AN ASSESSMENT OF INTRARUMINAL CHROMIUM CONTROLLED RELEASE CAPSULES FOR MEASURING FEED INTAKE IN DAIRY COWS

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of the requirements for the degree of

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SATRIA NUSANTARA NASUTION

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TABLE OF CONTENTS

CHAPTER THREE

GENERAL DISCUSSION AND CONCLUSIONS

APPENDICES

TABLES

FIGURES

ABBREVIATIONS

cm	= centimetres
cm^3	= cubic centimetres
Cr	= chromium
CV	= coefficient of variation
^oC	= degrees Centigrade
Cr_2O3	= chromium sesquioxide
d	= day
DGLS	= Directorate General of Livestock Services
DM	= Dry Matter
DMD	= Dry Matter Digestibility
DMI	= Dry Matter Intake
eg	= example
eqn	= equation
g	= gram
ha	= hectare
h	= hour
kg	= kilogram
km	= kilometres
l	= litre
m^2	= square metres
mg	= milligram
mm	= millimetres
NS	= not significant
OM	= organic matter
OMD	= organic matter digestibility
OMI	= organic matter intake
ppm	= part per million
SE	= standard error
μg	= micrograms
%	= percentage

CHAPTER ONE

INTRODUCTION

BACKGROUND

Indonesia is a tropical country that has an east-west length of 5000 km from 92^{o} to 141^{o} east longitude and a width of 1500 km from 6^{o} to 11^{o} south latitude (Indonesia Official Year Book 1989). Approximately 60% of total employment is related to the agricultural sector and 70% of non-oil exports are attributable to agriculture (The Fourth Five Year Development Plan 1984). The livestock industry is closely integrated with crop production, particularly in Java and Bali. In other areas, livestock plays a significant role in family life as draft power, particularly in the transmigration areas. In East Indonesia (East Nusatenggara and South Sulawesi) conventional grazing systems are of greater importance. The 1987 Indonesian livestock population comprised approximately 7 million draft cattle, 0.3 million dairy cattle, 10 million goats, 5 million sheep, 2 million buffaloes and 211 million poultry (Table 1.1).

As the fifth largest populated country in the world in 1990 (180 million) Indonesian people consumed 2.24, 4.18, 5.34 kg of egg, milk and meat per capita per annum. This is projected to increase by 1993 to 2.52, 4.54 and 6.79 kg/person of egg, milk and meat respectively (Djarsanto 1990), but this will still remain the lowest consumption of animal protein among South East Asia countries (FAO 1989). To meet the projected increase in consumption of animal products the population and production of Indonesian livestock will need to be increased to meet the needs of a population which is growing at the rate of 2.60% per annum (The Fourth Five Year Development Plan 1984).

Table 1.1 Livestock numbers and agriculture production in New Zealand and Indonesia.

		New Zealand	Indonesia
Total area (000, sk km)		268[a]	914
Forest (000,000 ha)		1.1[b]	121[c]
Pasture	improved (000,000 ha)	8[c]	negligible
	native (000,000 ha)	6	4
Foodcrop	Rice (000,000 ha)	negligible	31
Topography	Flat (%)	5[d]	No data
	Hill (%)	90	No data
	Steep (%)	5	No data
Human population (000,000 ha)		3.3[a]	180
Livestock population[a,b,e,f] (000,000 heads)			
Dairy cattle		3.3[a]	0.3
Beef/draft/cattle		4.5	7
Buffalo		negligible	2.5
Sheep		60	5
Goat		1.2	10.7
Deer		0.78	No data
Pigs		0.41	2.6
Poultry		3	211

Sources:

[a] MAF (1990)

[b] New Zealand Official Year Book (1988-1989).

[c] Whiteman (1979)

[d] Langer (1977).

[e] Livestock Sector Review-DGLS (1986).

[f] Indonesian Agriculture Statistics (1983).

In contrast, New Zealand is a temperate country (1600 km long and 450 km width) which lies from 33° to 53° south, and from 169° east to 173° west (New Zealand Official Year Book 1988-1989). The land area is 29% of that of Indonesia, but the area of improved and native grasslands are substantially greater (Table 1.1). The improved pastures consist predominantly of perennial ryegrass (Lollium sp.) and white clover (Trifolium repens). These species are relatively easy to manage and are symbiotic in the sense that legumes fix nitrogen which boosts ryegrass growth (Korte et al. 1987). In 1989 the New Zealand livestock population was approximately 3.3 million dairy cattle, 4.5 million beef cattle, 1.2 million goats, 0.78 million deer and 60 million sheep (MAF 1990) (Table 1.1).

New Zealand's agriculture is orientated towards export of the products from livestock farming, while these products contributed very little to Indonesian export earnings, in fact Indonesia is a net of importer of these products (Table 1.2).

Table 1.2 The value (NZ$ million) of agricultural exports from New Zealand and imports ('000 tonnes/'000 head) to Indonesia.

	New Zealand	Indonesia
Dairy and dairy products	2,234	448
Beef and veal	1,279	1.1
Live animals	2.1	3
Lamb and mutton	851	none
Poultry	none	none
Pigs	none	none
Forestry	786	No data
Cereals	237	No data
Fruit vegetables	824	Negligible

Sources:

MAF (1990).

Statistics on Livestock-DGLS (1987).

New Zealand's favourable environment for pasture production substantially reduces the cost of production compared to those incurred under intensive Northern Hemisphere indoor concentrate and roughage feeding systems (Scott 1981). This provides a comparative export advantage to New Zealand for pastoral products.

Indonesian livestock production, which is increasing by approximately 3% per annum (The Fourth Five Year Development Plan 1984), can be broadly classified into two systems. The largest system (in numerical terms) is livestock integrated with a cropping system (mainly rice); the second is a ranch grazing system based on native (unimproved) pasture. The average number of animals owned by individual farmers is low (e.g. for dairy cows 3 to 4 animals/farmer and for draft cattle 2 animals/farmer) (Livestock Sector Review-DGLS 1986). A small area (less than 5%) of improved pasture mainly elephant grass (Pennisetum purpereum), brachiaria (Brachiaria decumbens) and a tropical legume (Leuecaena leucocephala) have been developed primarily in Java, Bali, Nusatenggara, South Sulawesi and Sumatera (Livestock Sector Review-DGLS 1986). The crop-based system can be found in regions where the human population density is high (e.g. Java). A twice daily cut and carry feeding system for livestock is used. Feed is harvested from the terraces and embankments of the rice fields. In addition, residue from secondary vegetable crops (e.g. maize stovem) is used. Some feed is also harvested from the banks of floodways and irrigation canals (Livestock Sector Review-DGLS 1986). Concentrates such as soybeans, peanuts and coconut by-products are sometimes added to the herbage to improve animal growth rates. For the most part the herbage yield and quality of the grasses, weeds and other native vegetation consumed by livestock is low, with a dry matter digestibility of between 55% and 60% (Livestock Sector Review-DGLS 1986). The ranch grazing systems are mainly located outside of Java where the human population is lower e.g. Nusatenggara and South Sulawesi. The animals are grazed throughout the year under a set stocking system at an average stocking rate of 2 beasts/ha. Pasture availability and quality are heavily dependent upon seasonal conditions. The predominant native grass Imperata cylindrica is of poor quality with an average crude protein content of 6% DM (compared to 30% in white clover (Holmes and Wilson 1984)). The annual production of this native grass is around 6800 kg DM/ha/year (Chadhokar 1977). Animal production on these pastures is typically low (e.g. a mature Bali bull may only reach a live weight o 220 kg at 4 years of age (Bamualim 1986)). Mining and forestry activities are more dominant than agriculture with areas of native grassland supporting less than 0.5 million cattle on the less developed islands of Kalimantan and Irian Jaya.

One of the main constraints to Indonesian farmers who wish to improve their animal production efficiency is lack of capital e.g. in 1983 a farmer's income from East Nusatenggara was $US 136/year (Bamualim 1986). Government funds for investment in agriculture are limited but so far approximately 200,000 cattle have been credited to smallholders throughout Indonesia (Statistical Book on livestock-DGLS 1987). These farmers are supported by 9 forage stations in east, central and west Indonesia that provide and sow improved grass and legume pastures for the farmers.

The approximate distribution of cattle breeds and their respective populations in Indonesia are shown on the Table 1.3.

Table 1.3 The distribution and population of cattle breeds in Indonesia (Everitt 1978).

Breed	Population (million)	Area (location)
Ongole	5.50	Java and Sumba
Bali	1.60	Bali, Nusatenggara, South-Sulawesi
Madura	0.30	Madura and Java
North Sumatera	0.40	North Sumatera
Grati	0.01	Java
Friesian[a]	0.30	Java

aThis is the main exotic dairy breed, the remaining breeds are indigenous breeds.

Table 1.3 indicates that the Ongole is the most common cattle breed accounting for two thirds of all Indonesian cattle. This breed is mostly located on the Java and Sumba Islands. Bali cattle, which account for 20% of the cattle population, are involved in a New Zealand Indonesia breed development programme (Pane and Packard 1986). These cattle are tolerant of high temperatures and external parasites (Packard et al. 1990). In 1989 the Indonesian Government decided to use the improvement programme for Bali cattle in Bali and Sumbawa as a "model" for the national improvement of Bali cattle in Indonesia (Packard et al. 1990). The Friesian breed is found only in Java and contributes to less than 5% of the total cattle population. However, Friesians are becoming more popular as the demand for milk increases. Friesians have been imported from several countries over the past 15 years, including New Zealand. The Grati breed is the only indigenous breed suitable for milk production but their productivity is still low compared to that of Friesians (Table 1.4).

Table 1.4 Performance of Indonesia cattle and exotic breeds under Indonesian management.

Breed Main use	Ongole draft/ beef	Bali draft/ beef	Madura draft/ beef	Grati milk	Friesian milk
Birthweight (kg)	20	16	14	22	24
Mature liveweight					
male (kg)	430	400	300	500	450
female (kg)	320	300	200	400	375
Average daily gain					
(kg/day)[a,c]	0.75	0.66	0.60	0.90	no data
Dry matter intake					
(kg/day)[c]	6.42	6.02	5.33	7.97	no data
Milk yield					
(kg/lactation)	400	250	200	2200	2900[b]

Sources:

[a]Everitt (1978).

[b]Soewardi (1986).

[c]Fed with high concentrates (Livestock Sector Review-DGLS 1986).

The average milk production of Friesians in Indonesia (c. 9.5 litres/d) is much lower than for Friesians in New Zealand (14.5 l/d; Holmes and Wilson 1984). Nevertheless, if native breeds are managed on high quality feed (improved grass-legume pastures and high quality concentrates), Bali, Madura and Ongole bulls are able to reach liveweights of 546, 520 and 745 kg respectively at a mature age (4 years) while Bali, Madura and Ongole cows reach 424, 452 and 562 kg respectively (Livestock Sector Review-DGLS 1986).

The number of farms with dairy cows is relatively small. These are mostly managed under stall feeding systems where commercial concentrate feeds are used. Dairy farms are mainly located near to large urban centres. The development of the dairy industry is concentrated in Java because of the availability of surplus crop residue, and high per capita milk consumption (compared to the rest of Indonesia). The milk processing industry is also relatively well developed and has the capacity to absorb additional milk production. There are also credit programmes, husbandry and extension services available to farmers. In the Java environment a "cut and carry" system with improved pastures and limited concentrate feeding integrates best with the main use of land for food crops.

In regions where the human population is growing rapidly (e.g. Java) the requirement for food crops on a declining area of land is increasing. To increase the land area available for crops, less profitable livestock are being sold. This is increasing the rate of livestock depopulation. Thus, the Indonesian Government needs to prepare other islands to support the livestock population otherwise farmed in the highly populated, intensively cropped regions. This will require the development of native grasslands if increased and profitable levels of animal production are to be achieved (Tillman 1981).

The form of livestock feeding influences local research priorities. In Java, the main interest in livestock research is the use of surplus crop residues as a feed source. There have been relatively few grazing studies conducted in Indonesia due to the difficulty in obtaining feed intake measurements in grazing animals (Rika et al. 1981; Rudolf et al. 1988). The situation is similar in Malaysia (Dunsmore and Ong 1969) and Thailand (Hongyantarachai et al. 1989), where stall feeding experiments are also more common than grazing studies. In contrast, New Zealand, has adopted the metabolisable energy system from Agricultural Research Council (ARC 1980), to develop a quantitative estimates of energy and protein requirements for pastoral systems. This has been supplemented with intake and production data from grazing experiments (e.g. Nicol 1987). Indonesia has not yet developed a standardised set of feed requirements for native livestock on tropical pastures. However, several systems have been used. This includes the TDN (Total Digestible Nutrient) system and the Kellner method (Lubis 1963; Livestock Sector Review-DGLS 1986). Information on the nutritive value for most of the improved grass and legume species is available (DGLS 1986), but the Nutrient Research Council (NRC) is still used as the main reference. There is virtually no feed intake data for Indonesian pastures available to

extension personal. As a consequence, relatively little is known about the efficiency of livestock production on tropical pastures (both "improved" and "unimproved") in Indonesia or in other Southeast Asian countries.

METHODS OF MEASURING FEED INTAKE.

Several intake measurement techniques have been developed in agricultural industrialised countries, including New Zealand, over the past 40 years. New Zealand's pioneer workers included Wallace (1950), Lancaster (1950), Coup (1950) and Percival (1950). Measurement techniques can be broadly classified into animal and pasture-based methods. Animal-based methods can be conducted indoors under a "cut and carry" system or outdoors under field grazing conditions. The former is mainly related to direct measurement of intake for feed evaluation studies while the latter is based on indirect measurement, usually with a faecal marker.

Indoor measurement of feed intake is unable to replicate outdoor grazing conditions because the effects of the animal on diet selection and plant preference, and of climate and grazing activity (walking) are removed (Hodgson 1971; Stobbs 1975; Poppi et al. 1987). Nevertheless, the technique does have advantages. These include simplicity of measurements (i.e. feed offered less feed remaining) and sometimes lower costs, especially if sheep are used in place of cattle. Also an accurate measure of in vivo digestibility and other feed characteristics can be obtained. The animal's environment can be controlled and the use of animals and facilities are not restricted to the forage growing season. Indoor trial results frequently provide the only values which can be compared with those obtained from outdoor studies (Heaney et al. 1969). However, the chemical composition and the nutrient availability from forages may change during storage and the energy requirement for animal activity, particularly for harvesting and walking, are reduced (Heaney et al. 1969).

Outdoor measurement of feed intake is based on the relationship between faecal output and feed digestibility as shown in the following equation (1.1):

$$\text{Intake} = \frac{\text{Faecal output (g DM/d)}}{\text{(1-digestibility)}} \qquad \textbf{(eqn 1.1)}$$

where digestibility (D) is defined as:

$$\frac{\text{Feed intake - faecal output}}{\text{Feed intake}} \times \frac{100}{1} \qquad \textbf{(eqn 1.2)}$$

Faecal output can be measured directly by total collection using faecal collection bags and harnesses or indirectly through the concentration of an indigestible marker in the faeces (Meijs 1981). Total collection is laborious, and the harness and bag equipment disturbs the animal's grazing behaviour, particularly if inadequate training has been provided prior to the collection period (Corbett 1978). In addition, total collection of faeces from female animals is difficult, unless the urinary tract is catheterised.

Indirect measurement of faecal output using markers is based on the ratio between the quantity of marker administered daily and the average daily concentration of the marker in a sample of the faeces (Coup 1950). Thus daily faecal output is calculated as follows:

Faecal output (g DM) =

$$\frac{\text{weight of the marker given (g/d) x RR}}{\text{mean concentration of marker in faeces (g/d DM)}} \qquad \textbf{(eqn 1.3)}$$

where,

RR is the recovery rate of the marker. RR is defined as:

$$[A \times B] / C \qquad \textbf{(eqn 1.4)}$$

Where

A = mean concentration of marker in the faeces (g/g DM).
B = total daily faecal output(g DM/d).
C = total weight of the marker administered (g/d).

The marker technique reduces labour requirements for the collection of faeces, but preparation, administration and analysis of the marker involves considerable work, especially if the marker is administered twice daily.

Substances which are suitable for use as a faecal marker are inert, non-toxic to the animal, can be quantitatively recovered in the faeces, have no appreciable bulk, mix uniformly in the digesta, have no influence on digestion, are indigestible and preferably should be inexpensive (Raymond and Minson 1955; Kotb and Luckey 1972). Two categories of marker are in use. The first are referred to as natural markers. These are a naturally occurring components of the feed (e.g. lignin, chromogen, silica and n-alkanes, Meijs 1981; Mayes 1986). The second group, referred to as external markers, are added to the ration (e.g. in concentrate feeds) or are administered orally to the animal (e.g. chromium sesquioxide (Cr_2O_3), polymers and mineral salts)) (Kotb and Luckey 1972). Chromium sesquioxide is the most commonly used faecal marker (Meijs 1981).

Recovery of faecal markers

Compared to the total collection of faeces only a small proportion of the total faecal output per day is sampled (2 - 5 g fresh sample in sheep) when markers are used. Special care needs to be taken to ensure that:

1. The recovery of the marker from the faeces is consistent and preferably 100% (Coup 1950; Raymond and Minson 1955).

2. The faecal sample collected yields a marker concentration which corresponds to the mean 24 hour concentration of the marker in the faeces (Lancaster 1950; Lambourne 1957a; Brisson 1960; Nicoll and Sherington 1984).

The recovery of the marker depends on both the reliability of the marker assay (Lee et al. 1987) and the accuracy with which faeces are sampled (Lambourne 1957a; Nicoll and Sherington 1984). Factors which can contribute to a recovery of less than 100% of Cr_2O_3 include absorption of soluble chomium compounds (Le Du and Penning 1981), retention of the marker in the digestive tract (Curran et al. 1967), loss of Cr_2O_3 with rumen liquor, and regurgitation of the marker (Meijs 1981). Failure to collect all of the faeces, particularly if bags are used may also result in an apparent incomplete recovery of the marker (Langlands 1987).

Moran et al. (1987) found that the recovery of Cr_2O_3 was 94% in lactating cows, 84% in non-lactating cows and 85% in sheep when gelatin capsules were administered twice-daily in an indoor feeding trial. The reason why recoveries were different between these groups of animals was unclear. It was speculated that a faster rate of feed passage through the higher DM intakes of the lactating dairy cows compared to the non-lactating dairy cows and sheep could have been a contributing factor. This supports the work by Corbett et al. (1958) who stated that the rapid transfer of powdered Cr_2O_3 though the digestive tract would reduce the opportunity for Cr_2O_3 to settle in the ventral sac of the rumen.

To avoid the incomplete recovery of the marker, faeces have to be collected from the animal after a steady state concentration of the marker has been achieved and over a period which is sufficiently long to overcome the day to day variation in the concentration of the marker in the faeces (Brisson 1960). Faecal samples must also be collected at appropriate times within each day of the collection period (see next Section on Diurnal Variation). Hardison et al. (1959) showed that a steady state concentration of Cr_2O_3 was achieved in the faeces after 4-5 days for most grazing situations if Cr_2O_3 was given either in capsule form or as sustained release pellets. Similarly, Lambourne (1957b) demonstrated that a 3-4 day preliminary dosing period was required

before a steady state level of Cr was achieved in sheep when twice daily drenching with gelatin capsules was adopted. Lambourne (1957b) later suggested that this might be further shortened if triple or more doses of the marker were administered each day. A longer period (5-10 days) of preliminary dosing was required in a trial by Crampton and Lloyd (1951) where Cr_2O_3 was mixed in the daily ration of stall fed cows.

Diurnal variation

This is defined as the within-day variation in the concentration of the faecal marker over a 24 hour period (Raymond and Minson 1955; Brisson 1960; Nicoll and Sherington 1984). The magnitude of diurnal variation is influenced by the form of Cr_2O_3 administration (Pigden and Brisson 1957; Corbett et al. 1958; Corbett 1960), the frequency of dosing (Brisson et al. 1957, Langlands et al. 1963 and Kotb and Luckey 1972), the pattern of the feeding (Raymond and Minson 1955; Langlands et al. 1963) and the quality of the feed consumed (Lambourne 1957a).

In most grazing experiments the lowest and the highest daily concentration of the marker in the faeces occur in the morning and afternoon (Lambourne 1957b; Elam et al. 1959). Averaging the concentration of the marker in faecal samples obtained at these two times of the day (e.g. 0900 and 1430 h) has been found to provide a satisfactory estimate of the mean daily concentration of the marker (Kane et al. 1952; Wanyoike and Holmes 1981). Earlier Kane et al. (1952) had found the highest concentration of Cr_2O_3 in dairy cows occurred at 0900 h, but the peak concentration for the natural marker (lignin) was not achieved until 2000 h. Later Elam and Davis (1961) reported that the diurnal variation of lignin excretion was less than that of Cr_2O_3. However, the application of natural markers (such as lignin and chromogen) often give unsatisfactory results due to incomplete recovery of the marker in the faeces (Kane et al. 1953; Elam et al. 1962), loss of material during digestion, or uneven distribution of the natural marker through the plant (Elliot and Fokkema 1960). An effect of drying and heating during the preparation of samples for laboratory analysis could also be a source of error with natural markers (Lancaster 1954; Soest 1964).

Form of Cr2O3 administered

The form in which Cr_2O_3 is administered influences the degree of diurnal variation. Sustained release pellets, made up by mixing of Cr_2O_3 and plaster of paris at different proportions (50:50) and 20:80) were reported by Pigden and Brisson (1957). These pellets reduced the diurnal fluctuations of Cr_2O_3 to between 94 and 117% of the 24 h mean concentration in faeces, compared to 50 to 180% when a gelatin capsule was given once daily. Later it was found that the pellets were easily regurgitated by animals both at pasture and indoors (Corbett et al. 1960). By impregnating Cr_2O_3 into a shredded paper, Corbett et al. (1960) and Langlands et al. (1963a)

found that within-day variation of faecal chromium when administered to duodenum-cannulated sheep was more regular than when Cr_2O_3 was administered in gelatin capsules due to the slower release of Cr_2O_3 from the paper. Variation in the concentration of Cr_2O_3 in faecal samples obtained at different times of the day from cattle was also found when Cr_2O_3 was administered in the form of gelatin capsules (Raymond and Minson 1955). This was confirmed by Moran et al. (1987) who found that the Cr_2O_3 concentration varied between 75% and 115% of the mean daily concentration in the faeces of non-lactating cows dosed twice-daily with gelatin capsules. Diurnal variation was less in sheep and lactating cows under the same dosing regime (Moran et al. 1987). The more irregular excretion of Cr_2O_3 when administered in gelatin capsule form was probably due to the rapid passage of Cr_2O_3 through to the omasum before being properly mixed with the food in the reticulo-omasum.

Balch et al. (1957) found that Cr_2O_3 used in the form of "macaroni" (a flour paste in long tubes) resulted in diurnal variation which was intermediate between the level for paper and gelatin capsules recorded by Langlands et al. (1963a).

Frequency of marker dosing

The magnitude of diurnal variation is influenced by the frequency of dosing. Brisson et al. (1957) showed that Cr_2O_3 administered six times daily, resulted in the marker being released into the faeces in a more constant and uniform manner, compared to once or twice daily dosing. However, dosing more than twice-daily is impractical for most experimental situations. Smith and Reid (1955) found that variation in the concentration of Cr_2O_3 in the faeces of grazing cows, sampled at 0600 and 1600 h on seven consecutive days, was significantly less when twice- rather than once-daily dosing of Cr_2O_3 in gelatin capsules, or a mordanted concentrate feed (offered at the same times as dosing) was used. Elam et al. (1959) also found a significant within-day variation in marker concentration when Cr_2O_3 was administered with mixed pelleted rations to heifers whether they were fed once or twice a day or had free access to the feed throughout the day.

Pattern of feed intake and quality of feed consumed

The rate of passage of inert markers through the digestive tract is influenced by the quality of the feed consumed (Lambourne 1957a). When high quality feed is consumed, the rate of passage of digesta through the animal is increased and the level of diurnal variation in the faeces also increases (Raymond and Minson 1955). The variation is further increased if the pattern of feeding is irregular (Langlands et al. 1963a). This situation is reversed when poor quality feeds, such as hay, are used (Lambourne 1957a). This could also be influenced by the level of feed intake, but Lambourne (1957b) suggested that this was of only secondary importance compared to feed quality.

In summary, it is unlikely that diurnal variation can be eliminated by frequent or continuous administration since it occurs even when natural markers (e.g. lignin) are used in a continuous feeding regime as a faecal indicator (Kane et al. 1952).

Controlled release capsule technology

Intraruminal controlled release capsules (CRC) are the most recent technological development for administering Cr_2O_3 to the ruminant (Laby 1980; Laby et al. 1984). Cr_2O_3 is tabletted in a matrix with a sucrose monostearate. The tablets are inserted into a plastic cylindrical barrel. The present device for cattle has a diameter of 3 cm and are 12.5 cm long (Figure 1.1). The matrix core is extruded through a multi-orifice end plate by the action of a compressed spring plunger.

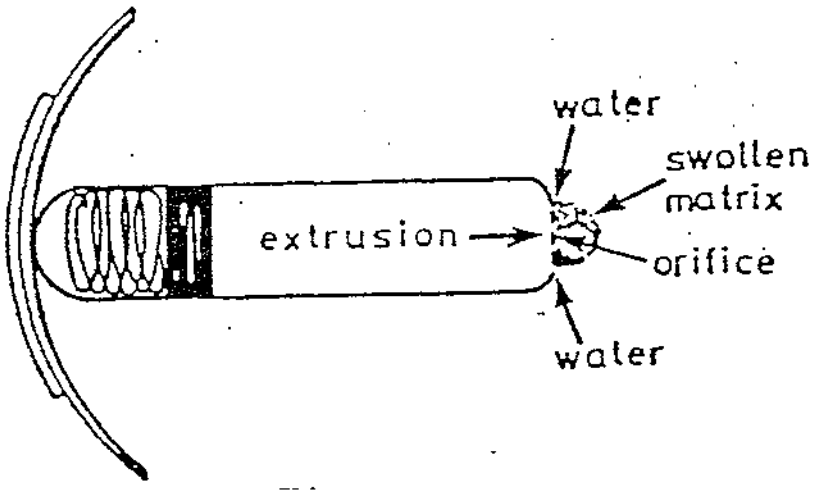

Figure 1.1 Intraruminal controlled release capsule for administration of Cr_2O_3 to cattle.

An elastic strip of plastic, folded against the cylinder barrel for oral dosing, opens into a "T" configuration in the rumen to prevent regurgitation of the capsule. The rate of Cr_2O_3 release is controlled by a combination of the effects of spring strength, orifice diameter and matrix composition (Laby et al. 1984).

Intraruminal CRC have been used to estimate the faecal output of sheep (Ellis et al. 1981; Laby et al. 1984; Lee et al. 1989; Parker et al. 1989; Parker et al. 1990) and cattle (Ellis et al. 1982; Bird et al. 1984; Grainger et al. 1987; Graham et al. 1988; Barlow et al. 1988; Hirschberg et al. 1990). Results from these trials show that the uniform release of Cr_2O_3 provides an excretion pattern of Cr_2O_3 into the faeces which is closely associated with the pattern of feed intake (Ellis et al. 1988). Diurnal variation is reduced to one third of that achieved with twice-daily drenching by gelatin capsules (Ellis et al. 1981). This increases the flexibility of the sampling time within a day (Parker et al. 1989). In sheep a steady state concentration of chromium is established in the faeces between 5 and 8 days after dosing (Parker et al. 1989). Since only a single application of Cr_2O_3 is required, interference to animals while dosing is lower, reducing the possibility of abnormal grazing behaviour, compared to twice daily administration of Cr_2O_3 in the form of impregnated paper or gelatin capsules (Ellis et al. 1981; Lee et al. 1989; Parker et al. 1989).

Ellis et al. (1981) found that the mean release of Cr_2O_3 from cattle CRC was 926 mg/d in 20 grazing steers and 5 rumen-fistulated cows. A steady state of Cr_2O_3 was established on d 2 after dosing and was linear up to d 17. Approximately 11.20 and 12.75 g Cr_2O_3 was released into the digestive tracts of the steers and cows, respectively, over the 17 d period. The difference in Cr_2O_3 released could be due to differences in the rumen environment of the types of cattle (steers and cows). Also disturbance to the releasing surface of CRC in the rumen-fistulated cows could have contributed to a greater loss of Cr_2O_3. The coefficient of variation (CV) of Cr_2O_3 release rate in the cows was smaller (6.5%) than that in the steers (8.5%). It was suggested that a significant proportion of the between-device variability resulted from fluctuations in release rate during the first few days.

Grainger et al. (1988) found that the difference between faecal output of indoor fed dairy cows estimated using CRC Cr_2O_3 was not significantly different from the actual faecal output measured by total collection. Estimates of faecal output derived from the chromium concentration of twice-daily grab samples of faeces were also not significantly different from a sample taken from the total collections bulked over 7 days. Grainger et al. (1988) estimated from these results that CRC could be used to detect small differences (5-10%) in faecal output. Barlow et al. (1988) used CRC to estimate the faecal organic matter output of 287 beef cows of different breeds and hence feed intake at three pasture allowances (high, medium and low) during and after lactation. The CRC used, linearly released Cr_2O_3 between d 3 and d 18. The mean daily release was between 930 and 1250 mg Cr_2O_3/d. The authors concluded that CRC eliminated many of the problems associated with twice daily dosing of Cr_2O_3.

However, the satisfactory result for the CRC in the study of Barlow et al. (1988) contradicts the findings of Bird et al. (1984) who found that there was still considerable variation in delivery rate between capsules (14-22 d of CRC life) and that diurnal variation in the chromium concentration

of faecal samples collected directly from the sward were significantly different. The chromium content in grab samples collected at 0900 h were also 15% lower than the mean of the sward samples collected during the day.

Kassano (1988) found no significant differences in the release rate of Cr_2O_3 from sheep CRC in calves offered different rations (grass plus soy bean, grass and milk powder, and grass and carbohydrate). Faecal output estimated from the concentration of chromium in the faeces did not differ significantly from the actual faecal output of the calves. Hirschberg et al. (1990) used cattle CRC to estimate the feed intake of steers and bulls continuously grazed at three different pasture allowances. Predicted intakes corresponded to those which had been previously published for animals of this liveweight, indicating that CRC had potential for studying feed intake in beef cattle at pasture.

The disadvantages of CRC have been identified in several experiments. First, an accurate measure of Cr_2O_3 release rate is important if absolute (rather than relative) differences in feed intake are required. Release rates can be obtained by three methods : recovery of CRC from slaughtered animals, periodic recovery of capsules from rumen-fistulated animals and the pattern of Cr_2O_3 disappearance from the faeces at matrix expiration (Ellis et al. 1988). In vivo estimates from slaughtered animals are impractical if the animals have a high commercial value or are required for future work. There are some indications that release rate may be decreased in rumen-fistulated animals (Parker et al. 1990), probably because of temperature fluctuations and disturbance to the matrix orifice when capsules are removed for measurement. The pattern of marker disappearance can identify the end point to within ± 0.5 d when twice-daily grab samples are used (Ellis et al. 1988). Endpoint determination considerably increases assay costs. An alternative is to bulk daily samples within treatments to determine an average release rate for the group, since group rather than individual intakes are usually being assessed (Rooden et al. 1988). Incorporation of a distinctive second marker in the final tablet of the matrix would also improve the identification of the endpoint of Cr_2O_3 release (Parker et al. 1990).

A second problem with CRC is absolute failure due to regurgitation of the capsule. Kassano (1988) found that three out of twelve calves (liveweight between 64 and 72 kg) regurgitated their sheep capsules. However, the incidence of capsule regurgitation in sheep is negligible (Parker 1990). Wilson (1989) recorded a 15% regurgitation rate of cattle CRC from Friesian dairy cows (c. 550 kg liveweight). Most capsules were lost from the cows on the low herbage allowance suggesting that CRC loss may be a function of liveweight and level of feed intake.

Methods of faecal collection

The method used to collect samples of faeces is an important part of the procedure for measuring faecal output by the marker technique, particularly when external markers such as Cr_2O_3 are used. Faecal samples can be obtained either per rectum (grab sampling) or from the sward (sward sampling) (Meijs 1981). For animals that are yarded twice daily (e.g. dairy cows), rectal grab sampling can be applied relatively simply because sampling can be conducted with other activities (e.g. milking). When animals are under continuous grazing management, or animal handling is restricted through lack of yarding facilities or by animal factors (e.g. nervous behaviour or young at foot) sward sampling is a practical alternative (Raymond and Minson 1955).

Sward sampling is best suited to situations where a natural marker is used or where a single application system for the external marker (e.g. controlled release capsules) is employed. To identify individual animals when sward sampling is adopted, coloured polysterene granules can be used to mark the faeces (Minson et al. 1960).

Methods of determining Cr concentration in the faeces

Marker techniques for estimating faecal output require an efficient laboratory assay for quantitatively recovering the marker from the faeces. The assay should preferably have few steps and be relatively inexpensive. In this section alternative assays for measuring faecal chromium are discussed.

The common procedures for digesting chromium into solution fall into three categories. These include sodium peroxide (Na_2O_2) fusion (Fenton and Fenton 1979), perchloric acid ($HClO_4$) digestion (Day 1954) and oxidation with a bromate and phosphoric acid solution (Christian and Coup 1954; Williams et al. 1962). Perchloric acid is not favoured because it is potentially dangerous (Fisher at al. 1972).

Three methods of measuring the concentration of Cr in solution are commonly used. These are atomic absorption spectroscopy (AAS), inductively coupled plasma emission spectroscopy (ICPES) and the titration method. The sensitivity of all three methods is similar (Lee et al. 1986), but AAS has the advantage of greater simplicity. An AAS instrument is also available in most nutrition laboratories. The concentration of chromium that can be determined by AAS ranges from 0.8 and 70 ppm with a lower detection limit of 0.15 ppm (Williams et al. 1962). The titration method is less suitable for CRC because of lower Cr concentrations in the faeces compared to either the gelatin capsule or paper methods of Cr_2O_3 administration (Costigan and Ellis 1987).

Digestibility determination

The common measure of nutritive value of herbage is digestibility because it represents the total amount of digestible nutrients per unit of feed intake (Graham 1988). Digestibility (see eqn 1.2) can be defined in terms of DM or OMD (Poppi 1983).

The accurate determination of feed digestibility is critical if it is used to indirectly estimate feed intake because feed intake is calculated by dividing faecal output by the ingestibility of the feed (Langlands 1987) (eqn 1.1). An error of 1% in estimating food digestibility equates to errors of 5, 3.3 and 2.5% respectively in estimating intake when digestibility is 80, 70 and 60%, respectively (Langlands 1987). The digestibility of feed can be determined by in vivo feeding trials or by in vitro laboratory techniques.

To determine digestibility in vivo, animals are fed exact quantities of a feed for a 4 to 8 day adjustment period prior to the measurement of faecal output and feed intake over a 5 to 20 day period.The preliminary period ensures that a steady state of faecal excretion for the feed type consumed is reached prior to measurements commencing (Blaxter et al. 1956; Minson 1981).

The amount of feed that is offered in digestibility trials is important because bias in calculating the feed digestibility coefficient can occur due to the level of feeding used (Minson 1981; Langlands 1987). Two levels of feeding are commonly used, maintenance and ad libitum. Ad libitum feeding occurs when the amount of feed offered per day is approximately 10% greater than the animal's requirement (Hamilton 1981). Maintenance feeding may result in under estimation of feed digestibility due to an inadequate quantity of feed being consumed, while an ad libitum level may overestimate digestibility because the animals are able to select the more digestible portion (e.g. leaf in preference to stem) of the feed on offer.

Sheep are often used in place of cattle in in vivo digestibility studies because of the smaller quantity of the feed required and the higher number of replicates which can be handled (Lancaster 1950; Minson 1981; Rooyen and Merwe 1983). Blaxter and Wainman (1961) found no significant differences between sheep and cattle in the digestiblity of different feeds over a range of feeding levels. In contrast, researchers from the United States have stated that feed digestibility data derived from sheep could not be used for cattle (Moe et al. 974). Schneider and Flatt (1975) found both significant differences and similarities in the data published for most of the sheep and cattle digestion trials conducted in the United States. If there is a difference in the digestive capacity of cattle and sheep, it could be related to the difference in the size of the reticule-omasal orifice, through which digesta passes to enter the lower digestive tract. In sheep it is so small that it is difficult for larger particles to pass through. As a result, the particles are regurgitated and broken down into small pieces, resulting in a higher digestibility after rumination (Rooyen and Merwe 1983).

Rooyen and Merwe (1983) found that sheep consistently digested energy (77.1 vs 68.3%) and crude fibre (55.8 vs 43.9%) more efficiently than cattle at the maintenace levels of feeding that equated to each of the species' body mass, but digestion of crude protein was slightly lower in sheep than cattle (81.8 vs 84.04%) at the low and medium level of feeding. At the high level of feeding, 76% of crude protein was digested by the sheep compared with 75% by the cattle. Diet selection and plant preference between sheep and cattle may also contribute to differences between the two species. Sheep are more selective of green material than cattle when grazed together (Van Dyne and Heady 1965; Hodgson 1982; Collins and Nicol 1986). The larger jaw in cattle and the use of the tongue to prehend feed prevents the precise selection of pasture components that occurs with sheep. As a result, cattle eat a higher ratio of stem to leaf material and more dead material than sheep, resulting in a lower feed digestibility value for cattle compared with sheep (Hacker and Minson 1981). However, sheep can graze closer to the ground because of their biting action and may consume more low quality herbage in some situations. It is concluded that in outdoor situations, sheep are more selective than cattle, even though this is not apparent when cut herbage is fed (Minson 1981; Collins and Nicol 1986).

Laboratory methods

Since the in vivo determination of digestibility is laborious and consumes large amounts of feedstuffs (Minson 1981), three laboratory methods for estimating feed digestibility have been developed (Omed et al. 1989). First is the two-stage technique based on the use of rumen liquor followed by acid pepsin (Tilley and Terry 1963). Second is an enzymatic method where the rumen liquor is substituted by fungal cellulose (Jones and Hayward 1975; Roughan and Holland 1977) and third is the use of the faecal micro organisms obtained from a filtered suspension of sheep faeces and pepsin (El Shaer et.al 1987).

The two stage-technique is the most extensively used method. However its application has the disadvantage of the need to surgically prepare animals to provide rumen liquor. While the second method (enzymatic method) obviates the need for a surgical animal, there may be considerable variation in the activity of enzymes from one batch to another, therefore herbage of known in vivo digestibility must be used as standards with each batch of samples. The new microbial method based on the use of the faecal micro organisms contained in a filtered suspension of sheep faeces is simple and cheap. In countries that do not have a regular supply of cellulose, this method is favoured. All three methods can give a satisfactory estimates of in vitro dry matter digestibility of forages, but some researchers question their reliability for predicting in vitro of concentrate feeds (Omed et al. 1989).

A representative sample of herbage to that consumed by animals must be submitted for the laboratory in vitro determination of feed digestibility. There are three methods for obtaining a representative herbage samples while the animals graze at pasture. These include hand plucking, oesophageal- and rumen-fistulated animal techniques. Corbett (1978) stated that hand plucking is laborious and difficult due to uncertainty of knowing what diet is being selected by the grazing animal. The method however, is simple and faster than using oesophageal- or rumen-fistulated animals.

The use of oesophageal-fistulated animals reduces interference to grazing behaviour (Arnold et al.1963). It is the most reliable method for getting a representative sample of herbage consumed from grazing animals (Hodgson and Rodriguez 1970). Rumen-fistulated animals are generally easier to establish compared to oesophageal-fistulates, but care should be taken during the collection period when contamination of ingested herbage can occur from ruminal contents (Meijs 1981).

Hand plucking is probably the best method to obtain a representative herbage samples in those countries where equipment used for oesophageal- and rumen-fistulated animals are unavailable.

Pasture measurement techniques

Sward methods for measuring daily feed intake are based on the difference between the amount of daily pasture mass offered (kgDM/ha) and residual herbage mass (kgDM/ha) (Meijs et al. 1982; Hodgson 1984). The difference between pre- and post- grazing pasture mass is divided by the number of animals grazed per day to provide the average daily intake per animal (kg DM/head/day). This technique only provides an estimate group mean intake, and because of the effect of pasture growth between grazings is only valid for grazing intervals of up to three days (Walter and Evans 1979).

The methods for estimating herbage mass can be classified into two categories; pasture cuts (a destructive technique) and non-destructive techniques (Brown 1954; 't Manetje 1978; Frame 1981).

The pasture cuts technique involves sampling, harvesting and weighing of a measured proportion of the area of pasture allocated to animals. The technique requires a high labour input but the herbage cuts may be bulked to reduce labour for washing and drying (Frame 1981). Standardization of the height to which pasture is cut is important although this may be difficult to achieve due to the variability in animal grazing height within a paddock. There may be bias in the cuts taken between treatments by a single operator (e.g. different residual heights on low and high allowance and treatments) and between operators (Michael 1982). Some workers have provided

guidelines for pasture cutting height, but such definitions are only valid for certain pastures due to the large variation in pastures between seasons ('t Mannetje 1978). For example, cutting at ground level is used only for high grazing pressure situations with either sheep or cattle, while a 3 cm stubble is used to simulate moderate to high grazing pressure with cattle or low grazing pressure with sheep and a 6 cm residual is used where herbage is being conserved (Frame 1981). Researchers at Massey University prefer to cut all swards to ground level using a sheep shearing handpiece.

Non-destructive techniques involve the measurement of one or more sward characteristics in the grazing area before and after grazing. The advantages of non-destructive methods over the cutting technique include a reduction in the amount of labour, equipment and time, and hence the cost of measurement (Earle and McGowan 1979; Parker 1981; Michael 1982).

A rising plate meter (Ellinbank pasture meter (EPM)) originally developed at Massey University (Holmes 1974) and later refined at Ellinbank in Australia (Earle and McGowan 1979) can provide quick and reasonably accurate estimates of pasture yield (Parker 1985). Pasture height, measured by a height meter as the distance between the ground and the plate, varies according to sward structure. Meter readings are calibrated to pasture yield for different seasons and sward compositions. To calibrate the EPM the average pasture height for 10 -15 quadrats are recorded prior to cutting. DM yield (y) is then regressed on pasture height (x) to provide a linear or non-linear calibration equation of the form $y = a + bx$.

Visual assessment of pasture mass is based on operator's eye appraisal ('t Mannetje 1978). The technique is subjective, but can be made quickly and at a low cost by experienced operators (Frame 1981).

PURPOSE AND SCOPE OF THE INVESTIGATION

Initial studies with CRC indicate that they could have considerable potential for improving current knowledge of feed intake at pasture. CRC increase labour efficiency for measuring feed intake compared to twice-daily administration of Cr_2O_3 in the form of either gelatin capsules or impregnated paper(Barlow et al. 1988). They would be particularly suited to the extensive grazing situation such as in Indonesia where it is not practical to handle cattle each day to administer faecal markers.

The purpose of this investigation was therefore to validate cattle CRC for indirectly estimating faecal output and hence feed intake in dairy cows fed indoors. A secondary objective was to compare pasture intake outdoors estimated indirectly from Cr dilution in the faecal sample and by the pasture cuts technique.

CHAPTER TWO

EXPERIMENTAL

AN ASSESSMENT OF INTRARUMINAL CHROMIUM CONTROLLED RELEASE CAPSULES FOR MEASURING FEED INTAKE IN DAIRY COWS

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ABSTRACT.

Feed intakes from 16 cows in the mid-lactation period were estimated using controlled release capsules (CRC).

The method involved an 18 day indoor feeding period when cows were fed ryegrass (Lolium perenne) and white clover (Trifolium repens) followed by a 7 day period outdoors on the similar type of pasture. During the outdoor period, intakes measured by CRC were compared to intakes measured by the pasture cuts technique.

Results (kg DM/d and kg OM/d) showed that estimates of faecal output (FO_{cr}) using CRC was not significantly different to those faecal output (FO_e) estimated by actual feed intake and feed indigestibility determined by the in vitro method. Estimates of intake (kg DM/d and kg OM/d) using FO_{cr} and feed indigestibility was not significantly different to actual intakes. Estimates of intake using CRC was similar to those intakes estimated by using pasture cuts technique. The intake values obtained are generally reliable for maintaining a daily milk production of 14 kg/day.

The CRC offers the flexibility of a single daily faecal sample and thereby reduces labour content compared with twice daily administration of Cr in an impregnated paper or gelatin capsule form. Current methods of measuring feed intake could be improved by using the intra-ruminal chromium controlled release capsules.

INTRODUCTION

A knowledge of herbage intake is critical to investigations into animal nutrition, pasture utilisation and the evaluation of efficiency of animals (Lee et al. 1990 and Morris et al. 1990). These parameters can be measured either directly at pasture or indirectly in the animals (Walters and Evans 1979). Measuring feed intake at pasture requires estimates of the amount of feed offered (pre-grazing herbage mass) and the amount of feed remaining (post-grazing herbage mass) (Meijs 1981). These parameters can be measured directly by the pasture cut method (Brown 1954) or indirectly using the Ellinbank Pasture Meter (Holmes 1974 and Earle and McGowan 1979). The accuracy of each method will depend on such factors as the variability of the pasture, between operator variance, and herbage growth over the measurement period (Michell 1982 and Korte et al. 1987). The pasture cuts technique has the disadvantage of greater labour requirement associated with cutting samples and subsequent dry matter determination (Michell and Large 1983).

Indirect intake measurements on animals can offer the possibility of providing individual estimates of intake (instead of group mean intakes from the pasture cuts technique) (Walters and Evans 1979). The indirect technique would be much less labour intensive if total faeces collection was replaced by the use of an indigestible marker (e.g. Cr_2O_3) (Hodgson and Rodriguez 1970; Michael and Large 1983; Lee et al. 1990).

A recently developed technology for administering the indigestible marker (Cr_2O_3) into ruminants is the controlled release capsule (CRC) (Ellis 1980). This method of Cr_2O_3 administration is less labour intensive and more flexible in the timing faecal collections. The technique also causes minimum disturbance to animals whilst they are grazing. This new technology is being studied by Massey University researchers in sheep and cattle under grazing conditions.

This study reports the results of an experiment at Massey University using controlled release capsules to assess faecal output and hence feed intake in dairy cows fed pasture either indoors or outdoors under grazing conditions. Estimates of feed intake using the CRC were compared with measurement of intake using before and after grazing measurements on pastures.

MATERIALS AND METHODS

Experimental design

The experiment was designed to validate the use of cattle CRC for estimating feed intake in lactating dairy cows. A secondary objective was to compare herbage intakes estimated from the concentration of faecal chromium with direct measurement by the pasture cuts technique of cows on a 24 hour rotational grazing system. The trial involved 16 cows fed indoors for 18 days before being grazed outdoors for 7 days on pastures of similar composition to the cut feed.

A companion trial was conducted with sheep to estimate the in vivo digestibility of the feed offered to the dairy cows.

Indoor trial

Sixteen Friesian cows between 2 and 5 years of age were individually housed in stalls at Massey University's Animal Physiology Unit on 29 November 1988. The average liveweight of the cows was 430 $\pm$ 22 kg (mean $\pm$ SE). A single Captec (Nufarm Ltd, Auckland) chromium CRC (65% Cr_2O_3 matrix, 40.6 mm core length, and multi-orifice endplate) was administered orally by a Captec balling gun to each cow on the same day (d 0 of CRC life). Pasture was cut twice a day, at 0830 h and 1500 h, with a drum mower (Vicon, New Zealand), collected in a silage wagon (Strautment, West Germany), and offered ad libitum to the cows for an 18 day period. Pasture was harvested from a mixed ryegrass (Lolium perenne) and white clover (Trifolium repens) sward.

Feed was supplied to the cows twice daily (morning and afternoon). The feed offered in the morning was weighed, while in the afternoon a known weight of "afternoon" cut feed was added to the remaining morning allowance; refusals that had scattered to the floor were returned to the bin. Refusals, weighed for individual cows prior to refeeding, were disposed of each morning. Duplicate samples of the daily feed offered and refused were oven dried at 80 ^{0}C to determine their respective dry matter contents. Dry matter intake (DMI/d) was calculated as the differences between DM offered and DM refused. The stalls were washed down twice daily while the cows were being milked in the morning (0700-0800 h) and evening (1500-1600 h).

Feed digestibility

To estimate the digestibility of the feed offered to the cows a daily sample of the cut herbage was taken from the bin immediately prior to feeding in the morning and afternoon. These samples were frozen (-12 ^{o}C) until required for in vitro analysis. The frozen samples were thawed, freeze dried and ground through a 1.00 mm sieve prior to being assayed by the method described by Roughan and Holland (1977) (see appendix 1 for a full description of the method). Six standards of known in vivo digestibility were used to calibrate the in vitro estimates of digestibility for the cut herbage samples.

Faecal sampling

Morning and afternoon faecal samples (approximately 20 g wet weight) were collected into pottles from the top of the faeces which had accumulated behind each cow between milkings, for the 18 days of indoor feeding. The faecal samples were oven dried at 80 ^{o}C for a minimum of 72 h or until a constant a dry weight was reached. The concentration of chromium was then determined using the phosphoric acid-potassium bromate digestion and atomic absorption spectrophotometry outlined by Costigan et al. (1987) but with modifications described by Parker et al. (1989). (See Appendix 2 for a full description of the method). An equal weight of the morning and evening faecal samples were combined to form a daily composite of 2.0 g DM for each cow. Duplicate samples were assayed for individual cows for days 4 to 18 of indoor feeding. Single samples per cow were assayed for days 1, 2 and 3 to reduce costs. The attainment of steady state levels of chromium in the faeces was estimated to be between d 5 and d 7 of CRC life (Graham 1988). Faeces samples were therefore combined over two 5-day periods (d 9 - 13 and d 14 - 18) to estimate the group mean intake of the cows. Equipment was not available for measuring total collections of faeces from the cows. Feacal output (FO_e) was therefore estimated from the digestibility of the feed consumed as follows

$$FO_e = I\,(1-D) \qquad \textbf{(eqn 2.1)}$$

where I = Intake (kg DM/head/d)

D = in vitro digestibility of the feed consumed (% DM)

This estimate (FO_e) was compared with faecal output estimated from the concentration of chromium in faeces (FO_{cr}). The FO_{cr} value was derived by dividing the average daily release of Cr from the CRC by the average daily concentration of Cr in the faeces as shown in equation 2.2:

$$FO_{cr} = \frac{\text{Cr released (mg/d).}}{\text{Cr in faeces (mg/gDM).}} \qquad \textbf{(eqn 2.2)}$$

Diurnal variation

To determine whether a diurnal pattern of chromium concentration occured in the faeces, faeces samples were collected at 6 h intervals over a 48 h period on d 13 and d 14 of the trial. A single sample (approximately 1 g DM) of faeces was assayed for each sampling time.

Grazing trial

On d 19 of CRC life the cows were returned to normal grazing at pasture. From d 19 to d 25 the cows were rotational grazed on 24 h breaks, set up using temporary electric fences. The grazing area required for each break, was determined by the herbage allowance method;

$$\text{Area (ha/d)} = \frac{\text{the number of animals x allowance (kg DM/cow/d)}}{\text{pre-grazing pasture mass (kg DM/ha).}} \qquad \textbf{(eqn 2.3)}$$

It was assumed that a herbage allowance between of 2 and 4 times the daily intake (approximately 40 kg DM/cow/d) was necessary for cows in mid-lactation (Glassey et al. 1980; Bryant 1980; Leaver 1985). Herbage intake was measured on the same basis as the indoor experiment by measuring the difference between herbage offered and that remaining after grazing, as shown in the equation 2.4.

$$\text{Daily intake (kg DM/cow/d)} = A - B / C$$

where A = Pre-grazing yield (kg DM/ha).
B = Post-grazing yield (kg DM/ha). **(eqn 2.4)**
C = Number of cows grazing per effective area (cows/ha/d).

Two methods of pasture mass measurement were used to determine pre- and post-grazing herbage mass; pasture cuts and a non-destructive technique based on calibrated pasture height. For the pasture cut technique 10 quadrats (0.18 m2) of both pre- and post-grazed herbage were cut each day to ground level with a sheep shearing handpiece (Frame 1981). The cut herbage samples were thoroughly mixed and bulked across samples before being washed and oven-dried in the oven at 80 ^{o}C for 24 h. Herbage mass per hectare (kg DM/ha) was estimated by multiplying the weight of herbage (10 quadrats) (kg DM) by 5555. Hand-separation of grass, clover, weed and dead material was carried out to determine the botanical composition of the of the swards pre-, and post-grazing and also of the herbage cut from the exclusion cages used to obtain digestibility

samples (Brown 1954).

For the non-destructive technique, an Ellinbank Pasture Meter was used (Earle and McGowan 1979). The meter height readings were calibrated separately against pre- and post-grazed herbage mass cuts by a linear regression ($y = a + bx$) of pasture mass (y;kg DM/ha) on the average meter reading (x;cm). Fifty readings were taken prior to and after grazing each day. Feed intake while grazing was estimated from the concentration of faecal chromium and an in vitro estimate of the digestibility of the herbage consumed. Samples for the in vitro digestibility assay were collected from three exclosure cages placed on each 24 h break. Herbage was cut from the exclosure cages to a height similar to that on the surrounding sward after grazing.

A second estimate of the digestibility of herbage consumed was obtained by analyzing a sample of herbage from the pre- and post-grazing swards separately and including these values in equation 2.5 (Walters and Evans 1979).

$$\text{OMD of herbage grazed (\%)} = \frac{\text{DOM yield before grazing} - \text{DOM yield after grazing}}{\text{OM yield before grazing} - \text{OM yield after grazing}} \times 100\% \qquad \textbf{(eqn 2.5)}$$

where,

OMD (%) = Estimated (Organic Matter) digestibility of herbage consumed (%).
DOM yield before grazing (kg OM/ha) = Organic Matter digestibility pre-grazing (%) x OM yield before grazing (kg OM/ha).
DOM yield after grazing (kg OM/ha) = Organic Matter Digestibility post-grazing (%) x OM yield after grazing (kg OM/ha).
OM yield before grazing (kg OM/ha) = Yield pre-grazing (kg DM/ha) x (1 - ash content of the herbage sample pre-grazing).
OM yield after grazing (kg OM/ha) = Yield post-grazing (kg DM/ha) x (1 - ash content of the herbage sample post grazing).

Determination of the endpoint of chromium.

Faeces samples were collected by observing individual cow defaecations for 2 hours prior to morning and afternoon milkings on d 19, 20, 21 and 22 and prior to the morning milking only on d 23, 24, 25 of CRC life. The experiment was completed on December 24. This faecal sampling procedure was only moderately successful with a minimum and maximum of 5 and 12 cows sampled, respectively, per day. It was anticipated from Australian data (Rodden pers comm) that Cr_2O_3 release would cease between d 23 and d 25 of CRC life. The pattern of chromium concentration in the faecal samples from d 19 to d 25 should therefore have enabled the endpoint of Cr_2O_3 release to have been identified within $\pm$ 1 d for individual cows.

Milk production

Daily milk production of the cows (kg milk/d) was recorded during both the indoor and outdoor phases of the experiment. Estimates of daily feed intake could therefore be related to milk production.

Feed digestibility trial with sheep.

The in vivo sheep digestibility trial started on d 2 of the cow indoor feeding trial when six-20 month Romney wethers were introduced to indoor feeding for a 4 day adjustment period. The wethers were weighed on d 2 and housed individually in metabolism crates. A relatively short adoption period was posssible because the wethers had previously been used for an indoor feeding experiment.

A known weight of fresh pasture, taken from that harvested for the cows, was fed ad libitum to the wethers twice daily at 0830 h and 1500 h for a 10 day period. Feed refusals were weighed for individual animals prior to refeeding each morning. Duplicate samples of the feed offered and refused were oven-dried at 80 ^{o}C to determine feed DM content as described for the dairy cow trial. The total output of faeces was collected each day and the fresh weight recorded each morning. Duplicates samples (c.100 g wet weight) of faeces were taken for each sheep and oven-dried to a constant weight. The digestibility of the feed was calculated according to the equation 1.2 (see Chapter One). To check the in vivo value, a sample of the feed offered each day (approximately 200 g fresh weight) was bulked over two 5-day periods, and thoroughly mixed before sub-sampling for in vitro digestibility analysis (Roughan and Holland 1977). A sub-sample from this was also used to determine the herbage botanical composition (Brown 1954).

Statistical analysis

The estimated faecal output (FO_e) and the faecal output derived from chromium (FO_{cr}) were compared on a DM and OM basis by paired T-tests for the two 5-day periods (period 1 and period 2). Differences in average values between period 1 and period 2 were tested by the same method. Intake, estimated from the FO_{cr} value and the in vitro feed digestibility, were also compared with the actual intakes for both periods by paired T-tests.

The estimates of feed intake by pasture cuts technique and by Cr_2O_3 dilution were subjected to ANOVA.

All analysis were undertaken using the 'SAS' statistical package (SAS 1985).

RESULTS AND DISCUSSION

Herbage digestibility

The mean in vitro feed digestibilities of herbage offered to the cows were 0.69±0.09 DM, (0.74±0.06 OM) and 0.66±0.06 DM, (0.72±0.04 OM) for period 1 (d 9 - 13 of CRC life) and period 2 (d 14 - 18), respectively. The mean in vitro digestibility values for the feed offered to the sheep were 0.72±0.02 DM (0.77±0.01 OM) and 0.69±0.02 DM (0.75±0.02 OM) for period 1 (d 7 - 11 of CRC life) and 2 (d 12 - 16), respectively. The corresponding mean in vivo feed digestibility measured with the sheep were 0.73±0.01 DM (period 1) and 0.72±0.01 DM (period 2), respectively.

The in vivo values were statistically higher than the values that were derived by the in vitro procedure for DMD confirming the results reported by Holmes (1980). The difference between the dairy cow and sheep values supports the theory that sheep are much more selective feeders than cows (Dudzinski and Arnold 1973; Mulholland et al. 1977; Collins and Nicol 1986). The cows apparently exercised very little preference for components of the herbage offered to them. There is evidence that when the two species graze the same pasture together significant differences exist, even though the diet selected is not apparent when cut herbage is fed (Langlands et al. 1963; Dudzinski and Arnold 1973).

The lower in vivo digestibility values for period 2 were not significantly different from the digestibility values for period 1. The lower clover and higher stem content in the second period than during the first period (Table 2.1) is likely to be the main reason for the difference between the two periods.

Table 2.1 **Botanical composition (% of dry weight) of feed during period 1 (d 7-11) and 2 (d 12-16) of the sheep digestibility trial.**

	White clover	Stem	Leaf	Weed	Dead
day 7-11	10	17	31	31	11
day 12-16	7	20	27	34	12

Regurgitation of CRC

Two cows regurgitated their capsules during the first day after administration. One capsule was recognized to have come from cow 195. The other capsule could not be identified to a cow until the results of the first run of the chromium assay were known. The second regurgitated capsule was then identified to cow 182. The CRC was reinserted to cow 195 on d 1 of CRC life, but none of the results for cow 195 (or 182) were suitable for inclusion in the analysis of faecal output.

Recovery of chromium in the faeces

In addition to the cows which regurgitated their CRC unsatisfactory recoveries of chromium from the faeces were achieved with a further three cows. Inspection of the pattern of daily concentration of chromium in the faeces indicated that problems with Cr_2O_3 release occurred on d 9 (cow 135, 136) and d 10 (cow 139) of CRC life (Figure 2.1). The reason for the atypical pattern of Cr_2O_3 release in these cows was not identified, but the chromium data for these cows were exluded from further analysis because it was not possible to estimate a release rate of Cr_2O_3 for the capsules concerned.

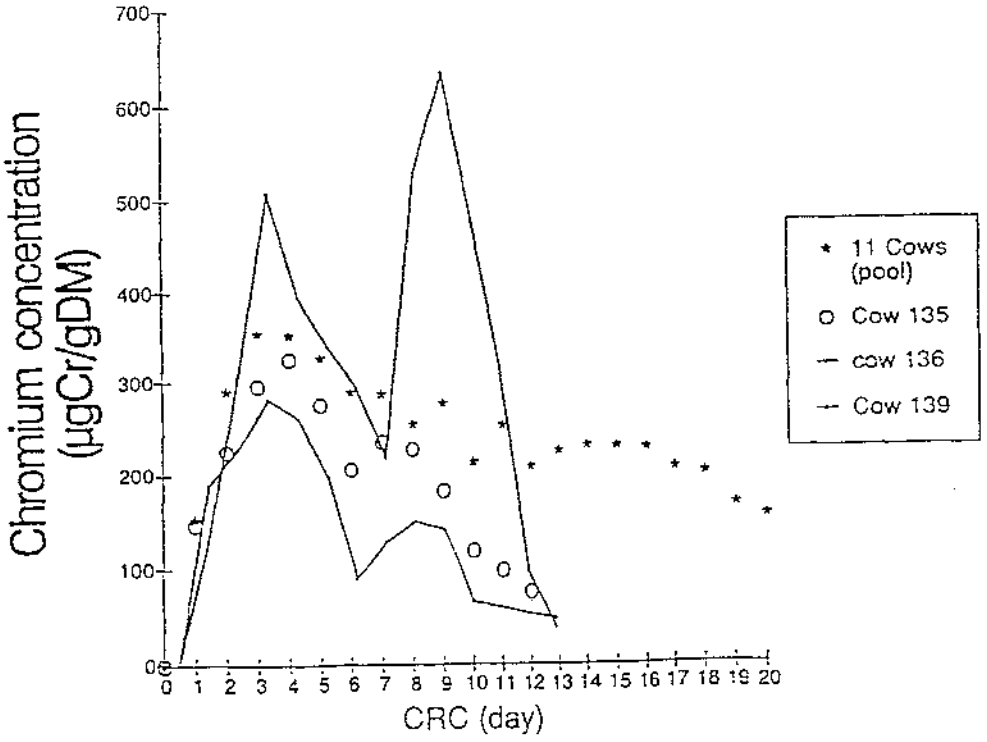

Figure 2.1 Comparison of mean Cr release rate in the faeces (11 cows) with those of three cows with atypical release rate characterictics.

Inspection of faecal chromium concentration for the remaining 11 cows showed that a steady state concentration of chromium was achieved on d 8 of CRC life (Figure 2.1). At this stage the recovery value was 116 $\pm$ 8 %. Chromium concentrations for some capsules began to decline on d 19 when the overall recovery was reduced to 68% $\pm$ 6%. The average endpoint of chromium release, as determined by the disappearance of Cr from the faeces was d 23. The average Cr release rate was therefore 1172 mg/d for the estimated average plunger travel of 1.65 mm/d and a matrix density of 2.064 mg/cm^3 (as specificied by the manufacturer) compared favourably with the data supplied by Captec of between 1400 and 1800 mg Cr_2O_3/d (1150 and 1400 mgCr/d). However, this result is higher than the release rate from cattle Cr capsules of 900 mg/d in an experiment conducted by Ellis et al. (1982). However, these capsules had cast core rather than pelleted matrix formulations and are therefore not strictly comparable.

The mean recovery of chromium from the 11 cows with satisfactory capsule performance was not significantly different from 100% from d 9 to 13 (period 1) and from day 14 to 18 (period 2) (Table 2.2).

Table 2.2 **Group mean faecal recovery of Cr (%) estimated using either in vitro or in vivo digestibility values to determine faecal output (FOe) of the cows.**

	Period 1	Period 2
in vitro digestibility	$99.07 \pm 5.19^{aA*}$	99.03 ± 4.75^{bB}
in vivo digestibility	86.85 ± 4.82^{aB}	81.25 ± 3.54^{bB}

*Different lower case letters indicate that differences between period were significant at the 5% level while different upper case letters indicate that differences between treatments(columns) were significant at the 5% level.

Chromium recoveries were significantly higher and close to 100% (>99%) when the in vitro measure of herbage digestibility rather than the in vivo value from the sheep was used to derive the estimated faecal output (FO_e) by the cows. Since no total collection faeces of were carried out, it is difficult to estimate the actual errors for Cr recovery in the faeces. On the other hand, total collection of faeces with bags may have influenced the behaviour and performance of the animals (Meijs 1981).

The indirect faecal output estimate, is dependent upon the in vitro values used. The reliability of these values could have been checked better if separate in vitro values of feed offered and refused had been obtained. Alternatively, oesophageal fistulated cows could have been used.

Diurnal variation

Morning and evening concentrations of chromium in the faeces varied by 3.15% but did not differ significantly between samples collected every six hours during day 13 and 14 of CRC life (Table 2.3).

Table 2.3 **Diurnal variation in Cr concentration (mg/kg DM) in faeces taken 8 times over a period of 48 hours from individual cows (n = 11).**

Times	1[b]	2	3	4	5	6	7	8	Mean ± SE
125[a]	248.0	25.10	259.5	259.5	247.0	232.5	228.0	229.0	244.31 ± 4.56
126	235.0	222.0	250.5	218.0	252.0	246.5	250.0	266.5	242.56 ± 5.79
128	230.0	247.0	238.0	234.0	230.0	228.5	246.0	233.5	235.87 ± 2.56
131	304.0	288.0	314.5	301.5	320.0	320.0	322.0	311.5	310.19 ± 4.14
140	307.0	314.0	292.5	315.5	297.0	300.0	321.0	324.0	308.88 ± 4.09
169	172.0	164.0	172.5	185.0	188.0	174.0	193.5	171.5	177.56 ± 3.56
172	195.5	191.0	196.0	212.0	200.0	193.0	192.5	221.0	200.13 ± 3.80
179	164.5	172.0	183.5	184.0	183.5	175.5	189.0	194.0	180.75 ± 3.37
197	209.5	206.0	277.0	219.0	198.0	198.0	209.0	224.0	217.56 ± 9.07
206	191.5	181.0	185.0	200.0	227.0	213.0	231.5	216.0	205.63 ± 6.75
224	192.5	196.0	186.5	186.0	177.0	203.0	194.0	168.0	189.63 ± 2.95
Mean	222.68	221.09	232.32	228.59	229.05	225.82	234.22	233.91	228.46
±SE	± 14.50	± 14.70	± 15.00	± 14.00	± 14.10	± 14.30	± 15.10	± 15.00	± 2.23

a = cow number

b = sampling times : 1, 8 = 48 h.

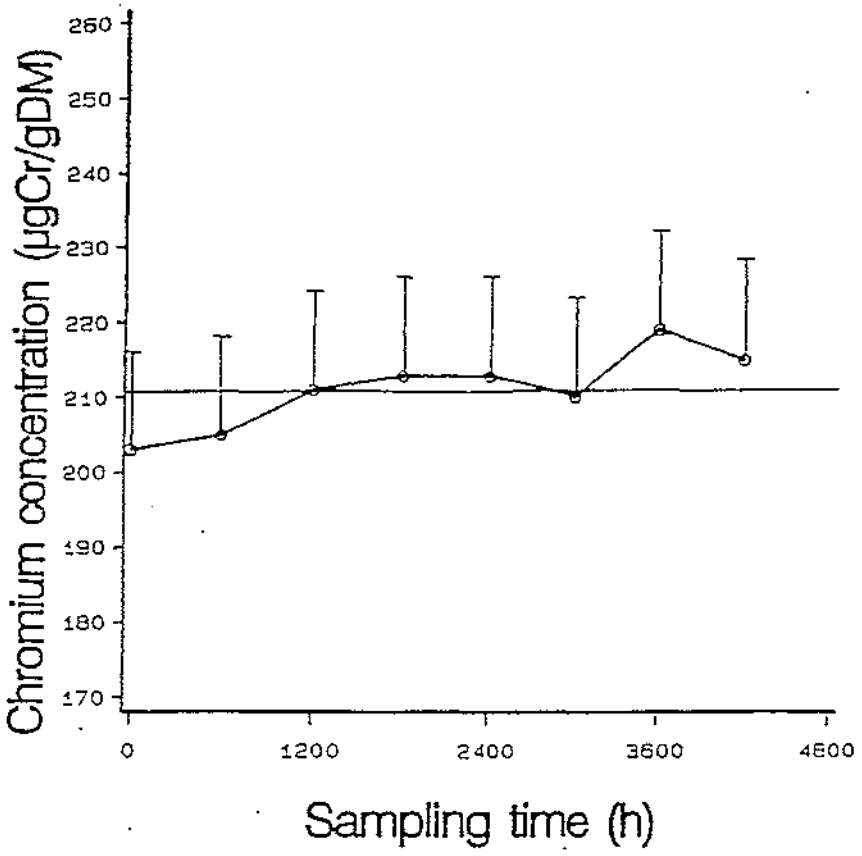

Figure 2.2 **Diurnal variation expressed as a percentage of the 48 h sample Cr concentration.**

Figure 2.2 indicates that the chromic oxide concentration taken at 2400 h and 0600 h was related to mean concentration within the 48 h (six h intervals) by the equation:

$$y = 0.66x - 2.98$$

where

y = the mean value of the concentration Cr in the faeces for the 8 sampling times.

x = sample number.

Diurnal variation of Cr concentration in the daily (two days) faecal sample CRC obtained using the method was lower than reported by other methods. For example, Coup (1950) found morning and evening concentrations of Cr in the faeces of dairy cows to vary by as much as 15%. Kane et al. (1952) found a similar level of diurnal variation and suggested taking samples at 1300 - 1500 h and 0400 - 0600 h, at which times the chromic oxide concentration was close to the 24 h average. Alternatively, these authors suggested taking samples from 1000 - 1200 h when chromic oxide concentration was below average and at 1400 - 0400 h, when the concentration was above average. The mean concentration of Cr at these two sampling times was close to the 24 h mean.

Estimation of faecal output

The comparison between the derived group mean faecal output (FO_e) and the group mean faecal output estimated from the concentration of Cr (FO_{cr}) in the faeces is shown in Table 2.4.

Table 2.4 A comparison between faecal output derived from the in vitro feed digestibility Fo_e with that estimated from the chromic oxide concentration in faeces Fo_{cr} during two 5 days periods (kg DM and kg OM ± SE).

	FO_e		FO_{cr}		FO_{cr}/FO_e(%)	
Period	DM	OM	DM	OM	DM	OM
1	4.80±0.12	3.64±0.10	5.12±0.29	4.15±0.28	7.9	14.23
2	5.14±0.21	3.84±0.10	5.36±0.15	4.30±0.12	4.2	11.91

Faecal outputs (DM and OM) estimated from the concentration of chromium were consistently, but not significantly, higher than the derived faecal output in period 1 and period 2 and did not differ significantly between periods.

The accuracy of the FO_e value is critical because total collections of faeces were not obtained for individual cows. The in vivo values from the sheep were the only comparable data to the in vitro results obtained for the cows. However, the chromium recovery analysis suggests that the in vitro values were more appropriate.

Intake Estimation

The group mean intakes estimated by equation 1.1 using in vitro feed indigestibility is shown on Table 2.5.

Table 2.5 Actual and Cr estimated intake (kg DM (± SE) and kg OM) (± SE) of cows during period 1 and 2.

	Actual intakes		Estimated intakes		Sign
Period	DM	OM	DM	OM	
1	15.23±0.39[a]	13.94±0.39	16.43±0.89	15.92±1.00	NS
2	15.28±0.48	13.87±0.47	15.93±0.46	15.51±0.43	NS

[a]Differences between estimated and actual intakes between period 1 and 2 were not significant.

Feed DM intakes (estimated by Cr) were overestimated compared to actual values in period 1 by 7.8% (P=NS) and by 2.6% in period 2 (P=NS). Similarly, there was no significant difference between periods (period 1 and 2) in terms DM and OM intake. The corresponding differences for the estimated OM intakes were 14.23 and 11.84% respectively (P=NS).

The OM difference (13%) is higher than the DM difference (5%) for period 1 and 2. This could be due to the OMD values (0.74 and 0.72) used. The actual OMD values were not calculated in this study. If the OMD values were reduced to 70 and 69% for period 1 and 2 respectively, the OM values would be similar to the difference in DM values. This situation indicates that intake estimated by this method (Eqn 1.1) is greatly influenced by the digestibility of the feed consumed.

Outdoor grazing

From d 19 to d 25 of CRC life, cows grazed paddocks with perennial ryegrass (Lolium Perenne) white clover (Trifolium repens) pasture. The mean concentration of chromium in the faeces (ugCr/g DM) was measured on d 19, 20, 21, 22, 23, 24 and 25 of CRC life for 7 cows which had CRC that were still in the linear phase (Table 2.6).

Table 2.6 **Mean Cr concentration (ug/g DM) in the faeces of the cows (n=7) with CRC still in a linear release phase during the grazing study**

day	125[a]	126	140	169	172	179	224
19	nfs[b]	266.5	nfs	260	182.3	180.3	nfs
20	268.5	nfs	nfs	nfs	185.0	274.5	190.2
21	279.2	299.5	255	nfs	211.5	205.0	193.5
22	nfs	217.5	226.5	nfs	264.5	218.5	198.5
23	44.7[c]	18[c]	nfs	nfs	234.0	nfs	nfs
24	nfs	nfs	nfs	207	12[c]	75[c]	38[c]
25	13.5	nfs	nfs[c]	nfs[c]	nfs	35	nfs

a = cow number.

b = no faeces sample.

c = endpoint.

Even though daily faeces samples from each cow were collected with only moderate success from d 19 to d 25, the endpoint for individual CRC can still be determined. The endpoint varied between cows. For example, the capsule in cow 125 expired on d 21 while the CRC in cows 172, 179 and 224 were finished on d 24 of CRC life. No cow had a CRC which was still in the steady state 25 days after administration.

Pre- and post-grazing pasture mass determined by pasture cuts and the area of each pasture break are shown in Table 2.7. Corresponding pasture heights determined by an EPM for each of the 4 days period is also shown on Table 2.7. The pasture height data is the only information that can be provided for the EPM because individual pasture heights corresponding to each pasture cut were not recorded. An alternative calibration, estimated indirectly by regressing the mean daily pasture mass (y; kg DM/ha) on the average pasture height (x;cm) recorded each day for the pre- and post-grazing swards, was as follows:

$$y = 196 + 267\,x,$$

$$r = 0.93$$

where y = pasture mass (both pre- and post-grazing pasture mass; kg DM/ha).

x = pasture height (cm).

Table 2.7 **Area grazed, pre- and post-grazing pasture mass and corresponding mean EPM pasture heights.**

Day	Area grazed (ha)	Pre-grazing (kgDM/ha)	Pasture height (cm)	Post-grazing (kgDM/ha)	Pasture height (cm)
1	0.15	4682	16.8	2877	10.0
2	0.17	3885	13.8	2438	8.4
3	0.14	4417	15.8	2194	7.5
4	0.16	4575	16.4	2753	8.8
Mean	0.15	4390	15.70	2566	8.8
SE	0.007	176	0.67	154	0.52
CV	9.18	8.10	8.48	12	11.0

The difference between daily allowance pre-grazing (41.15 kgDM/head/d or 15.7 cm of pasture height) and residual herbage mass (24 kgDM/head/d or 8.8 cm of pasture height), was equivalent to an average cow consumption of 17.1 kg DM/head/d . The daily utilization of pasture was 41.55% (the efficiency of grazing in terms of DM intake/DM allowance).

The estimated digestibility of herbage consumed, obtained by the in vitro and pasture difference technique (herbage disappearance calculated by the equation 2.5) is shown on Table 2.8.

Table 2.8 Digestibility (OMD %) of herbage consumed during grazing trial.

Parameter	Method	
	In vitro	Pasture cuts
Herbage consumed[a]	76.19	78.12[c]
SE	0.11	0.44
CV	0.20	1.13
Pre-grazing[b]	74.61	-
SE	0.01	-
CV	19	-
Post-grazing[b]	71.27	-
SE	0.05	-
CV	8.0	-

[a]Sample cut from under cages at a similar pasture height to the post-grazing mass.
[b]Sample cut from quadrat prior to grazing (pre-grazing) or after grazing (post-grazing).
[c]Estimated from equation 2.5.

Table 2.8 indicates that the OMD value, calculated by pasture difference was 78.12 (% OMD). The in vitro OMD value taken from the cage was 76.19 (% OMD). The reason for the difference between these two figures is not clear. It is speculated that the pasture difference between pre- and post-grazing pasture mass (kg DM/ha) obtained, and hence OMD (see equation 2.5) could be one reason. However this could not be checked because the other method of pasture mass identification using EPM (as rival data) was not recorded.

As expected, the mean organic matter digestibility value for pre- grazed herbage (approximately 75%) was 3% units higher than those for the post- grazed herbage (approximately 71%). The digestibility of the herbage that was cut inside the cage was (approximately 2%) higher indicating that a considerable selection had occurred during grazing (table 2.8). The exclosure cages indicated that the cows consumed leaf in preference to stem and green herbage in preference to dead material. The results are in agreement with Hughes (1983) who stated that pastures of white

clover and perrenial ryegrass with height ranging between 10 to 20 cm have an OMD between 75 and 78%.

The mean DMI of the cows estimated by the CRC method was similar (17.53±0.28 kg DM vs 17.38±0.86 kg DM/cow/d) to the intake estimated by the pasture cuts technique. Corresponding values for OMI were 16.90±0.34 kg OM and 16.10±0.69 kg OM/cow/d) (Table 2.9).

Table 2.9 Comparison of DMI cows estimated by the pasture difference and CRC chromic dilution techniques.

	Pasture cuts	Cr dilution
DM intake		
kg/d	17.38	17.53
SE	0.86	0.28
CV (%)	1.97	7.43
OM intake		
kg/d	16.10	16.90
SE	0.69	0.34
CV (%)	8.67	4.02

The higher intake (15%) measured by both techniques in the outdoor situation compared to the indoor (table 2.5) suggest that the cows required more energy for walking and grazing activities when outdoors. The ARC (1980) handbook states that prehending and grazing activities require at least 10% more energy, but, obviously, other factors have to be considered, including liveweight change, stage of lactation, milk yield and non-nutritional factors (Holmes and Wilson 1984).

The higher intake resulted in higher milk production outdoors (14.16±0.1 kg/day) compared to indoors (13.12±0.14 kg/day. The intake data obtained is reliable according to the energy requirements for grazing dairy cows (ARC 1980). The metabolisable energy of the pasture (Summer pasture) has been assumed to be 10 MJME/kg DM in this trial (Holmes and Wilson 1984), therefore approximately 16 kg DM/d pasture is required to produce 14.16 kg milk/d with no weight gain or loss (ME maintenance, gain, mobilisation and lactation requirements are 0.60 MJME/kg $LW^{0.75}$/d, 39 MJME/kg gain, 32.4 MJME, 4.96 MJME/kg milk/d respectively) (ARC 1980, Holmes and Wilson 1984). The results are similar to that published in the literature. For example, Bryant et al. (1980) offered daily herbage allowance of 52.7, 33.2 and 13.5 kgDM/cow for a period of 5 weeks in early lactation. These allowances resulted in apparent intakes of 16.3, 14.3 and 9.6 kgDM/cow. The low allowance cows produced significantly less milk and lost live weight and body condition.

It should be noted that intake estimated by the pasture cuts method in this trial did not include a measure of herbage produced during grazing. Pasture growth over short time periods is extremely difficult to measure since it involves assumptions about the growth rate of the sward whilst it is being continuously defoliated by the grazing animal. Most authors agree that any bias introduced by excluding growth during grazing over a period of less than 3 days is likely to be minimal and can be ignored (Green 1949; Linehan et al. 1952; Lowe 1959; Carter 1962; Pigden and Minson 1969).

CHAPTER THREE

GENERAL DISCUSSION AND CONCLUSIONS

GENERAL DISCUSSION

The results of the experiment have indicated that the use of CRC (for administering Cr_2O_3 to dairy cows to estimate faecal output and hence feed intake) can improve the current understanding of feed intake under grazing and indoor feeding conditions. A single daily faecal sample has provided estimates of faecal output similar to those obtained using actual feed intake and in vitro feed digestibility measurements (eqn 2.1).

This experiment has also shown that faecal samples from individual animals can be collected at any time of the day without reducing the degree of accuracy of Cr recovery. Collections can occur at milking and therefore the CRC has the advantage of being less labour demanding than twice daily administration of chromium in the form of either gelatin capsules or impregnated paper.

However, several problems still exist with the validation of CRC. No total collection of faeces was obtained to validate the accuracy of faecal output (FO_e) values estimated from the actual intake and feed indigestibility (eqn 2.1). There was a problem of capsules being regurgitated by cows. Kassano (1988) found a similar problem when he administered sheep CRC into calves. Wilson (pers. comm.) reported in his experiment that 15% regurgitation occurred when grazing cows were administered the same type of CRC as used in this trial. An irregular pattern of Cr release was found in 3 cows over the first 10 days after administering the capsules. Overall only 11 out of 16 capsules (69%) performed to a satisfactory standard.

A solution to the regurgitation and the irregular pattern of Cr release could not be identified in this trial as the animals were not slaughtered. It is speculated that the design of the capsule may need to be modified. Parker et al. (1990) and Hirschberg et al. (1990) reported no regurgitation in CRC administered to sheep and 200 kg steers respectively. Captec are currently modifying their cattle CRC design and this may overcome the regurgitation problem.

The accuracy of feed intake estimation (when eqn 1.1 is used) could also be limited by the inaccuracy of digestibility measurement. Providing representative samples of herbage consumed for digestibility determination using the in vitro method is crucial. It is suggested the samples of herbage that more accurately reflect the diet of animal can be provided by oesophageal fistulates rather than cutting herbage inside cages placed over herbage prior to grazing as was used in this trial. The in vivo feed digestibility values from the sheep are the only comparable values for the in vitro digestibility values obtained (in vitro values from feed offered to sheep and cows), but are

not really comparable because sheep are more selective feeders than cattle.

The estimation of intake by pasture cuts and Cr dilution methods were not significantly different. However the Cr dilution technique is less time consuming and less labour intensive than the pasture technique. A minor disadvantage in the Cr dilution technique is obtaining faecal samples from each individual cow under paddock grazing conditions, especially if labour is limiting. Often cows, once disturbed, would simultaneously defecate making identification of faeces to individual cows difficult and hence difficulty in determination of the endpoint of Cr release for individual cows.

There are problems associated with measuring pasture intake using the pasture cuts technique. These are associated with variation in pasture height, species composition, water content, contamination and bulk density of pasture. Variation between operators when cutting the grass could also influence herbage mass determination. The Ellinbank Pasture Meter used in this trial could not give a daily feed intake estimation because no calibration between pasture height (cm) and pasture mass (kg DM/ha) was recorded.

To date, the CRC has not been assessed in tropical regions such as Indonesia. Australian workers reported that the CRC gave satisfactory results when administered into beef cows under sub-tropical pasture but there still remains an unanswered question about the suitability of CRC for tropical pasture (Barlow et al. 1988). Benefit from this new technology may be applied into several types of experiments such as stocking rates, feed availability and quality, use of dietary supplements, breed differences and disease treatments. Further investigations to examine different capsule design (to prevent regurgitation and irregular Cr release patterns) are required, e.g. a series of experiments using CRC to measure intake followed by serial slaughtering of the animals.

As New Zealand production systems are based on seasonal pasture production e.g., spring, summer, autumn and winter, it would be interesting to asses the CRC under different seasons or stages of production e.g. early, late lactation, and the dry period. Another topic that deserves investigation is measurement of feed intake using CRC in animals fed supplements while at pasture or under feedlot conditions.

CONCLUSION

Controlled release capsules for administering Cr_2O_3 in ruminant animals does increase the flexibility for measuring faecal output, and hence, feed intake in grazing and indoor feeding situations. Some refinements to capsule design are required to prevent malfunctions. The capsule should be tested under a wide range of conditions including tropical pastures such as found in Indonesia or where animals are fed supplements either while grazing or under feedlot conditions.

APPENDICES

APPENDIX 1. PROCEDURE FOR IN VITRO ANALYSIS AS DESCRIBED BY ROUGHAN AND HOLLAND (1977).

Principle

Laboratory determination of in vitro digestibility involves comparing samples of unknown digestibility with samples of known in vivo digestibility (standards). Known amounts of freeze-dried, ground (1 mm mesh) samples and standards are first subjected to a hot neutral detergent solution to remove soluble cell contents. The samples are then washed once in hot distilled water and twice in cold distilled water. The cell wall is then hydrolysed with fungal cellulase solution (derived from Trichoderma sp.) for 5 hours at 50°C. The remaining undigested material is filtered, weighed and ashed. The total percentage ash by weight of the samples and standards is determined simultaneously. Laboratory results for the in vivo standards are used to derive a regression relating the laboratory in vitro digestibility to their known in vivo digestibility values. This regression is then used to estimate in vivo digestibility for the unknown samples. The procedure is able to estimate DMD, DOMD, and OMD. The procedure follows closely that of Roughan and Holland (1977).

Equipment

72	30 ml Pyrex beakers
72	20 ml culture tubes with screw caps
1-2	rack(s) to hold 72 tubes above
72	Gooch crucibles 40 mm i.d. porosity 1
1	muffle furnace
1	1 litre wash bottle
1	10 ml dispenser 1 litre reservoir
1	1 litre measuring cylinder
3	2 litre erlenmeyer flasks
5	boiling beads (5 mm diameter)
1	bunsen burner
1	tripod
1	gauze mat
1	pH meter
1	centrifuge heavy duty general purpose
1	filter pump (aspirator)
2	large glass dessicators 250 mm i.d.
2	dessicator plates 200 mm i.d.
1	powder funnel
1	2 litre filter flask
1	filter adapter rubber to fit filter flask above
1	stirring rod
1	100 ml beaker
1	analytical balance accurate to 0.1 mg
1	drying oven 40-200°C
1	incubator 10-120°C with rotisserie motor attached
1	rotating stage with 72 20 ml tube capacity
1	10 ml weighing boat

Reagents

Neutral Detergent Solution

Sodium dodecyl sulphate 30.0 g ethylene diamine tetra acetic acid - disodium salt (EDTA) 18.6 g di-sodium tetraborate (Borax) 6.8 g di-sodium hydrogen orthophosphate 4.6 g sodium sulphite 10.0 g 2-ethoxyethanol 10.0 ml. Make up fresh before use. Place all of the above reagents in a 2 litre erlenmeyer flask. Add 5 boiling beads. Add 1 litre of distilled water to the above and (heat to) dissolve. The pH should be between 6.9-7.1. Add concentrated HCl (about 2.5 ml) to achieve this.

Buffered Cellulase Solution

Sodium acetate ($C_2H_3NaO_2$ AR grade) 2.04 g acetic acid glascial 0.9 ml cellulase T-2 (Trichoderma derived) powder 18.0 g distilled water 1.5 l. Make the acetate buffer (pH 4.8) solution first. Weight the sodium acetate into a weighing boat. Transfer to a 2 litre erlenmeyer flask and dissolve in 500 ml of distilled water. Add the acetic acid followed by 1 litre of distilled water.

Weigh cellulase powder into a 100 ml beaker. Add approximately 30 ml of the buffer solution to the cellulase powder and mix to a paste. Add another 30 ml and mix well. Pour off the dissolved cellulase into a clean 2 litre erlenmeyer flask. Add another 60-70 ml of buffer solution to the cellulase and repeat the process until all the cellulase is dissolved. This solution can be made up the day before and stored covered in a refrigerator overnight.

The acetate buffer (pH 4.8) is used to keep the cellulase T-2 powder in its optimum pH range 2.5-7.0.

Cellulase T-2 powder has an optimum temperature range of 30-60°C. Be careful to keep the cellulase solution within this range during the laboratory procedure.

NOTE: Cellulase T-2 powder contains mainly cellulase (Cl-ase 20,000 u/g), and hemicellulase (36,000 u/g xylanase). Cellobase, avicelase, CMC-ase, amylase, and protease are also present in small quantities. Cellulase T-2 powder is available through Pfizer Chemicals Division, a division of Pfizer Laboratories Limited, Auckland.

Procedure

This laboratory procedure requires 6 working days to complete. Days 3,4 and 5 must be run consecutively with no break between them. Consecutive runs of this procedure can be overlapped if a large number of samples are to be analysed.

Day One (Monday or Thursday)

1. Choose at least 6 in vivo standards over the greatest range possible of those available. It is necessary to choose standard types (ie hay, lucerne, silage, fresh pasture, etc) to correspond with those of the unknowns (Goto and Minson, 1977; McLeod and Minson, 1982). The standards are placed in the last available spaces in the run, eg no.s 61-72 for 6 in vivo standards.

2. Label 72 30 ml pyrex beakers from 1 to 72. Place beakers on a tray and into alternate beakers (starting with beaker no. 1) place approximately 2.0 g (half fill the beaker). Write the sample or standard name next to the appropriate numbers on the result sheet. Next to the name of each standard record the in vivo DMD, DOMD, and OMD values in the appropriate columns.

3. Place all the beakers in oven at 105°C overnight.

Day Two (Tuesday or Friday)

1. Remove beakers from the oven and transfer to a dessicator to cool. Cool to room temperature.

2. Label 72 20 ml screw cap test tubes from 1-72. Please them in order in the rack(s). Accurately weigh out 0.19 - 0.21 g of each sample or standard into a weighing boat. Record the weight (to 0.1 mg) on the result sheet in the column labelled 'WS' (Weight of Sample). Quantitatively transfer the contents of the weighing boat into the appropriate test tube. Duplicate this for each sample and standard.

N.B. This section of the procedure is determining the total percentage ash of the samples and standards.

3. Accurately weigh (to 0.1 mg) the alternate empty beaker (starting with beaker no. 2) and record on the result sheet in the column labelled 'WB' (Weight of Beaker). Transfer the remainder of the dry sample from the beaker immediately prior into the weighed beaker. Weigh the beaker and sample (to 0.1 mg) and record in the column labelled 'WBND' (weight of Beaker and Non-Digested dried sample).

4. Place weighed beakers containing the dry sample in a muffle furnace at 500°C overnight. (This will have to be done Monday night for a Thursday start batch).

5. Make up neutral detergent solution as given in the section on 'Reagents" above. Cover and leave to cool overnight.

6. Check the pH of the neutral detergent solution is 6.9-7.1. If not, add concentrated HC1 to get the right pH (about 2.5 ml).

Day Three (Wednesday or Monday)

1. Turn rotisserie oven on at 100°C.

2. Remove ashed samples from the furnace and transfer to a dessicator to cool. Cool to room temperature.

3. Weigh beaker and ashed sample (to 0.1 mg) and record in the column labelled 'WBNA' (Weight of Beaker and Non-Digested Ashed sample). Discard ash, wash and dry beakers.

4. Heat the neutral detergent solution to boiling.

5. Add 10 ml of just-boiled neutral detergent solution to each test tube containing the weighed samples. Cap each tube and shake vigorously to thoroughly mix sample and detergent solution.

6. Place tubes on the rotating stage for the rotisserie oven. Be careful to balance the rotating stage to reduce wear on the rotisserie motor. The rotating stage does not balance, so the test tubes are arranged to compensate for this.

7. Place rotating stage and test tubes in the rotisserie oven for 1 hour at 100°C.

8. Remove rotating stage and test tubes from the oven and quickly transfer the tubes to the centrifuge tube holders. Centrifuge while hot at 2000 rpm for 15 mins.

9. Aspirate the supernatant, being careful not to remove any of the sample or standard.

10. Heat 1 litre of distilled water to boiling (use white jug).

11. Add 10 ml of boiled distilled water to each test tube. Cap each test tube and shake vigorously to thoroughly mix sample and water.

12. Place rotating stage and test tubes in the rotisserie oven for 30 mins at 100°C.

13. Remove rotating stage and test tubes from the oven and quickly transfer the tubes to centrifuge holders. Spin at 2000 rpm for 15 mins.

14. Aspirate the supernatant, being careful not to remove any of the sample.

15. Add 10 ml of cold distilled water to each test tube, cap each tube and shake vigorously to thoroughly mix.

16. Centrifuge the tubes at 2000 rpm for 15 mins.

17. Repeat steps 14 and 15.

18. Centrifuge the tubes at 2000 rpm for 30 mins.

19. Aspirate the supernatant, being careful not to remove any of the sample.

20. Make up the cellulase solution as given in the "Reagents" section. Cover, and place in the refrigerator overnight.

Day Four (Tuesday or Thursday)

1. Turn rotisserie oven on to 50°C.

2. Add 10 ml of cellulase solution to each test tube. Cap each tube and shake vigorously to thoroughly mix sample and cellulase solution.

3. Do (2) and (3) of Day Three if not already done.

4. Place tubes on the rotating stage as in Step 3, Day Three.

5. Place rotating stage and test tubes on rotisserie oven for 5 hours at 50°C.10.45 - 3.45 9.45 - 2.45

6. Number 72 Gooch crucibles porosity 1 from 1-72. Place on a tray in order and dry in a furnace for 3 hours (500°C).

7. Remove crucibles from furnace and transfer to dessicators to cool. Cool to room temperature.

8. Accurately weigh (to 0.1 mg) the dried crucibles and record on the result sheet in the column 'WC' (Weight of Crucible).

9. Remove the rotating stage and test tubes from oven. Quickly transfer the test tubes to centrifuge tube holders while still warm.

10. Centrifuge the tubes at 2000 rpm for 15 mins.

11. Aspirate the supernatant being careful not to remove any sample.
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12. Add 10 ml of cellulase solution to each test tube. Cap each tube and shake vigorously to thoroughly mix sample and cullulase solution,.

13. Place tubes on the rotating stages as in Step 3, Day Three.

14. Place rotating stage and test tubes in rotisserie oven overnight (15 hours - 5 pm to 8 am) at 50°C.

Day Five (Friday or Wednesday)

1. Remove the rotating stage and test tubes from the oven.

2. Place tubes in centrifuge holders, spin at 2000 rpm for 15 mins.

3. Aspirate cellulase off. Resuspend in room temperature distilled water, 10 mls.

4. Place crucible no. 1 in filter adapter in filter flask. Filter contents of test tube no 1 through crucible. Rinse screw cap and test tube with distilled water until no fine pieces of sample remain. Repeat until all samples are filtered.

5. Place tray of crucibles and filtered samples in oven at 105°C overnight.

6. Wash and dry test tubes and lids.

Day Six (Monday or Thursday)

1. Remove crucibles from oven and transfer to dessicators to cool. Cool to room temperature.

2. Accurately weigh (to 0.1 mg) the crucibles and dried filtered samples and record on result sheet in column 'WCUD' (Weight or Crucible and Undigested Dried sample).

3. Place the dried crucibles in muffle furnace at 500°C overnight.

Day Seven (Tuesday or Friday)

1. Remove the crucibles with ash from the furnace and transfer to dessicators to cool. Be careful not to lose any of the fine ash when moving the crucibles. Cool to room temperature.

2. Accurately weigh (to 0.1 mg) the crucibles and the ashed samples and record on result sheet in column 'WCUA' (Weight of Crucible and Undigested Ashed sample).

3. Wash and dry crucibles rinsing them well.

APPENDIX 2 CHROMIUM ASSAY METHOD OUTLINED BY COSTIGAN ET AL. (1987) MODIFIED BY PARKER ET AL. (1989).

1. Weigh out approximately 1 g sample of faeces for each cattle/treatment in duplicate.

2. Dry samples in oven for 3-4 hours and reweigh.

3. Place dried samples in furnace overnight at 500°C for 12 h ashing. Allow samples to cool until they can be handled, then place in dessimating chamber until beakers are at room temperature. Reweigh to record ash weight.

4. Digestion:

   (a) Acid = 97: 3 v/v/
   85% phosphoric acid. 10% v/w Mn SO_4.4 H_20 (1940 mls 85% phosphoric acid added to 60 ml Mn SO_4 solution).

   (b) Bromate = 4.5% w/v $KBr0_3$ in distilled H_20 (00 g - in 2000 ml)

   (i) Pre heat block to 140°C - turn control box to 265°C for 20 minutes before starting.

   (ii) Add 4 ml of acid to ashed sample and heat to 140°C. This should take 15-20 minutes.
   Cover with heat proof glass. Remove samples and cool under 100°C. Turn control box up to maximum of 399°C.

   (iii) Add 6 ml of bromate and reheat until block thermometer is reading 210°C.

   (iv) Remove samples from block and allow to cool. Approximately 10 ml of warm-hot water (distilled H_20 (70°C)) should be added to each beaker to prevent gel formation.

   (v) Quantitatively transfer beaker digest to 50 cm^3 volumetric flask. Thoroughly flush beaker using distilled water. Make up volumetric flask to 50 cm^3.

   (vi) Stand digest overnight, then pour off 10-15 ml into plastic pottles.

APPENDIX 3 REGRESSION BETWEEN PASTURE HEIGHT (X:CM) AND PRE- AND POST-GRAZING PASTURE MASS (Y:KG DM/HA) ESTIMATED BY ELLINBANK PASTURE METER (EPM).

Pasture height (x = cm)	Pasture mass (y = kg DM/ha)
15.56	4682.6
15.42	3885.9
13.72	4417.0
16.88	4575.6
10.64	2877.0
10.10	2438.0
7.48	2194.0
8.42	2753.0

$y = 196 + 267 x$

$r = 0.93$.

y = pasture mass (kgDM/ha).

x = Pasture height (cm).

APPENDIX 4 ORGANIC MATTER DIGESTIBILITY ESTIMATES (OMD) OF PASTURE GRAZED BY COWS.

Day	OMD (%)
20	78.29
21	78.28
22	76.90
23	79.01
Mean ± SE	78.12 ± .44

APPENDIX 5 DMI (± SE) ESTIMATED BY PASTURE CUTS AND CR DILUTION FOR 4 DAYS GRAZING PERIOD (KG DM/DAY)

Day	Pasture Cuts	Cr dilntion
20	16.47	17.02
21	15.71	17.61
22	19.68	17.97
23	17.69	-
Mean	17.38± .86	17.53± .28

APPENDIX 6 OMI (± SE) ESTIMATED BY PASTURE CUTS AND CR DILUTION FOR 4 DAYS GRAZING PERIOD (KG OM/DAY)

Day	Pasture Cuts	Cr dilution
20	15.16	16.32
21	14.47	17.16
22	18.12	17.45
23	16.29	-
Mean	16.10± .69	16.97± .34

APPENDIX 7 BOTANICAL COMPOSITION OF FEED OFFERED TO SHEEP IN IN VIVO TRIAL AND COWS WHILE GRAZING

	white clover	stem	leaf	weed	dead
sheep					
day 7-11	10	17	31	31	11
day 12-16	7	20	27	34	12
Grazing cows					
pre grazing	9	4	40	17	31
Pasture consumed	11	6	45	26	12
post grazing	4	28	7	21	40

APPENDIX 8 COMPARISON BETWEEN INDOOR AND OUTDOOR DAILY MILK PRODUCTION (kg/day ± SE)

Day of CRC	Indoor Milk Production (kg/day)	(% fat)	Outdoor Milk Production (kg/day)	(% fat)
9	13.63± .91	0.61±0.4		
10	12.78± .31	0.55± .04		
11	13.29± .61	0.59± .03		
12	13.39± .83	0.59± .04		
13	13.54± .41	0.60± .05		
14	13.02± .20	0.55± .02		
15	12.25± .90	0.52± .04		
16	13.03± .80	0.53± .03		
17	12.72± .78	0.55± .03		
18	13.58± .88	0.56± .04		
19				
20			14.15± .78	0.62± .04
21			14.06± .80	0.60± .04
22			13.96± .84	0.57± .04
23			14.46± .88	0.60± .03
Mean ± SE	13.12±0.14	0.57± .009	14.16±0.10	0.60±0.01

APPENDIX 9 MEAN (± SE) DAILY FAECAL OUTPUT (FO_e) DERIVED FROM MEAN ACTUAL MEAN INTAKE (± SE) USING THE IN VITRO FEED INDIGESTIBILITY FOR THE TWO PERIODS (D 9-13 AND D 14-18)

Period	Day	Actual Intake		FO_e	
		kg DM	kg OM	kg DM	kg OM
	9	14.09±0.80	12.84±0.70	4.44±0.30	3.35±0.20
1	10	15.50±0.80	14.20±0.70	4.89±0.30	3.71±0.20
	11	16.51±0.80	15.24±0.70	5.21±0.30	3.98±0.20
	12	14.91±0.62	13.73±0.60	4.78±0.20	3.52±0.20
	13	15.12±0.39	13.68±0.50	4.78±0.20	3.57±0.20
Overall Mean		15.23±0.39	13.94±0.39	4.89±0.12	3.64±0.10
	14	14.53±0.60	14.53±0.60	4.89±0.20	3.58±0.10
	15	15.11±0.90	15.11±0.90	5.08±0.30	3.29±0.20
2	16	16.24±0.70	16.24±0.70	5.46±0.20	4.10±0.20
	17	16.51±0.80	16.51±0.80	5.55±0.30	4.18±0.20
	18	13.99±0.60	13.99±0.60	4.70±0.20	3.55±0.20
Overall Mean		15.28±0.50	13.87±0.50	5.15±0.20	3.84±0.10

APPENDIX 10 MEAN (± SE) DAILY FAECAL OUTPUT (FO_{cr}) AND MEAN (± SE) DAILY INTAKE ESTIMATED BY THE CR-DILUTION AND IN VITRO FEED INDIGESTIBILITY FOR THE TWO PERIODS (D 9-13 AND D 14-18).

Period	Day	FO_{cr}		Estimated intake	
		kg DM	kg OM	kg DM	kg OM
	9	4.36±0.40	3.28±0.30	13.81±1.20	12.56±1.10
	10	5.57±0.40	4.46±0.30	17.65±1.10	17.09±1.10
1	11	4.78±0.40	3.86±0.30	15.17±1.10	14.80±1.00
	12	5.7 ±0.60	4.90±0.50	18.92±1.80	18.77±1.80
	13	5.2 ±0.30	4.28±0.30	16.62±0.90	16.39±0.90
Overall Mean		5.20±0.30	4.15±0.30	16.43±0.90	15.92±1.00
	14	5.11±0.30	4.15±0.20	15.20±0.90	15.00±0.80
	15	5.10±0.30	4.07±0.20	15.18±0.70	14.71±0.72
2	16	5.09±0.20	4.12±0.20	15.15±0.60	14.87±0.70
	17	5.67±0.30	4.41±0.40	16.85±0.90	15.91±1.40
	18	5.80±0.30	4.72±0.30	17.24±0.90	17.05±0.90
Overall Mean		5.36±0.20	4.30±0.70	15.93±0.50	15.51±0.40

APPENDIX 11 DAILY IN VITRO FEED DIGESTIBILITY OFFERED TO THE COWS FOR TWO PERIODS (D 9-13 AND D 14-18) (% DMD AND % OMD)

Day of CRC life	DMD	OMD
9	69.19	74.34
10	70.44	75.33
11	69.42	74.46
12	67.79	71.43
13	65.51	71.99
Mean ± SE	68.47±0.09	72.31±0.04
14	64.42	71.04
15	66.09	72.02
16	67.43	73.02
17	66.56	72.35
18	67.46	73.12
Mean ± SE	66.29±0.06	72.31±0.04

APPENDIX 12 DAILY FEED DIGESTIBILITY FROM IN VIVO DIGESTIBILITY TRIAL WITH SHEEP (% DM)

Day of cow's CRC life	Means ± SE
7	77.08±1.66
8	72.06±3.26
9	69.95±1.31
10	72.98±0.59
11	73.96±1.22
Mean ± SE	73.21±1.21
12	72.28±0.88
13	73.48±1.00
14	70.39±1.90
15	70.41±1.60
16	75.52±1.40
Mean ± SE	72.42±0.97

APPENDIX 13 PAIRED T-TEST FOR THE DIFFERENCE BETWEEN FO_e AND FO_{cr} FOR PERIOD 1 (D 9-13) AND PERIOD 2 (D 14-18) (KG DM/D AND KG OM/D).

	Period 1		Period 2	
	Kg DM	Kg OM	Kg DM	Kg OM
Mean difference	0.40	0.51	0.21	0.45
Observation (n)	5	5	5	5
SE	0.30	0.27	0.24	0.2
T	1.28	1.90	0.90	2.26
Prob > | T |	0.268	0.13	0.41	0.08

APPENDIX 14 PAIRED T-TEST FOR THE DIFFERENCE BETWEEN PERIOD 1 (D 9-13) AND PERIOD 2 (D 14-18) FOR FO_e AND FO_{cr} (KG DM/D AND KG OM/D).

	FO_e		FO_{cr}	
	kg DM	Kg OM	kg DM	Kg OM
Mean difference	0.33	0.20	0.17	0.15
Observation (n)	5	5	5	5
SE	0.15	0.11	0.24	0.26
T	2.16	1.91	0.73	0.55
Prob > | T |	0.09	0.13	0.51	0.613

APPENDIX 15. THE ESTIMATED DAY OF EXPIRATION OF CHROMIUM MATRIX (11 CONS)

Day	125	126	128	131	140	169	172	179	197	206	224
8	4.72	5.33	5.49	7.36	5.74	2.81	5.19	5.47	4.32	4.29	2.43
9	5.10	5.17	5.98	7.06	6.35	2.87	4.68	5.56	4.83	5.26	3.68
10	5.18	4.37	4.64	4.39	5.09	2.90	3.33	2.82	3.75	3.98	3.70
11	4.97	4.14	3.10	4.89	3.62	3.29	3.34	3.39	3.16	4.18	4.31
12	4.91	3.97	3.94	4.75	4.18	3.35	4.81	3.06	2.88	4.12	3.50
13	4.69	4.25	4.39	5.58	5.70	3.17	3.65	3.24	4.0	3.49	3.53
14	4.32	4.69	4.36	5.91	5.80	3.61	3.71	3.41	3.89	4.03	3.44
15	3.93	4.35	4.06	6.67	5.20	3.95	3.73	4.06	3.97	3.79	3.58
16	5.24	4.25	4.16	5.66	3.50	3.53	3.39	3.73	4.47	4.31	3.93
17	3.93	3.86	4.27	4.24	4.32	3.10	3.89	3.31	2.61	4.05	4.46
18	3.83	4.21	3.14	4.25	4.26	2.93	2.93	3.74	4.07	4.24	3.40
19	-[a]	4.08	0.39	3.07	-	4.02	3.40	3.36	2.61	2.05	2.84
20	4.98	-	0.18	0.44	-	-	3.45	5.06	2.07	0.62	3.94
21	5.18	4.85	0.26	0.59	4.79	3.21	3.95	3.88	-	-	3.59
22	-	4.0	-	0.59	4.26	-	4.98	4.03	-	0.48	3.70
23	0.83	-	0.36	0.28	-	-	4.43	-	-	0.34	-
24	-	0.79	-	0.46	-	3.88	0.24	1.50	-	0.25	0.76
25	0.25	-	0.15	0.96	-	-	-	0.70	0.42	0.23	-
Mean	4.65	4.44	4.32	5.42	4.84	3.37	3.93	3.87	3.81	4.16	3.60
Endpoint(d)	23	23	19	20	25	25	24	24	18	20	24
CV (%)	11.11	10.46	19.85	24.00	18.56	16.35	17.33	21.92	17.78	10.49	13.92
SE	0.15	0.12	0.26	0.39	0.24	0.15	0.17	0.22	0.20	0.13	0.13

[a] no faeces collected

APPENDIX 16 ESTIMATION OF FAECAL OUTPUT FROM CHROMIUM ATOMIC ABSORPTION READING

Faecal output (FO) = $\frac{\text{Chromium release d (mg Cr/d)}}{\text{Chromium recovered/gram faecal dry matter (DM)}}$

(i) Chromium release rate = $\frac{\text{length core (mm)}}{\text{No. of days to endpoint}}$ = mm/d.

(ii) Chromium released/d = $\pi.r^2.d.t.p.mw$

where r^2 = radius of CRC

d = Density of chromium (2.064 g/cm^3)

t = (i)

p = Active concentration of Cr_2O_3 (0.65)

mw = Molecular weight (0.6843)

(iii) Chromium in faeces = AA x 1/W x 1.0416 x 0.05

where AA = Atomic reading

W = weight of faeces analysed

1.0416 = recovery factor

0.05 = dilution factor

(iv) FO = $\frac{\text{Chromium released/d (ii)}}{\text{Chromium in faeces (iii)}}$ = g DM/d

APPENDIX 17 CONVERTING DM INTO OM BASIS

DM intake = DM intake + (OM intake x Ash %)

Ash = Ash contents in the herbage sample for in vitro analysis (17.1)

FOM = FO (1-Ash) (17.2)

where the FOM = Faecal organic matter

FO = Faecal dry matter

Ash = Ash content with faecal sample

$$\text{DM intake (kg OM/d)} = \frac{\text{FOM}}{\text{1-DOM}} \quad (17.3)$$

FOM = Faecal organic matter (17.2)

DOM = Digestible organic matter (%) by in vitro analysis.

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