1984). Variation in chromium excretion in suckled cows at pasture. *Anim. Prod.* 39: 1.

Nitsan, Z.; Volcani, A.; Hasdai, A. and Gordin, S. (1971). Soya protein substitute for milk protein in milk replacers for suckling calves. *J. Dairy Sci.* 55: 811.

Pigden, W.J. and Brisson, G.J. (1956). Effect of frequency of administration of chromic oxide on its faecal excretion pattern by grazing wethers. *Can. J. Agric. Sci.* 36: 146.

Pigden, W.J. and Brisson, G.J. (1957). Note on a chromic oxide pellet to provide uniform release of this indicator in the rumen of cattle. *Can. J. Anim. Sci.* 37: 185.

Preston, T.R.; Whitelaw, F.G.; MacLeod, N.A. and Philip, E.B. (1965). The nutrition of the early weaned calf. VII. The effect of nitrogen retention of diets containing different levels of fish meal. *Anim. Prod.* 7: 53.

Raymond, W.F. (1954). Studies in the digestibility of herbage. III. The use of faecal collection and chemical analysis in pasture studies. a) Ratio and tracer methods. *J. Brit. Grassld. Soc.* 9: 61.

Raymond, W.F. and Minson, D.J. (1955). The use of chromic oxide for estimating the faecal production of grazing animals. *J. Brit. Grassld. Soc.* 10: 282.

Reid, J.T.; Woolfold, P.G.; Richards, C.R.; Kaufman, R.W.; Loosli, J.K.; Turk, J.I. and Blaser, R.E. (1950). A new indicator method for the determination of digestibility and consumption of forage by ruminants. *J. Dairy Sci.* 33: 60.

Reid, T.C. (1986). Comparison of autumn/winter with spring pastures for growing beef cattle. *Proc. N.Z. Soc. Anim. Prod.* 46: 145.

Roofayel, R.L. and Lyons, D.J. (1984). Determination of Marker Chromium in Faeces using Inductively Coupled Plasma Emission Spectrometry. *Analyst* 109: 523.

Schurch, A.F.; Lloyd, L.E. and Crampton, E.W. (1950). The use of chromic oxide as an index for determining the digestibility of a diet. *J. Nutr.* 41: 629.

Shelton, D.C. and Reid, R.I. (1960). Measuring the nutritive value of forages using *in vitro* rumen technique. *Proc. 8th Int. Grassld. Congr.* pp. 524.

Smith, A. and Reid, J.T. (1955). Use of Cr_2O_3 as an indicator of faecal output for the purpose of determining the intake of pasture herbage by grazing cows. *J. Dairy Sci.* 38: 515.

Steel, R.G.D. and Torrie, J.H. (1980). Principles and procedures of statistics: A Biometrical Approach. McGraw-Hill Book Company, N.Y.

Stevenson, A.E. (1962). Measurment of feed intake by grazing cattle and sheep. VIII. Some observations on the accuracy of the chromic oxide technique for the estimation of faecal output of dairy cattle. *N.Z. J. Agric. Res.* 5: 339.

Stobbs, T.H. and Cowper, L.J. (1972). Automatic measurements of the jaw movements of the dairy cows during grazing and rumination. *Trop. Grassld.* 6: 67.

Stobbs, T.H. (1973a). The effect of plant structure on the intake of tropical pasture. 1. Variation in the bite size of grazing cattle. *Aust. J. Agric. Res.* 24: 809.

Thomas, D.B. and Hinks, C.E. (1983). A note on optimum level of roughage inclusion in the diet of the early-weaned calf. *Anim. Prod.* 36: 299.

Tilley, J.M.A. and Terry, R.A. (1963). A two-stage technique for the *in-vitro* digestion of the forage crops. *J. Brit. Grassld. Soc.* 18: 104.

Torell, D.T. (1954). An oesophageal fistula for animal nutrition studies. *J. Anim. Sci.* 13: 878.

Troelsen, J.E. (1965b). Sustained release of chromium oxide in the rumen of sheep from a Cr_2O_3 paper pellet. *Anim. Prod.* 7: 239.

Tyrell, H.F. and Reid, J.T. (1965). Prediction of energy values of cows milk. *J. Dairy Sci.* 48: 1215.

Uden, P.; Colluci, P.E. and Soest, P.J. van (1980). Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. *J. Sci. Food Agric.* 31: 625.

Valderrabano, J. (1979). Techniques of measuring intake by grazing sheep. M. Phil. Thesis, University of Reading.

Van Dyne, G.M. and Meyer, G.H. (1964a). A method for measurement of grazing livestock using micro-digestion techniques. *J. Range Mgmt.* 17: 204.

Van Es, A.J.H. (1978). Feed evaluation for ruminants. The system in use from May 1977 in the Netherlands. *Livst. Prod. Sci.* 5: 331.

Van Soest, P.J. (1963). Symposium on nutrition, forage and pasture: New chemical procedures for evaluating forages. *J. Anim. Sic.* 23: 838.

Van Soest, P.J. (1982). In: "Nutritional ecology of ruminants". O & B. Books, Inc. Corrallis, Oregon. U.S.A.

Vermorel, M. (1978). Feed evaluation for ruminants. 2. The new energy system proposed in France. *Livst. Prod. Sci.* 5: 347.

Williams, C.H.; David, D.J. and Ismaa, O. (1962). The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. *J. Agric. Sci. (Camb.)* 59: 381.

Wittayanuparpyuenyong, K. (1987). The comparison of supplements for young calves grazing autumn pasture. M. Agric. Sci. Thesis Massey University.

Yanagisawa, M.; Suzuki, M. and Takechi, T. (1970). Cationic interferences in the atomic absorption spectrophotometer of chromium. *Anal. Chim. Acta* 52: 386.

A P P E N D I X

Table 1 Analysis of variance of Cr concentration in faeces by using High Bromate (H and Low Bromate (LB) for different rations

SOURCE OF VARIATION	DF	SS	MS	F-value	Significance
Treatments	3	249.731	83.244	32.326	***
Method	1	242.409	242.409	94.134	***
Interactions	3	14.055	4.685	1.819	NS
Residual	32	82.404	2.575		
Total	39	588.598	15.092		

Table 2 Analysis of variance on Cr release rate for three blocks

SOURCE OF VARIATION	DF	SS	MS	F	Significance
Blocks	2	1446.33	723.17	2.947	NS (0.1284)
Treatments	3	1874.22	624.74	2.546	NS (0.1522)
Error	6	1472.37	245.39		
Total	11	4792.92			

Table 3 Analysis of variance on Cr Recovery for three blocks

SOURCE OF VARIATION	DF	SS	MS	F-Value	Significance
Treatments	3	1182.57	394.19	1.96	NS (0.199)
Error	8	1610.24	201.28		
Total	11	2792.81			

Table 4 Analysis of variance for within day times of faecal sampling for Cr concentration for Block II Period 2

SOURCE OF VARIATION	DF	SS	MS	F-Value	Significance
Treatments (calves)	3	19.89	6.63	2.67	NS (0.0565)
Times	2	0.06	0.03	0.01	NS (0.9873)
Error	54	134.04	2.48		
Total	59	153.99			

Table 5 The relationship between NR (g d^{-1}) and DEl (MJ d^{-1}) for the three Blocks (NR figures are means of two periods within a Block)

	TREATMENT							
	S		P		L		G	
Replicates	NR	DEl	NR	DEI	NR	DEl	NR	DEl
I	13.28	26.70	-0.13	20.36	10.63	19.42	5.24	24.44
II	-0.13	13.63	-4.42	13.71	17.26	20.79	-3.24	13.31
III	17.01	25.99	14.91	25.50	16.99	28.34	3.63	22.90

Table 6 Energy utilisation by calves fed different rations during the experimental period (5-day Means)

Block		TREATMENTS S	P	L	G
I	Total EI (MJ/calf/d)	(26.70)	(20.36)	(19.42)	(24.44)
	Faecal EO (MJ/calf/d)	(5.64)	(3.47)	(3.15)	(4.75)
	Apparent DEI (MJ/calf/d)	(21.06)	(17.09)	(16.27)	(19.69)
	DE Concentration	(.79)	(.84)	(.84)	(.81)
	Urinary EO (MJ/calf/d)	(1.33)	(1.59)	(2.10)	(1.71)
	Est. Methane Loss (MJ/calf/d)	(2.53)	(2.05)	(1.95)	(2.36)
	Est. MEI (MJME/calf/d)	(17.20)	(13.45)	(12.22)	(15.62)
	ME Concentration (MJME/kgDM)	(12.06)	(13.81)	(13.62)	(11.94)
II	Total EI (MJ/calf/d)	12.37(24.28)	12.64(23.82)	19.84(27.75)	13.53(21.73)
	Faecal EO (MJ/calf/d)	3.20(6.20)	3.85(5.20)	2.69(3.33)	3.09(5.57)
	Apparent DEI (MJ/calf/d)	9.17(18.08)	8.79(18.62)	17.15(24.42)	10.44(16.16)
	DE Concentration	.74(.74)	.70(.78)	.86(.88)	.77(.74)
	Urinary EO (MJ/calf/d)	1.05(1.38)	1.09(1.57)	1.02(0.98)	1.42(1.08)
	Est. Methane Loss (MJ/calf/d)	1.10(2.17)	1.05(2.23)	2.06(2.93)	1.25(1.94)
	Est. MEI (MJME/calf/d)	7.02(14.53)	6.65(14.82)	14.07(20.51)	7.77(13.14)
	ME Concentration (MJME/kgDM)	10.69(11.46)	10.30(12.37)	14.98(15.51)	10.89(11.73)
III	Total EI (MJ/calf/d)	31.04(34.22)	30.72(34.28)	32.53(36.41)	27.92(35.47)
	Faecal EO (MJ/calf/d)	5.87(7.42)	6.50(7.51)	5.92(6.35)	8.01(9.58)
	Apparent DEI (MJ/calf/d)	25.17(26.80)	24.22(26.77)	26.61(30.06)	19.91(26.89)
	DE Concentration	.81(.78)	.79(.78)	.82(.83)	.72(.73)
	Urinary EO (MJ/calf/d)	1.57(1.67)	1.78(1.97)	1.43(1.48)	2.15(1.88)
	Est. Methane Loss (MJ/calf/d)	3.02(3.22)	2.90(3.21)	3.19(3.67)	2.39(3.11)
	Est. MEI (MJME/calf/d)	20.58(21.91)	19.54(21.59)	21.99(24.98)	15.37(20.90)
	ME Concentration (MJME/kgDM)	12.68(12.32)	12.95(12.92)	13.84(14.27)	10.71(11.58)
+ Averages:					
	Total EI (MJ/calf/d)	21.71(29.25)	21.68(29.05)	26.19(32.08)	21.73(28.60)
	Faecal EO (MJ/calf/d)	4.54(6.81)	5.18(6.36)	4.31(4.83)	5.55(7.58)
	Apparent DEI (MJ/calf/d)	17.17(22.44)	16.51(22.70)	21.88(27.24)	15.18(21.03)
	Urinary EO (MJ/calf/d)	1.31(1.53)	1.44(1.77)	1.23(1.23)	1.79(1.48)
	Est. Methane Loss (MJ/calf/d)	2.06(2.70)	1.98(2.72)	2.63(3.30)	1.82(2.53)
	Est. MEI (MJME/calf/d)	13.80(18.22)	13.10(18.20)	18.03(22.75)	11.54(17.02)

\+ Blocks II and III
Bracketed figures = Period 2
Unbracketed figures = Period 1