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# APPLICATION OF INTRARUMINAL CHROMIUM CONTROLLED RELEASE CAPSULES TO THE MEASUREMENT OF HERBAGE INTAKE OF SHEEP AT PASTURE

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Animal Science at Massey University

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### ABSTRACT

Experimental evidence obtained since 1950 suggests that New Zealand sheep farm production and financial returns could be increased by adopting separate grazing management for ewes of different pregnancy and rearing status from 6 weeks before lambing until weaning. Progress in developing management systems for the differential allocation of pasture, favourable lambing paddocks and labour during this period has been restricted by the absence of equipment for diagnosing ewe pregnancy status and a lack of data relating pasture conditions to feed intake and ewe and lamb productivity. Accurate pregnancy diagnosis by realtime ultrasound scanning has been available to farmers since 1985, but research into ewe grazing management continues to be hampered by the absence of techniques for measuring feed intake. This thesis addressed the latter issue, first by validating controlled release capsule (CRC) technology for measuring feed intake and second by examining feed intakes of ewes differing in pregnancy and rearing status and relating intakes to productivity.

A series of 11 experiments were conducted with sheep CRC to validate this technology for measurement of intake and to develop appropriate systems for using the technology in experimental situations. These studies examined: the linearity and period of Cr<sub>2</sub>O<sub>3</sub> release; the effect of presence of capsules in the rumen on voluntary feed intake; the effect of feed type and feeding level on Cr<sub>2</sub>O<sub>3</sub> release rate; and the accuracy of faecal Cr<sub>2</sub>O<sub>3</sub> concentration in predicting faecal output of sheep dosed with CRC when alternative sampling regimens were applied. These experiments, conducted under both indoor feeding and outdoor grazing conditions, established that CRC released Cr2O3 into the rumen in a uniform manner once initiation of matrix extrusion had been completed 2 to 3 days after capsule insertion. The subsequent period of linear release (25 to 100 days) was found to be primarily dependent upon characteristics of the capsules controlled at manufacture (i.e. orifice diameter, matrix composition and length of pressed tablet matrix core). In comparison, environmental factors, both within and outside the sheep, had relatively small effects on the rate or linearity of Cr2O3 release. Release rate decreased by c. 4% if daily feed intake was at 0.7 maintenance compared to an <u>ad libitum</u> level, increased by c. 2% if hay rather than fresh pasture was consumed and decreased by 10 to 13% if capsules were placed in rumen-fistulated sheep rather than in intact animals. Adoption of feeding level below 0.6 maintenance for 4 to 7 days reduced Cr<sub>2</sub>O<sub>3</sub> release rate and could cause capsule failure. Between-capsule variation in release rate from CRC recovered from the rumen by slaughter was low (coefficient of variation 2.0 to 6.5%). Variation between capsules within sheep was usually lower still. Voluntary herbage intake was significantly reduced if sheep were dosed with prototype CRC with inflexible wing designs. Under indoor conditions, correlations of 0.90 to 0.99 between daily faecal output derived by Cr<sub>2</sub>O<sub>3</sub> dilution and actual faecal output for individual sheep were obtained. The correlation between estimates of mean 3-day faecal output of sheep at pasture predicted from the Cr2O3 concentration in morning and evening grab samples and from total collections was 0.87.

Prediction of individual animal intakes (indoors) appeared less accurate (r=0.74) because of variation in capsule release rate and in the animal's own ability to select

and digest its diet. Group mean estimates, which are appropriate for practical grazing conditions, were usually within  $\pm 10\%$  of the actual value. Low diurnal variation in faecal Cr<sub>2</sub>O<sub>3</sub> concentration (non-significant) allowed flexible faecal sampling regimens to be applied. In summary CRC were demonstrated to be superior to existing feed intake measurement techniques and to be well suited to the estimation of mean intakes of sheep, provided that suitable faecal sampling regimens were applied.

A pilot study investigating the feed intakes and productivity of ewes of different pregnancy and rearing status indicated that intakes of twin-bearing ewes were reduced in comparison to those of single-bearing ewes during late pregnancy, when the two groups were grazed together under "commercial" farming conditions. During lactation, intakes exhibited a curvilinear relationship with time and were generally higher (by up to 32%) in twin-rearing ewes than in single-rearing ewes. This pattern of feed intake was less clear in a subsequent nine-week lactation study. In that trial, experimental groups comprising equal numbers of ewes rearing single or twin lambs were continuously grazed on five different pastures maintained at fixed sward surface heights (2.5, 4.0, 6.0, 7.0 and 9.0 cm). Herbage intakes by both single- and twin-rearing ewes were maximised at a sward surface height of approximately 5.0 cm (1000 to 1100 kg dry matter/ha). Lamb growth rates were not affected by sward height during the first six weeks of lactation because the ewes mobilised body reserves to maintain milk production. All ewes lost liveweight during the first 6 weeks of lactation but only the ewes on the 2.5 cm sward failed to regain lost liveweight from weeks 6 to 9 of lactation. Wool production, strength and colour were not affected by sward conditions in either the ewes or lambs over the lactation period. These results suggest that New Zealand farmers would gain little benefit from differential management of ewes post-lambing where a minimum grazing height of 5.0 cm could be maintained provided that ewes were in good condition (i.e minimum condition score 3.0) at lambing.

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Miss Christine Andricksen patiently typed most of the manuscript, with assistance from Mrs Denise Stewart and Mrs Kathy Hamilton. Graphs were prepared by Miss Yvette Cottam.

I have achieved each of the goals I set myself at the commencement of this study: to maintain my family and church responsibilities; to continue my normal teaching requirements; and to enjoy the PhD study programme. It was not always easy and I am particurlarly grateful to Vivienne, my wife, for her loyal support during the last four years. Without her help this thesis would never have eventuated.

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# ABBREVIATIONS

	stanic charaction another hotomator
AA	atomic absorption spectrophotometer
ANOVA	analysis of variance
C.	circa (approximately)
CIDR	controlled internal drug release device
Cr	chromium (III)
Cr <sub>2</sub> O <sub>3</sub>	chromium sesquioxide
CRC	controlled release capsule
CS	condition score
CSIRO	Commonwealth Scientific and Industrial Research
	Organisation
CV.	cultivar
d	day
DM	dry matter
DMD	dry matter digestibility
DMI	dry matter intake
DOMD	digestible organic matter in the dry matter
	(D-value)
DOMI	digestible organic matter intake
DSIR	Division Scientific and Industrial Research
EPM	Ellinbank pasture meter
F.O.B.	free on board
h	hours
ha	hectare
HFRO	Hill Farming Research Organisation (Scotland)
ICPES	Industively coupled plasma emission
	spectrophotometry
L	lactation
М	maintenance
MA	mixed age
min.	minute
MJME	megajoules of metabolisable energy
NHA	net herbage accumulation
NA	not applicable
N/ktex	Newtons per kilotex
OM	organic matter
OMD	organic matter digestibility
OMI	organic matter intake
Р	pregnancy
RDM	residual dry matter
RH	relative humidity
RMT	rumen mean retention time
X	tristimulus value (red)
Y	tristimulus value (green)
Z	tristimulus value (blue)

### Weights, volumes and measures

μg	microgram
mg	milligram
g	gram
kg	kilogram
ml	millilitre
nm	nanometre
µm	micrometre
mm	millimetre
km	kilometre
mA	milliamp
<sup>o</sup> C	degrees centigrade
	0

### (xviv)

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#### Statistical terms

CV	coefficient of variation
df	degrees of freedom
n	number
r	correlation
RSD	residual standard deviation
SD(s)	standard deviation
sem	standard error of the mean
TSS	total sum of squares
var	variance
x	mean
NS	not significant
+	significant at P<0.1
*	significant at P<0.05
**	significant at P<0.01
***	significant at P < 0.001

#### **CHAPTER ONE**

### INTRODUCTION

#### BACKGROUND

The estimated New Zealand sheep population of 60.8 million (NZMWBES 1989) includes some 44 million breeding ewes. These are run on 22000 farms and contribute, through exports of lamb, mutton, live animals and wool, to approximately \$NZ 2.5 billion (F.O.B) export earnings (or c. 20% of total produced exports; Department of Statistics 1989). The New Zealand sheep industry is pastoral based. Most flocks are grazed at moderate to high stocking rates (7-20 ewes/ha), only limited supplementary feeding (mainly in the form of hay and silage) is employed and very few animals are housed during winter. Much of New Zealand's applied sheep research has therefore been focussed on improving the supply and quality of pasture feeds and on increasing the efficiency with which pasture is utilised by the animal. Evaluation of alternative management systems for the breeding cwe has been particularly important because productivity of the ewe impacts significantly upon national wool and lamb production.

The direction of sheep farming research has been significantly affected during the last two decades by fundamental changes in Government agricultural policy (Ulyatt 1989). From 1975 to 1981 farmers were actively encouraged, via subsidies and supplementary payments for produce, to increase livestock production (Bushnell et al. 1982). Sheep numbers increased quickly during this period to reach a peak of 70.3 million in June 1982. Sheep research tended to emphasize increasing productivity through higher stocking rates. This was often associated with farming systems which had higher costs because of increased inputs of items such as fertiliser, animal health and short-term capital improvements (e.g. subdivision, water supply and access).

This was to be quickly reversed by significant changes in economic policy following the election of a Labour Government in 1984 (Douglas 1984, 1985). The adoption of a floating exchange rate, removal of Government price support and input subsidies, and reduction of tariffs were all designed to create a less regulated, more market-orientated economy. These changes coincided with reduced demand and low international prices for sheep meats, and high domestic interest rates on borrowed finance. Most sheep farmers were placed under tight financial constraints. A common response was to reduce costs by adopting a less intensive farming system with increased emphasis on high per animal performance. Confidence to invest in farming, particularly on hill country where 45 % of the national flock is farmed, was sharply eroded and the large capital inputs associated with the development of New Zealand pastoral agriculture largely disappeared. Sheep numbers decreased rapidly and by 1989 had almost

contracted to the population size existing prior to the expansion of the late 1970's (NZMWBES 1989). Traditional priorities of pastoral and livestock research were now less relevant. Instead, research which would help farmers develop low input sustainable farming systems, or which contributed to improved utilisation of existing resources, while at the same time giving greater consideration to the requirements of the end-users of products became more applicable. It was expected that future improvements in output, efficiency and profit would be achieved as existing farm systems were 'fine-tuned' and technologies such as new plant varieties (MacKay et al. 1989), genetically superior animals (McEwen et al. 1988) and improved management information sytems (Baars 1988) were adopted.

However, because the provision of feed remains the major cost of pastoral farming, the production of pasture and the efficiency with which this is converted into saleable animal products must continue to be a primary focus of sheep research in New Zealand. While this poses new challenges, the need to design improved grazing management systems which ensure that a higher proportion of pasture grown is productively harvested by animals remains a high priority. This thesis is set within the context of designing management systems to improve the efficiency with which limited resources (mainly pasture, but also labour, shelter and farm topograghy) are allocated to breeding ewes during late pregnancy and lactation.

The common practice of farmers is to adopt a relatively fast (30-40 days) rotation or set stocking management during ewe pregnancy (Parker and Townsley 1986). Providing carly winter feed reserves are high, this usually results in moderate to high ewe body condition (condition score (CS) > 3.0) but low pasture reserves at the commencement of lambing (Smeaton et al. 1983). A similar situation occurs at lambing on farms where a slow (>70 days) winter rotation has been successfully used to transfer autumn and carly-winter pasture growth through the winter, but accumulated reserves of feed are used early by set stocking ewes three to six weeks prior to lambing (Smeaton et al. 1983). High ewe body condition in conjunction with low pasture reserves does not jeopardise lamb production if the onset of spring pasture growth coincides with the increased requirements of the lactating ewe and the ewe is exposed to only a short period (< 3 weeks) of underfeeding (Coop et al. 1972; McEwen et al. 1983). However, on properties where late summer-autumn pasture production is low or winter stocking rates are high (often through delayed sales of lambs), these management sytems usually result in underfeeding of ewes from mid- to late-pregnancy. The result at lambing is a combination of poor ewc condition (CS < 2.0), low ewe liveweights (< 50 kg) and inadequate farm pasture cover (400-700 kg DM/ha over the entire property). These conditions can be found on up to 50% of sheep farms in early spring (Parker 1984a). Under these circumstances ewe and lamb losses are high (Smcaton et al. 1983; Putu et

al. 1988), lamb growth rates are poor (Sheath and Bircham 1983), wool production and quality is depressed (Monteath 1971; Horton and Wickham 1979; Hawker and Thompson 1987) and further ewe liveweight losses, commonly of 5-10 kg by mid-lactation, are difficult to regain even if generous feeding allowances are made available post-weaning (Thompson et al. 1985). Furthermore, losing liveweight and refeeding to regain weight reduces the efficiency of feed utilisation compared to management systems which allow ewes to maintain liveweight (Keenan et al. 1969). Similarly, lambs with poor weaning weights rarely reached subsequent performance targets (McNeil 1982) and, if selected as flock replacements, have poorer lifetime performance (Hight and Jury 1976).

The effects of poor winter and spring grazing management are most pronounced in multiple-bearing ewes. Their higher nutritional requirement results in greater stress during periods of feed shortage than in their single-bearing peers. Separate grazing programmes for single- and twin-bearing(/rearing) ewes to account for their different feeding and physical requirements have therefore been recognised since the 1950's as a method of improving flock productivity (Richardson 1972). However, it was not until the late 1970's that the Department of Scientific and Industrial Research (DSIR) implemented a concerted research programme to develop equipment which would permit accurate identification of the pregnancy status of ewes at a modest cost to New Zealand farmers (Beach 1982). It was argued from research results and farmer experience that identification of ewe foetal status by 90 to 100 days of pregnancy would provide the following benefits (reviewed by Garrick (1984) and Blair (1984)):

- identification of non-pregnant ewes which could be sold at higher prices in midwinter;
- better utilisation of feed (and shelter) resources;
- reduced ewe losses through pregnancy toxaemia;
- improved survival of multiple lambs through better ewe condition at lambing and possibly increased lamb birthweights;
- reduced dystocia in single-bearing ewes by avoiding over-feeding and hence high lamb birthweights;
- improved nutrition of ewes rearing multiples, resulting in higher lamb growth rates, greater ewe wool production and higher ewe body condition at weaning;
- long term improvement in flock fecundity through the selection of twin-reared ewe lambs for flock replacements.

Because suitable equipment for pregnancy diagnosis had not been available up to this time (1981) no grazing management trials to quantify the effects of partitioning a fixed amount of pasture between single- and twin-rearing ewes had been conducted. There was conjecture as to whether the benefits identified from independent research of

components of the proposed management system would be realised in practice (e.g. Allison 1980). A series of management trials using prototypes of a truck-mounted X-ray system commenced in spring 1982 to evaluate, under commercial farming conditions, the effects of allocating twin-bearing ewes 30-35% of the pasture which would otherwise have been allocated to the single-bearing ewes, from one week prior to lambing until weaning (Parker 1984b). The trials demonstrated that the weaning weights of ewes rearing twins, and weights of their lambs, could be improved but this was obtained partly at the expense of weaning weights of single-rearing ewes. Most of the advantages of differential management were realised during the first 4-6 weeks of lactation. Pasture quality declined most rapidly on the paddocks with twin-rearing ewes at low stocking rates.

It was clear from this early work that the design of differential grazing management systems for the ewe was limited by the poor definition of relationships between ewe feed intake, pasture availability and production. Production responses for different methods of partitioning pasture between single- and twin-rearing ewes could be defined by conducting a series of farmlet trials, but this would be very expensive and limited to at most one or two environments. A more efficient approach would be to define the relationships between herbage intake, pasture conditions and animal production, and to use this information in modelling studies to identify the system which was most likely to be successful in practice (McCall 1984).

The most comprehensive New Zealand studies of herbage intake-allowance relationships for ewes of different bearing/rearing status (Rattray and Jagusch 1978; Rattray et al. 1982a,b) were derived by the difference technique with ewes shifted to fresh breaks of pasture every three days. These conditions are representative of commercial farming practice during pregnancy, but the majority of farmers adopt set stocking management from one to six weeks before lambing. Grazing management studies during lactation (Clarke and Lambert 1982; McEwen et al. 1983; Smeaton et al. 1983; Smeaton and Rattray 1984) suggest that the relationship between herbage intake, pasture availability and production are different under set stocking. In particular, intakes appear to be higher under conditions of low pasture availability. Earlier intake studies during lactation by Coop and Drew (1963) and in Britain by Bircham (1981) confirm this view.

In late 1985, when this research project commenced, each 1% improvement in sheepmeat and wool export production had an estimated F.O.B value of \$27 million (Anon. 1985), despite the unfavourable market conditions prevailing at that time (Taylor 1986). Further investigation of the effects of differential grazing of the ewe during late pregnancy and lactation to improve the efficiency and profitability of the

national flock was therefore warranted. However, before the partitioning of feed according to ewe pregnancy status could be successfully implemented on commercial sheep farms, basic research was still required in two main areas. First, further development of technology (and its application) for identifying the pregnancy status of the ewe accurately and at a low cost was essential. Second, better definition of feed intake-productivity relationships was needed to develop, even at a fundamental modelling level, alternative grazing management systems for the pregnant and lactating ewe. This thesis is concerned primarily with the latter need. It focuses on the validation of a new technology for measuring herbage intakes in ruminants and the application of this technology to the measurement of feed intakes of ewes at pasture.

# EFFECTS OF NUTRITION DURING PREGNANCY AND LACTATION ON PRODUCTIVITY OF THE EWE

Feed Requirements of Ewes of Different Pregnancy and Rearing Status

Feed requirements for the pregnant and lactating ewe under New Zealand pastoral conditions have been published by Coop and Drew (1963), Jagusch and Coop (1971) and Rattray (1974, 1986). A significant proportion of these feeding recommendations have been derived from indoor calorimetric studies rather than from measurements made on ewes at grazing. Estimates of the energy requirements associated with grazing, walking and the effects of climate are imprecisely defined and range from 25-80% of requirements measured indoors (Coop and Drew 1963; Hadjipicris and Holmes 1966; Russel et al. 1967; Langlands 1977). Coop and Drew (1963), for example, estimated that feed intakes of woolly ewes were increased by 10% due to a temperate autumn climate (60-80% in freshly shorn sheep depending on weather conditions (Joyce 1968)) and a further 20% when grazing was ad libitum or 50-80% when pastures were very short and grazing time was 6-8 hours per day. The typical adjustment for grazing sheep is to increase indoor requirements by 30-50% (Rattray 1986). Herbage allowance production relationships, derived mainly from rotational grazing trials conducted since 1975, supplement some of the feed intake recommendations (Rattray and Clarke 1984; Geenty and Rattray 1987). Herbage intakes obtained by the difference technique have been obtained with the herbage allowance - production relationships in some trials but, because of the limitations of this measurement technique (see p.15), these values can only be interpreted as being broad management guidelines. Thus, while textbook tables may give the impression that the feed requirements of the ewe are well defined, the absence of an easily applied and accurate method of measuring intake in grazing animals means that relatively few field trials have been conducted in New Zealand (or other temperate grassland countries for that matter) to validate the derived estimates of energy requirements of the ewe at grazing.

Feed requirements for both single- and twin-bearing ewes are reportedly similar to those of a non-pregnant ewe until 6 weeks before lambing (Foot and Russel 1979; Rattray 1986). A common recommendation is that twin-bearing ewes at this stage of lactation should be offered 1.3 maintenance (M) or 9% more than ewes with singletons. By weeks 3 and 1 pre-partum the difference increases to 21 and 24% respectively (Rattray 1986). However, in an indoor study of voluntary intake, Hadjipieris and Holmes (1966) found that intakes of twin-bearing ewes declined by 11% over the final six weeks of pregnancy while those of single-bearing ewes increased by 48% over the same period. A 12% decline in intakes of twin-bearing ewes between 7 and 2 weeks prior to parturition was measured by Foot and Russel (1979), although intakes were still higher at 2 weeks pre-partum than in mid-pregnancy. This suggests that reduced rumen volume, due to the rapidly increasing uterine volume over this period (Wallace 1948), limits the intake of twin-bearing ewes. A similar effect may be caused by a high level of abdominal fat (Forbes 1968). In practice then, and as acknowledged by Russel et al. (1967), the recommended levels of feed intake may not be physically possible in late pregnancy, particularly if grazing management to control feed intakes is imposed uniformly across the flock.

Feed intakes increase rapidly after parturition to meet the additional energy requirements of lactation and are estimated to be 50-100% greater than in dry ewes and 50-75% higher than the requirements of late pregnancy (Coop and Drew 1963; Hadjipieris and Holmes 1966; Langlands 1977; Foot and Russel 1979). Rattray (1986) reported that ewes rearing twins should consume 15, 17, 15 and 5% more than ewes with singletons at weeks 1, 3, 6 and 9 after parturition, respectively. For these recommendations peak intakes are equivalent to 3.5 M (3.1 kg DM/day for a 50 kg ewe) for twin-rearing ewes and 3.0 M (2.7 kg DM/day) for single-rearing ewes. During the later stages of lactation, energy is diverted to rebuilding body reserves (Coop and Drew 1963). Ewes, particularly those with a single lamb, are able to regain most of the liveweight lost in early lactation by weaning providing an <u>ad libitum</u> supply of high quality pasture is made available.

Production responses, in terms of ewe and lamb weaning weight, to increased nutrition are significantly greater during lactation than during pregnancy (Coop 1950; Rattray 1986), and effects of underfeeding during pregnancy can largely be compensated for by weaning at 12 weeks through a high level of nutrition during lactation (Smeaton and Rattray 1984). However, the loss of wool production and reduction in fibre strength through low winter feeding cannot be compensated for (Coop 1953; Monteath 1971; Hawker et al. 1982). The general recommendation, where pasture is in short supply, is to conserve pasture in late pregnancy by maintaining ewes on restricted intakes

(Rattray et al. 1982b; McCall 1984). Saved pasture can then be used more efficiently by the lactating cwe. This strategy should, however, be restricted to flocks of medium to low fecundity, especially if ewe condition is already low. Where the incidence of twinning is higher (>35%) more profitable options may be to differentially graze single- and multiple-bearing ewes or to introduce high quality supplements for twinbcaring cwes (Fowler and Thompson 1985; Earle and Male 1988) because of the potential detrimental effects of underfeeding on twin lamb survival (see p.8).

#### Effects of Grazing Management on Ewe and Lamb Performance

The ability to ration winter pasture supply means that farmers usually prefer some form of rotational grazing to set stocking during pregnancy. However, Smeaton and Rattray (1984), reported no differences in final ewe liveweight between winter grazing durations of 3.5, 14, 28 and 56 days. Ewe liveweights fluctuated more widely because of the greater extremes in daily nutrition associated with longer grazing durations but no information on lamb survival or overall productivity of the systems was provided (Sheath 1982). At high stocking rates, ewe and lamb weaning weights arc significantly reduced if set stocking replaces rotational grazing 4 weeks before, rather than at, lambing (Smeaton and Rattray 1984). A comparison of rotational and set stock grazing showed only small differences in annual flock productivity but lambing percentages were generally lower (by 0-34%) under rotational grazing (Clarke et al. 1986). Lamb deaths due to stillbirths, misadventure and dystocia were higher under rotational grazing, but starvation-exposure losses were much higher under set stocking. By contrast Scales et al. (1986) found that the incidence of dystocia was significantly higher in twin lambs from ewes set stocked from two days before the expected lambing date compared to twin lambs from ewes shedded daily as they lambed. Although a total of 2953 lambs were recorded in this study it is difficult to explain the cause of the shortterm dystocia response. The general consensus is that the benefits of rotational grazing during pregnancy in controlling feed intakes and ewe liveweight changes, and transferring pasture for consumption during lactation, are greatest where stocking rates are high (Smeaton et al. 1983) or winter pasture cover and ewe condition are low (Smeaton et al. 1985).

Set stock management from lambing to weaning is preferred by farmers because disturbance of the ewe at lambing is reduced, mismothering through shifting ewes is eliminated and control of spring pasture growth is more effective (Lambourne 1956; Sheath and Bircham 1983; Smeaton and Rattray 1984). At medium to high stocking rates rotational grazing during lactation generally increases ewe weights at weaning and produces pastures of poorer quality at weaning than does set stock grazing (Boswell et al. 1974; Milligan 1981), but has no effect on (Broadbent 1964; Newton et al. 1985) or

reduces (Smeaton and Rattray 1984) lamb wcaning weights. Lambourne (1956) found no difference between single- and twin-rearing ewes in liveweight, fleece production, wool quality, lamb survival or number of lambs drafted for slaughter at stocking rates of 10, 15 or 20 ewes/ha. but results may have been influenced by the use of cattle to control surplus pasture growth which was more prevalent on the rotationally grazed swards. Lamb growth rates are maximised at a daily allowance of about 8 kg DM/ewe/day under rotational grazing but both liveweight gain and wool production of single- and twin-rearing ewes continue to increase with greater daily allowances (Gibb and Treacher 1978; Rattray et al. 1982b: Munro and Geenty 1983; Penning et al. 1986). Rotational grazing may be adopted during early lactation (or continued from late pregnancy) to build pasture cover. However, the grazing interval should be reduced to 14 to 21 days once pasture cover approaches 1000-1100 kg DM/ha (when set stocking can be readopted) and mob sizes restricted (<400 ewes) to minimise mismothering of lambs (Sheath and Bircham 1983).

As mentioned previously, herbage intake-productivity relationships for ewes set stocked during lactation under New Zealand conditions are poorly defined. Although lambing date may have confounded the results. McEwen et al. (1983) estimated by the difference technique that intakes of ewes rearing single and twin lambs were not significantly different, but were 1.5 and 2.3 times greater on a residual herbage mass of c.500 and 900 kg DM/ha respectively than on 300 kg DM/ha. It is unlikely that the highest ewc intakcs (3.35 kg DM/cwe/day) would have been greater had residual herbage mass been increased further. This response pattern compares favourably with British research which has shown that intakes during lactation are maximised at a pasture height of 5-7 cm for single-bearing (Bircham 1981) and twin-bearing (Milne et al. 1981; Penning and Hooper 1985) ewes. This pasture height corresponds to a residual mass of c. 1100 kg DM/ha (Hodgson 1984). Langlands (1977) reported that under Australian conditions ewe intakes are maximised at residual herbage masses of around 1500 kg DM/ha. Lamb intakes arc less affected by extremes in herbage mass (Gibb and Treacher 1976) and are thought to be maximised at a sward height of > 3.0 cm (Wadsworth 1979). Thus the pasture requirements of suckling lambs for high production under set stocking appear to be similar to those of their dams.

#### Potential to Modify Lamb Birthweights by Nutrition

There has been considerable interest in the potential to modify lamb birthweights through ewe nutrition during pregnancy because lamb survival and lamb birthweight are highly correlated (Mullaney 1969; Dalton et al. 1980; Duff 1981). Lamb survival rates are highest when birthweights fall within a range of approximately 3.5 to 5.5 kg, depending on breed (Dalton et al. 1980). Lambs lighter than 3.5 kg are mainly born as

twins and are most prone to death from exposure, starvation or mismothering (Hinch et al. 1985; Scales et al. 1986). The common cause of death in lambs weighing more than 5.5 kg at birth, which are mainly singles, is injury during parturition (dystocia).

There is conflicting evidence concerning the degree to which lamb birthweights can be modified by nutrition during pregnancy, which can be considered in terms of three periods; early- (day 0-30), mid- (day 31-100) and late- (day 101-145) (Robinson 1983). The general recommendation is that ewe liveweight should be maintained during early pregnancy as embryo loss through reabsorption has been associated with both severe undernutrition and high planes of nutrition during this period (Bennet et al. 1964; Eddy 1976; Davis et al. 1981; Robinson 1983; Kelly et al. 1989).

Placental growth occurs mainly during mid-pregnancy, the placenta reaching its maximum size at about day 90 (Robinson 1983). Because placental weight is highly correlated with lamb birthweight (Mellor 1983) factors which influence placental development are important (Kelly and Ralph 1988). Coop and Clarke (1969) demonstrated that a ewe liveweight loss of up to 5 % during mid-pregnancy had only a minor effect on lamb survival (-0.5% in twins) compared to maintaining ewe liveweights, but no data on lamb birthweights or ewe intakes were provided. This result significantly influenced the development of winter grazing management systems in New Zealand. Moderate to severe undernutrition in mid-pregnancy can reduce lamb birthweight by up to 0.8 kg (Curll et al. 1975; Rattray and Trigg 1979; Kleeman et al. 1988) and cause foetal loss mainly through the reabsorption of one of a set of twin embryos (Kelly et al. 1989). The response in lamb birthweights may be dependent on the condition of the ewe at mating (Russel et al. 1981; Gunn et al. 1986), as well as other factors of animal origin (Russel et al. 1981). It is considered that the effect of midpregnancy nutrition on lamb survival and growth rates reflects changes to maternal body reserves and the mothering ability of the ewe, more than the effect of birthweight per se (McClymont and Lambourne 1960; Rattray and Trigg 1979; Russel et al. 1981; Kelly and Ralph 1988). For example, Dove et al. (1988a) found that level of nutrition did not significantly affect birthweights but that ewe milk production and lamb growth rates were greater in ewes which had been fed at a higher plane during mid-pregnancy. The effects of poor mid-pregnancy nutrition on lamb birthweight can be compensated for by increased feeding in late pregnancy (Parr et al. 1986) but the response is dependent on the severity of prior underfeeding (Rattray and Trigg 1979).

Foetal growth follows an exponential pattern (Rattray 1986) and approximately 80% of the final lamb birthweight is gained during during the last trimester of pregnancy. Ewe nutritional requirements correspondingly increase by 40 and 60% for single- and twinbearing ewes respectively (Rattray 1986). The potential to modify lamb birthweight

therefore appears to be greatest during the last six weeks of pregnancy. Low to moderate nutrition in late-pregnancy causes a larger reduction in the birthweights of twin than of single lambs (Wallace 1948; Coop 1950; Peart 1967; Russel et al. 1967; Smeaton et al. 1983; Kleeman et al. 1988), although zero or very small effects of low nutrition have also been recorded (Papadopoulos and Robinson 1957; Rattray et al. 1982a). These different nutritional responses may relate to placental development in early pregnancy (Davis et al. 1981; Robinson 1983), although it is difficult to compare between nutritional levels adopted in most grazing trials because feed intakes have not been recorded. Even with sub-maintenance feeding of the ewe from mid-pregnancy, lamb birthweights were reduced by only 0.5 to 0.7 kg on hill country pastures (Smeaton et al. 1985). The potential to increase birthweights of twin lambs by high levels of nutrition is limited (<0.5 kg) and may contribute to problems associated with overfatness in the ewe if a high level of nutrition is maintained throughout pregnancy (Forbes 1968; Rattray 1986). However, Earle and Male (1988) indicated that improved nutrition of twin-bearing ewes in good condition for only two weeks pre-partum significantly increased birthweights by 0.2 kg and lamb survival by 7%.

In summary, losses of large single lambs could be reduced by adopting moderate- to severe-underfeeding of single-bearing ewes from mid-pregnancy, but in practice this would only increase survival in flocks of medium fecundity by a maximum of 5-7%. There could, however, be significant reductions in ewe wool production and quality (Monteath 1971) and possibly carryover effects on ovulation rate at the next mating (Fletcher 1974; Cahill et al. 1984). Twin lamb birthweights could be increased by 0.5 kg with generous feeding in late pregnancy (Scales et al. 1986). This could improve lamb survival by up to 5% (Garrick 1984; Hinch et al. 1985), but associated gains in lamb survival from improved colostrum and milk production, and mothering ability, would probably be greater (McCance and Alexander 1959; Fowler and Thompson 1985; Putu et al. 1988).

#### **Ewe Milk Production**

The ewe lactation curve usually peaks one to four weeks after parturition and steadily declines from this point to a level which is between 20 and 40% of peak production after eight weeks (Barnicoat et al. 1949, 1957; Scales 1968; Geenty and Sykes 1986). Milk production is most influenced by the level and pattern of herbage intake but is also affected by carryover effects (e.g. ewe condition and feeding regimen) from pregnancy (Peart 1967; Foot and Russel 1979; Gibb and Treacher 1980; Gibb et al. 1981), ewe age and lamb birthweight (Rattray 1986). Twin-rearing ewes produce approximately 30% more milk at the peak of lactation than single-rearing ewes but differences in total production over the lactation may amount to only 10-15% (Corbett 1968; Geenty and

Sykes 1986). Normally 60-70% of total production is produced during the first 6 weeks of lactation (Peart 1967).

The production of colostrum at the commencement of lactation is earlier and more copius in cwes which have been fed to gain liveweight during the last 2-6 weeks of pregnancy than in ewes which have been undernourished during this period (McCance and Alexander 1959; Khalaf et al. 1979). This may significantly affect survival and subsequent growth of the lamb (McCance and Alexander 1959).

The reported correlation between milk production and lamb growth rate in ewes rearing singletons during the first 4 weeks of lactation is variable (r = -0.6 to 0.6), indicating that in many circumstances the lamb is unable to utilise all of the milk produced (Barnicoat et al. 1949; Slen et al. 1963; Peart 1967). Single-rearing ewes with the highest milk production therefore do not necessarily have the fastest growing lambs and factors other than ewe milk production greatly influence the growth of single lambs in early lactation. On the other hand, the growth rate of twin lambs shows a consistent positive correlation (r = 0.3 to 0.8) with milk production and ewes with highest milk production generally have the fastest growing lambs (Slen et al. 1963; Torres-Hernandez and Hohenboken 1980). The importance of milk production to the lamb progressively diminishes from 3 to 4 weeks after parturition, as development of the lamb's rumen allows pasture to be substituted for milk (Brown 1964). Herbage intakes increase most rapidly in twin lambs of ewes with low milk production and by week 7 of lactation are about 0.3-0.4 kg organic matter (OM)/day (Penning and Gibb 1979; Gibb and Treacher 1980; Geenty and Dyson 1986).

Milk production of cwes rearing twins is most responsive to increased pasture availability during the first 4-6 weeks of lactation (Barnicoat et al. 1957; Gibb and Treacher 1978). However, Gibb and Treacher (1980) indicate, that even with <u>ad libitum</u> feeding, twin-rearing ewes are unable to consume sufficient pasture in the early stages of lactation to meet daily energy requirements. Maternal body reserves are therefore utilised. This has the most pronounced effect when herbage availability is low. Thus ewes with higher body condition at lambing, because of more favourable feeding during or before pregnancy, generally have higher daily milk yields than thinner ewes (Gibb and Treacher 1980). However, if milk yields are expressed in terms of metabolic liveweight, ewes undernourished during pregnancy may be higher producers (Geenty and Sykes 1986). Delaying an increase in herbage availability until 4 weeks into lactation would have little effect through ewe milk production (Peart 1967), and lambs would gain greatest benefit from increased herbage intake.

It can be concluded that overall lamb productivity can be increased by ensuring that

twin-rearing ewes have high levels of body reserves (CS > 3.0) and <u>ad libitum</u> access to high quality spring pasture during the first 6 weeks of lactation. The timing of lambing in relation to the pattern of pasture production is therefore very important (Rattray et al. 1975; McEwen et al. 1983; McCall et al. 1986a). In situations where ewe condition and pasture availability were both low, worthwhile increases in twin lamb growth rates could be realised by using a high concentrate supplement (Earle and Male 1988). The amount and quality of herbage becomes increasingly important to the lamb as lactation progresses and, unless the lamb is weaned early (6-8 weeks), weaning weights of both the ewe and the lamb(s) will benefit from a good supply of high quality pasture (Gibb et al. 1981).

#### **Wool Production**

Few comparative studies of the wool production of non-mated ewes and ewes of different pregnancy and rearing status grazed together have been reported. Estimates of the relative wool production of dry and lactating ewes therefore mainly relate to situations where the groups have been grazed separately (Corbett 1979). Annual greasy wool production is estimated to be 0.0 to 0.45 kg (0 to 10% of annual production) lower in twin-rearing ewes than in those which which have reared a single lamb and 0.1 to 0.5 kg (3 to 10% of annual production) greater in dry ewes than in ewes rearing singles (Story and Ross 1960; Seebeck and Tribe 1963; Lewer et al. 1983; Langlands et al. 1984; Newman 1988). The relatively small proportion of any annual difference (5 to 8%) that can be attributed to the effects of rearing rank during lactation are dependent on the length of lactation, level of milk production and feeding level (Corbett 1979; Oddy 1985). Hawker et al. (1982) found that increasing herbage allowance from 2 to 10 kg dry matter (DM)/ewe/d would increase wool production by a maximum of 0.15 kg over a 12 week lactation. Pregnancy reduces annual wool production by 3-10% (Reid 1978; Corbett 1979). Most of this reduction occurs during the last trimester of pregnancy and is largest in twin-bearing ewes which are underfed (Corbett 1979; Rattray 1986). The effects of nutrition on wool growth are much more significant during both pregnancy and lactation than those caused by changes in the physiological status of the ewe (Coop 1953; Coop and Clark 1969; Monteath 1971; Corbett 1979; McCall et al. 1986a). It can be concluded that net wool production gains through preferential partitioning of pasture to ewes rearing twins during lactation are likely to be small.

#### Non-Nutritional Effects on Lamb Survival

Kilgour (1982) noted that on hill country a high proportion of lambs slip some distance from the lambing site at birth. This can lead to mismothering, particularly of multipleborn lambs, by disrupting the bonding process (Stevens et al. 1984). Lamb mortality on hill country properties may be reduced (possibly by more than 10%) if ewes are shifted to flat or easy contour (slopes  $< 20^{\circ}$ ) land for lambing (Knight et al. 1989). McMillan and Knight (1985) indicated that ewes and their lambs may be returned to steeper paddocks one day after lambing without further losses due to slope occurring.

Starvation-exposure mortality, which accounts for 25-50% of perinatal lamb deaths (Sykes 1982; Donnelly 1984), can be reduced by the provision of shelter. Bird et al. (1984a) summarised the effects of providing shelter on lamb mortality within 48 hours of birth in six trials and found that losses of both single and multiples were reduced by 30-50%. Planning feed supplies to ensure that the most sheltered paddocks are available for preferential use by twin-rearing ewes at lambing could therefore increase overall lamb survival by 6-10% (Garrick 1984).

### DEVELOPMENT OF SYSTEMS FOR DIAGNOSING EWE PREGNANCY STATUS

To implement differential grazing management of single- and twin-rearing ewes in commercial flocks an accurate, mobile, low cost, easily serviced and rapid (60-100 ewes/hour) method of diagnosing ewe foetal status is required. Diagnosis should be safe for both the ewe and her lamb(s) and the operator, and operational skills should preferably be able to be acquired with a modest level of training. A wide range of techniques for determining pregnancy status and diagnosis of multiple pregnancy have been tested. These have been reviewed by Lindahl (1971), Richardson (1972), Thwaites (1981) and Watt et al. (1984). The following section is restricted to a brief discussion of the use of laparoscopy, ultrasound scanning, X-ray fluoroscopy and blood metabolite levels for identifying groups of ewes for differential management during pregnancy and lactation.

#### Laparoscopy

The in situ observation of reproductive organs by laparoscopy allows pregnancy status to be determined from three days after mating (Kelly and Allison 1976). The technique requires skilled operators to prepare a small surgical incision and to operate specialised equipment for examining the ovaries. Up to 30 mature ewes can be examined per hour with four operators (McDonald 1989). The operation has a only a minor effect on ewe reproductive performance, even if repeated on the same ewe on several occasions (Kelly and Allison 1976). However it relies on the assumption that no fertilisation losses occur (i.e. that the number of foetuses equals the number of corpora lutea). Laparoscopy has mainly been adopted on commercial farms in New Zealand in association with embryo transfer programmes.

#### Radiography

Pregnancy diagnosis of ewes by X-ray was adopted in the 1940's for monitoring lamb foetal development (Wallace 1948) and was subsequently used for determining pregnancy status (e.g. Benzie 1951; Ardran and Brown 1963; Richardson 1972). Differentiation between foctal number with close to 100% accuracy could be achieved at 90-100 days, i.e. after foctal bone ossificiation (Richardson 1972; Wilson 1981). High capital costs, lack of portability of equipment, potential for exposure to radiation and relatively low throughputs (30-40 ewes per hour) were the main disadvantages of early X-ray pregnancy diagnosis systems (Rizzoli et al. 1976). In addition the dorso-ventral orientation adopted for the X-ray of sheep required considerable animal handling prior to diagnosis (Beach 1982, 1984). A transportable walk-through lateral-abdominal viewing system with a throughput of up to 250 ewes per hour and a diagnostic accuracy of  $\geq$ 95% at 90-110 days of pregnancy had been developed by the New Zealand DSIR by 1982 (Beach 1982). This equipment was leased to a veterinary practice and used to test about 100,000 ewes on commercial properties prior to each of the 1984 and 1985 lambings (Quinlivan 1986). Some machine failures occurred but the major difficulty proved to be hiring and training staff who could rapidly and accurately differentiate between single and multiple foetuses for prolonged operating periods (6 to 8 hours per day). Further refinements to animal handling equipment and more recent videofluoroscopy equipment would have enabled both the diagnostic rate and accuracy to be increased (Beach 1984). Fewer ewes were tested in 1986 when an accuracy of 95.8 to 98.8% was obtained, except on one property (71.2%), with throughput rates of 200-250 ewes/hour (Grace et al. 1989). Low returns on a large capital investment (\$100,000) and the rapid development of ultrasound scanning equipment has meant that X-ray technology for pregnancy diagnosis has not been adopted commercially.

#### Ultrasound Scanning

Ultrasound equipment for pregnancy diagnosis of farm animals ranges from simple pulse-echo and Doppler machines costing up to \$1,000, to considerably more expensive realtime scanning equipment (>\$10,000, Thwaites 1981). In general the low cost equipment, in the hands of a skilled operator, can provide 90-95% accuracy in determining whether the animal is pregnant at 40-60 days of pregnancy (Hulet 1969;

Stone and Fricker 1970; Hulet 1972; Lane and Lewis 1981), but reliable determination of single or multiple pregnancy status has usually been unsuccessful (Allison 1971).

During the early 1980's realtime ultrasound scanners with external probes, developed for human medicine, were evaluated for determining foetal status in sheep. A >95%accuracy in predicting foetal number at 40-60 days was achievable with skilled operators (Fowler and Wilkins 1982, 1984; Carter 1986). Recent experiments have indicated that this form of scanning provides a more accurate estimate of litter size at birth in high fecundity Merino ewes than does twice daily lambing observations (Smith et al. 1988). Wilkins (1988) has also reported a 95% accuracy in predicting the presence of single vs twin embryos from day 27 of pregnancy using a trans-rectal linear array probe attached to an ultrasound scanner. Diagnosis time and accuracy is highly dependent on operator skill, but test rates (for 0, 1 and 2 foetuses) of 80-200 ewes per hour are possible with experienced operators (Carter 1986; Duckworth 1986; Grace et al. 1989). Differentiation between two or more foetuses is less accurate and slower (Owens and Armstrong 1985). An ultrasound pregnancy diagnosis service is available to farmers in some regions of New Zealand (Duckworth 1986). With further refinements to the technology, and reductions in equipment costs, realtime ultrasound scanning by skilled operators is likely to remain the most effective method of pregnancy diagnosis on commercial properties.

## Metabolic Status of the Ewe

Studies of blood metabolite levels in ewes during the last two months of pregnancy indicate that the lamb(s) imposes considerable nutrient demands on the ewe (Russel et al. 1967). This requires the catabolism of fat stores if nutrient intake is inadequate (Mellor 1987). It is therefore possible to differentiate between ewes according to their level of nutrient stress as measured by blood metabolite levels. Feeding the ewe of the basis of her blood metabolite levels at 90-100 days of pregnancy has therefore been proposed (Russel et al. 1967; Parr et al. 1984). This approach acknowledges that management of the ewe on the basis of pregnancy status alone may not be appropriate because other factors such as her age, body size/condition and the inherent growth potential of her lamb(s) may also have an important influence on her nutritional requirements (Robinson et al. 1971; Mellor 1967).

Management systems based on blood metabolite levels in ewes grazed at pasture, as opposed to those under controlled indoor feeding conditions (Mellor 1987), have not been successful. For example, Kelly et al. (1985) failed to record differences in lamb birthweight or survival when ewes were allocated to different feeding treatments on the basis of blood glucose concentrations. Similarly, Gao et al. (1990) showed that

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measurement of blood metabolites at 110 days of pregnancy was not more effective in identifying ewes of medium fecundity requiring additional feed intake during late pregnancy than pregnancy diagnosis alone. Although a rapid blood metabolite test (e.g. for glucose) could be developed, the technique requires skilled animal handling to prevent disturbance of blood metabolite concentrations (e.g. through stress). More importantly, it would probably not provide any advantages in cost, accuracy or ewe testing rate compared to ultrasound scanning (Gao et al. 1990).

## TECHNIQUES FOR MEASURING HERBAGE INTAKE

The paucity of feed intake data for ewes at pasture can be largely attributed to the absence of an easily applied but accurate technique for measuring herbage intake. The characteristics and limitations of herbage intake measurement techniques are discussed in this section. More detailed reviews of this subject have been published by Pigden and Minson (1969), Hodgson and Rodriguez (1971), Langlands (1975, 1987), Cordova et al. (1978), Corbett (1978, 1981), Meijs (1981), Meijs et al. (1982) and Le Du and Penning (1982).

The techniques for measuring herbage intake in grazing animals can be broadly classified as being either sward- or animal-based. They are sometimes inappropriately referred to as direct and indirect methods of intake measurement. These terms are more correctly applied to animal-based estimates which are derived either directly (e.g. weight change) or indirectly (e.g. marker concentration in the faces) from measurements made on the animal.

## Sward-Based Techniques

## Pasture difference

The pasture difference technique is based on the equation:

# intake = (pre grazing herbage mass - post grazing herbage mass) number of animals

The accuracy of the technique is therefore heavily dependent on the precision with which herbage mass can be measured. It is not suitable for assessing herbage intake under continuous stocking management. The coefficient of variation of replicated pasture cuts to estimate herbage mass is 20 to 35% for most sward types (Campbell et al. 1962; Frame 1981). Accuracy can be increased by taking more sample cuts or increasing sample size, but this increases labour requirements, and may alter animal grazing behaviour, respectively. An alternative is to adopt a double-sampling method of assessing herbage mass based on a non-destructive technique (e.g. pasture height) which has a high correlation with pasture mass and which can be obtained with little additional effort (Back et al. 1969; O'Sullivan et al. 1987).

The difference method is most reliable if the grazing period is short because this minimises potential errors arising from herbage growth, senescence, decay or pest damage during the measurement interval. Most researchers agree that these factors have a minimal effect on intake estimates over a 2 to 3 day period (Meijs 1981), but this will be dependent on the type of pasture and the rates of herbage accumulation being achieved. Herbage accumulation is normally accounted for by an assessment of the growth of undisturbed pasture within an exclosure cage, and is assumed to be linear with time. This may not represent conditions on the grazed sward (Frame 1981). Within-plot variation in herbage conditions is usually ignored but may affect animal intakes and their estimation. An indication of the effect of factors such as herbage maturity, botanical composition and variation in sward spatial distribution can be obtained by recording a series of intake measurements over several days.

Estimates of intake arc more precise if a large amount of herbage is consumed per unit area (Meijs 1981). This is more readily achieved with groups rather than individual animals. Individual animal intakes are therefore usually not obtained when the difference technique is applied.

The coefficient of variation (CV) between estimates of intake obtained by 'difference' were reported by Walters and Evans (1979) to vary from 7 to 250 %. In contrast, Meijs (1981) suggested that, provided topped pre-grazed herbage could be used, average group mean intake could be estimated with a CV of about 6%. Factors affecting precision of estimates include level and variability of pasture yield (Green 1949), length and intensity of the grazing period (Linehan 1952; Penning et al. 1986), number and size of sample units (Green et al. 1952), type of machinery used for harvesting (Meijs 1981), pasture type and difficulty of recovering samples from post-grazed swards (Walters and Evans 1979). The general recommendation is that the difference technique is useful for making preliminary assessments of herbage intake but wherever possible these should be confirmed using animal-based estimates.

## Sward tissue turnover

Animal consumption under continuous grazing can conceptually be determined by changes in sward mass through time using the formula (Bircham 1981):

NP = G - S = C

where NP = net production of herbage, G = new herbage growth, S = senescence and C = animal consumption.

These parameters can be determined most easily if the sward is maintained at a fixed pasture mass. However, the required detailed and laborious measurement of sward tissue turnover make this approach impractical for most experiments.

## Direct Animal-Based Measurements

Short-term changes in animal liveweight.

Herbage intake can be estimated using short-term changes in liveweight according to the formula (Erizian 1932):

intake =  $(W_1 + L + F + U + R) - W_2$ 

where  $W_1$  and  $W_2$  are liveweight at time  $t_1$  and  $t_2$ ,

L = weight of water drunk

F = weight of faeces produced

U = weight of urine produced

R = weight loss due to respiration and transpiration (sometimes referred to as insensible weight losses (Penning and Hooper 1985)).

The technique was adopted by Allden (1962) and Allden and Whittaker (1970) to estimate the rate of intake in grazing sheep, this parameter being multiplied by grazing time to estimate average daily intake. No assessment of the accuracy of the method was made. Application of the technique requires considerable labour input and some animals must be fitted with harnesses to allow the total collection of facees and urine. There is no simple method for measuring R in grazing animals and estimating the dry matter content of herbage grazed by the sheep may be difficult. Penning and Hooper (1985) evaluated a modification of the technique in ewes using more accurate equipment for recording liveweight change (based on a 1 hour grazing period) and 24 hour grazing behaviour. They found a good agreement between daily mean intakes estimated by this approach and by the chromic oxide dilution technique, and concluded that it may be the most appropriate technique for estimating intake where pasture conditions are changing rapidly.

Electronic weighing (telemetry) allows very sensitive measurement of liveweight changes to be made. For example, Horn and Millar (1979) placed steel-encapsulated pressure transducers ('bovine boots') under the hooves of cattle to record weight changes as small as 0.5 kg/500 kg while the animals were grazing. This may improve the applicability of liveweight change for estimating intake, especially if combined with measurements of grazing behaviour.

## Grazing behaviour

Herbage intake is a function of grazing behaviour and in mechanistic terms can be defined as the product of bite size, biting rate and grazing time (Allden and Whittaker 1970). Meters to record biting rate (Stobbs and Cowper 1972) and vibro-recorders to measure grazing time (Penning 1983), that can be easily fitted to grazing animals with minimal disturbance to their normal behaviour, have made this approach to intake measurement more popular. An important advantage of the technique is that herbage intake can be related to behavioural aspects of grazing. Combining these data with information describing sward characteristics (c.g. sward height, spatial distribution and plant morphology) and animal production allows an improved definition of factors affecting herbage intake (Jamieson and Hodgson 1979; Penning and Hooper 1985). This information is important for improving the grazing management decisions of farmers (Hodgson 1984). However, Hodgson and Maxwell (1981) qualified the use of the technique as being '...a means of explaining observed effects on herbage intake rather than a means of estimating herbage intake itself'.

#### Water intake and turnover rate

Animals derive water from three sources: drinking water, water in food and water produced by metabolic processes in tissues (Bondi 1987). 'Metabolic' water comprises 5-10 % of total water intake in most domestic animals. The derivation of herbage intake by this method is based on the assumption that total water intake equals water which is drunk plus water consumed in the herbage (i.e. the metabolic fraction is assumed to be zero). Thus, if drinking water is measured or restricted, herbage intake can be derived from the water content of the herbage (Hyder et al. 1966). Indoor trials are required to define the relationship between water requirements, air temperature and intake (Benjamin et al. 1977) but these may not be representative of outdoor grazing conditions. The technique cannot be applied in some environments, for example those in which water in the feed exceeds the daily water requirements of the animals (Hyder et al. 1966).

Water turnover rate can be estimated by dosing grazing sheep with tritiated water or deuterium oxide (Benjamin et al.1977) and measuring the decline in radioactivity in the blood. If access to drinking water is restricted, herbage intake can be calculated by dividing water turnover by the water content of grazed herbage. The accumulation of isotopically labelled water from the dam has also been used to estimate milk intake of calves (Wright et al. 1974) and lambs (Dove 1988).

## Bolus size and swallowing

Stuth et al. (1981) demonstrated that the swallowing motion of goats could be easily identified from impulses provided by electrodes implanted in the oesophagus. Forwood (1985) adapted the technique to oesophageally-fistulated steers to monitor bolus swallowing events and observed a high correlation between DM intake and the number of such events (r > 0.9). Herbage intake could potentially be calculated by multiplying the number of bolus swallowing events by average bolus size.

## Feeding standards and animal productivity

Herbage intake can be estimated by dividing the energy requirements for maintenance and production by the nutritive value of the herbage available (Corbett 1978). Improved estimates of the energy requirements and better equipment for measuring production have increased the reliability of this approach, which is commonly used by farmers for feed planning (Forbes 1986).

#### Indirect Animal-Based Techniques

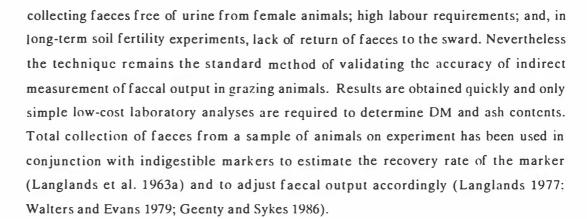
A common disadvantage of direct animal-based techniques for estimating herbage intake is that the animal's normal grazing behaviour is modified. Disturbance of grazing can often be reduced if herbage intake is estimated indirectly from the animal. Indirect animal-based measurements of herbage intake are based on the expression:

intake=faecal output/(1-digestibility) (Equation 1.1) where digestibility = (feed intake-faecal output)/feed intake

Separate estimates of faecal output and digestibility of herbage consumed are therefore required.

## Estimating faecal output

Faecal production can be measured directly by fitting animals with harnesses and collection bags. Meijs (1981) listed the disadvantages of this technique as being: reduced animal performance and modification of grazing behaviour; incomplete collection of faeces through incorrect fitting of bags or damage to bags; difficulties of



Alternatively faecal output is estimated from the concentration of an indigestible marker in the faeces using the equation;

faecal output = weight of marker ingested / concentration of marker in facces

The technique assumes that a known proportion of marker ingested is recovered in the faeces (normally 100%) and that the faecal sampling technique allows the mean marker concentration to be calculated. A substance is suitable for use as a marker if it is inert and non-toxic to the animal, is not absorbed or retained in the digestive tract, is of low bulk, mixes uniformly in the feed and digesta, is readily and quantitatively measured, and preferably is inexpensive (Kobt and Luckey 1972). Markers, or indicators, are classified as being 'external' if they are added to the diet, administered by infusion or taken orally (e.g. chromic oxide) or 'internal' if they occur naturally in the animal's diet (e.g. lignin).

When markers are used, faecal samples are collected either directly per rectum, grab sampling, or from the sward (CAB 1961; Kobt and Luckey 1972). Grab sampling allows samples to be obtained for individual animals but requires a high level of animal disturbance and is stressful to the animal. The commonly adopted twice-daily sampling routine cannot practically be maintained for periods of more than 5 days without allowing a time for recovery from potential damage to the rectum due to bruising or irritation of wall linings, particularly if immature animals are involved. In addition some animals become 'sampling shy' and defaecate immediately prior to sampling. This problem can be partially overcome by observing animals while grazing. Although this increases labour costs, animal disturbance is reduced. Sward sampling decreases animal disturbance and provides a more random procedure for obtaining samples (Raymond and Minson 1955) but individual animal records cannot be identified unless a second unique marker (e.g. polystyrene beads; Minson et al. 1960) is included in the faeces. For large grazing areas, and for animals with small pelleted faeces, less labour is required if sampling sites (rings) are marked randomly across the sward (Raymond and Minson 1955; Langlands et al. 1963b). There is limited evidence that marker concentration in sward samples can be affected in some environments by insect damage, leaching during heavy rain or sedimentation (Raymond and Minson 1955; Meijs 1981).

Chromic oxide has become widely accepted as a marker for estimating faecal output in grazing ruminants since it was first proposed by Edin (1918). More recently, increased interest has been shown in radioisotopic markers (Chamberlain and Thomas 1983), natural plant components such as N-alkanes (Mayes et al. 1986) and acid-insoluble ash (Van Keulen and Young 1977), and rare earths such as ytterbium chloride (Prigge et al. 1981; Krysl et al. 1988). This interest has been a consequence of the development of new methods for applying and assaying these markers. Most of the validation experiments for chromic oxide were completed during a 10-year period from 1950 including studies by Kane et al. (1950), Lancaster et al. (1953). Linkous et al. (1954), Raymond and Minson (1955), Kamcoka et al. (1956), Pigden and Brisson (1956), Lambourne (1957a,b), Corbett et al. (1960), Lambourne and Reardon (1963) and Langlands et al. (1963a,b). As a result of these trials, two sources of error associated with the use of chromic oxide, which apply to faccal markers in general, were identified. A short term error, commonly referred to as diurnal variation, arises from differences between marker concentration in the sample collected and a representative sample of the faeces, while a long term error (incomplete or over-recovery) occurs when the marker output differs from marker intake (Corbett et al. 1958). The long term error arises primarily from sampling procedures and the determination of faccal chromium. Le Du and Penning (1982) found the average recovery of chromium achieved in 55 experiments covering both sheep and cattle was 96.5% (SD 5.6%). A higher recovery and lower variation (100.7, SD 6.6%) of Cr<sub>2</sub>O<sub>3</sub> administered to sheep than of Cr<sub>2</sub>O<sub>3</sub> administered to dairy cows (83 to 98% depending on feed type and form of Cr2O3) was noted by Chamberlain and Thomas (1983). This could be due to differences in the distribution of feed particles in the rumen. Rumen contents of sheep are usually uniformly distributed whereas a raft formation of herbage floating on rumen liquids is found in cattle (Waghorn 1989). Meijs (1981) indicated that incomplete recovery of chromium may be explained by absorption of soluble chromium compounds, retention of the marker in the digestive tract, losses during sample preparation (particularly grinding), regurgitation of the marker, failure of the assay method or incomplete collection of the faeces (when deriving a recovery factor using total collection). In addition poor recoveries in some experiments can probably be attributed to sampling having occurred prior to steady state levels of chromium being achieved in the faeces (Pigden and Brisson 1956), to the form in which Cr<sub>2</sub>O<sub>3</sub> is administered (Chamberlain and Thomas 1983) and to an inadequate length of sampling period to account for between-day fluctuations in marker output (McRae and Armstrong 1969). Moran et al. (1987) also indicated that the magnitude of diurnal variation in feed intake (e.g. dry compared to lactating cows) and

the type of feed (which influences passage rate) affect chromium recovery. Recovery of additional chromium may occur if environmental levels of chromium are high and there is an associated high level of soil ingestion (Corbett 1981). Langlands et al. (1963a) therefore recommended that total faecal collections from an additional treatment group should be made to quantify any bias in the level of Cr2O3 recovery, but this assumes 100% reliability of the bagging technique and introduces extra animals to the experiment. Reducing the specific gravity of Cr2O3 to improve the distribution of the marker among feed particles in the rumen, by administering Cr2O3 as a component of shredded paper (Corbett et al. 1958) or coating with an inert polymer (Bruckental et al. 1989), can improve recovery rates by decreasing within-day variation in faecal marker concentration. Increasing the frequency of Cr2O3 administration has a similar effect (Pigden and Brisson 1956). Minimum sampling periods of three days, and preferably longer (Langlands et al. 1963b), are recommended because of the high between-day variation in faecal output (CV > 15%; Heaney et al. 1968; McRae and Armstrong 1969; Ulyatt 1972) even when feeding levels are controlled. Bias associated with the assay of faecal Cr<sub>2</sub>O<sub>3</sub> can be monitored by including standards with known levels of the marker.

Diurnal variation in faecal chromium concentration, which has been found in dairy and beef cattle (Smith and Reid 1955; Corbett et al. 1958; Hopper et al. 1978; Moran et al. 1987), goats (Kameoka et al. 1956), pigs (Clawson et al. 1955) and sheep (Raymond and Minson 1955; Brisson et al. 1957; Lambourne 1957b; Langlands et al. 1963a), has been shown to be primarily due to the method of chromium administration and the faecal sampling routine adopted. The level and pattern of feed intake, feed type and quality, physiological status of the animal and whether the animal is housed or free-grazing, also affect within-day variation in marker concentration. Efforts to reduce diurnal variation have been based mainly on one or more of the following: increasing the frequency of Cr<sub>2</sub>O<sub>3</sub> dosing (Pigden and Brisson 1956; Lambourne 1957b); changing the form in which Cr<sub>2</sub>O<sub>3</sub> is administered to slow its rate of passage through the digestive tract (Corbett et al. 1958; Pigden et al. 1964; Troelson 1966); increasing the frequency and changing the timing of faecal sampling (Raymond and Minson 1955; Lambourne 1957a,b; Corbett et al. 1958); or developing predictive equations which describe the natural circadian rhythm and the appearance of markers in the facces (Hopper et al. 1978; Nicoll and Sherington 1984). For example, Brisson et al. (1957) demonstrated that diurnal variation could be largely eliminated if gelatin capsules were administered six times rather than once daily, and Corbett et al. (1958, 1960) showed that twice daily drenching with Cr2O3-impregnated paper (which slowed the rumen passage rate and increased the opportunity for mixing with the digesta) rather than with gelatin capsules reduced errors by a third. These and other similar results have led to an accepted routine where Cr<sub>2</sub>O<sub>3</sub>-impregnated paper pellets are drenched twice daily and rectal grab samples are obtained concurrently (Hodgson and Rodriguez 1971; Le Du and

Penning 1982). This routine involves considerable disturbance of animal grazing patterns because, in addition to a 5 to 7 day pre-sampling period, drenching needs to be continued even on days when faecal samples are not required. Diurnal variation can be reduced by the continuous infusion of the marker from a pump mounted on the animal's back (Siebert et al. 1978) but this is costly, affects grazing behaviour, and limits both the number of animals and the grazing area which can be used. In trials where animals are receiving supplements,  $Cr_2O_3$ -mordanted feed can be used (Hopper et al. 1978; Lobato et al. 1980). Diurnal variation (10-15%) still occurs, however, because the feed is not usually consumed uniformly across the day or in proportion to the main feed source (Kamcoka et al. 1956; Hopper et al. 1978).

The above discussion indicates that a method to continuously release a uniform amount of  $Cr_2O_3$  (or other marker) into the rumen without daily drenching or back-mounted pumps, would significantly decrease diurnal variation (Lambourne 1957b), lessen the frequency of faecal sampling and reduce animal handling. The Laby intraruminal device (Laby 1980) has the potential to fulfill these requirements.

## Development of intraruminal chromium controlled release capsules: 1969-1985

The development of intraruminal controlled release capsules (CRC) commenced in 1969 when Dr R. Laby of the Biotechnology Division of CSIRO (Australia) patented a variable-geometry slow release device for the control of bloat in cattle (Ellis 1980; Laby 1980). During the 1970's, the devices were evaluated as a means of delivering: magnesium for the control of grass tetany in cattle (Laby 1980); iodine for prevention of goitre in sheep (Mason and Laby 1978); monensin for growth stimulation in cattle (Watson and Laby 1978); and anthelmintics for treatment of internal parasites in sheep (Anderson and Laby 1979; Anderson et al. 1980). The release period of active ingredients varied from 30 days in the cattle bloat capsule to a maximum of three to six years for the iodine capsule (Laby 1980; Lehane 1982; Ellis and Coverdale 1982).

The prototype CRC, also referred to as a controlled release device (CRD), for delivering  $Cr_2O_3$  into the rumen of sheep was described by Harrison et al. (1981). The test capsules were custom-made from a plastic syringe barrel into which a cast core matrix of  $Cr_2O_3$  sucrose mono-stearate (50:50 w/w) was inserted (Figure 1.1). This was followed by a plastic plunger and a compressed steel spring. The contents of the barrel were heat sealed into place with a rubber bung. A 9.53 mm orifice exposed the matrix to the rumen contents, forming a gel which was extruded through the force of the spring. In rumen-fistulated animals the capsules were suspended in the rumen by a nylon-filament attached to the cannula, but for intact animals this was substituted by a plastic strip which opened into a T-configuration to retain the CRC in the **rumen** 

(Figure 1.1). The matrix was released from the prototype capsules in an almost linear fashion over a period of 10 to 20 days (Harrison et al. 1981).

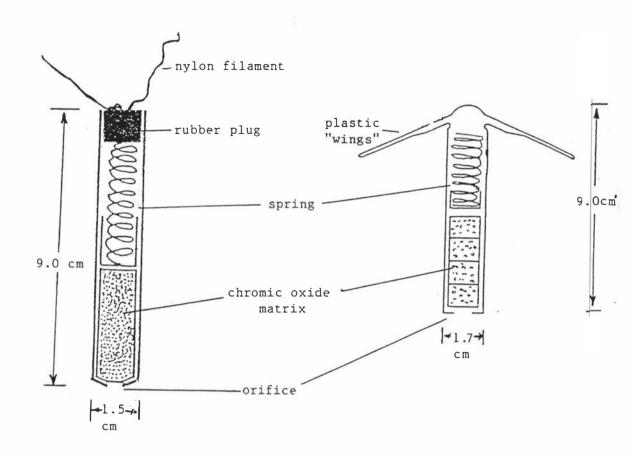


Figure 1.1 Prototype and commercial intraruminal chromium controlled release capsules.

Ellis et al. (1981) reported three experiments in which CRC were evaluated in sheep. In experiment one, an average release of 0.36 g  $Cr_2O_3/d$  over a period of 13.4 d to 15.1 d was achieved in rumen-fistulated sheep. In experiment two, the coefficient of variation (CV) of CRC chromium in faeces samples taken at four consecutive two-hourly intervals, from sheepfed lucerne pellets hourly, was 6.2%. This compared with a 20.1% CV where  $Cr_2O_3$  had been administered by twice-daily dosing with gelatin capsules. Between-day variation in faecal chromium concentration for the methods was similar (CV = 16 vs 18%). In the third experiment CRC were compared with twice-daily drenching of gelatin capsules in grazing sheep (n = 3 per group). Between-day variation in chromium concentration in the faeces collected per rectum twice (daily from d 7 to d 12 after administration of  $Cr_2O_3$  commenced) was substantially lower in the sheep with CRC (CV = 26 vs 50%). Ellis et al. (1981) concluded from these experiments that CRC with reliable release characteristics could be made for sheep and that the more uniform delivery of  $Cr_2O_3$  by CRC reduced the number of faecal samples which needed to be collected. In the following year Harrison et al. (1982) reported that a linear release of  $Cr_2O_3$  for up to 20 d (111 mg Cr/d) was achieved with CRC in rumen-fistulated sheep. Chromic oxide for these capsules had been finely ground (94% of the particles were <10 $\mu$ m) prior to forming the matrix. Steady state levels of  $Cr_2O_3$  in the faeces were reached 5-6 days after insertion of the capsule in the rumen.

The first details of  $Cr_2O_3$  CRC being used in grazing steers and rumen-fistulated cows were published by Ellis et al. (1982), although the device was already being developed commercially to deliver monensin in cattle (Schlink and Ellis 1982). Chromic oxide was incorporated in a solid core matrix. Plunger travel from d 2 to d 17 in CRC in rumenfistulated cows was linear ( $r^2 > 0.99$ ) and on average 13.8% faster than in intact steers slaughtered on d 15. Variation in plunger travel was the same in both groups (CV =8.4%), but the data from the rumen-fistulated cows indicated that 2-3% of this variation occurred during initiation of matrix extrusion from d 1 to d 3. Weston et al. (1983) used similar cattle  $Cr_2O_3$  CRC to estimate faecal output of grazing animals. Capsules containing Cr EDTA and ytterbium nitrate as markers for digesta flow studies were evaluated by Hynd and Ellis (1984). The CV of marker release from these capsules was 14% (including analysis and measurement error).

Cattle chromium CRC were used by Bird et al. (1984b), with only moderate success, to estimate herbage intakes of steers grazed at two stocking rates. Overall 33 of the 40 capsules provided useful data, but an irregular pattern of faecal marker concentration suggested that plunger travel may have been temporarily interrupted in some of the remaining CRC. The life of the CRC, as estimated by the time of marker disappearance from the faeces, ranged from 14 to 22 days (mean 16.3 days). Herbage intakes based on sward faecal sampling were 5 to 28% lower than those derived from once-daily sampling of the faeces per rectum. This was attributed to diurnal variation because chromium levels were significantly different in sward samples collected at 0900, 1400, 1700 and 2100 hours.

Laby et al. (1984) demonstrated by two experiments that  $Cr_2O_3$  release rate could be controlled by orifice diameter and matrix composition, but no data supporting the claimed third controlling factor (spring strength) were presented<sup>1</sup>. A linear (r>0.997) plunger travel rate of 0.787 to 0.797 mm/d was recorded in CRC recovered by slaughtering wether lambs grazing pasture. In an indoor trial, faecal output estimated from faecal chromium concentration equalled actual faecal output measured by total collection from sheep offered one of four different supplements. Sampling for faeces

<sup>1</sup>Laby (1986) subsequently argued that the passage of gas by osmosis into the barrel to relieve the vacuum formed as the matrix diminished was an important factor in maintaining a uniform release rate as the spring tension reduced.

commenced six days after CRC administration. The authors concluded "Release rate was found to be independent of the diets used, the age of animals, whether they were penned or free-grazing, fistulated or intact, dosed with one or three CRD's or whether the CRD's were ... [in sheep]... tethered or untethered."

The use of sheep chromium CRC for measuring herbage intake in fallow deer (<u>Dama</u> <u>dama</u>), which are comparable in liveweight to sheep (30-60 kg), was reported by Kelly et al. (1985). Chromium release rate was derived from plunger displacement of CRC fitted to rumen-fistulated sheep grazed with the deer, but because of "technical reasons associated with ....prototype continuous release devices" it was necessary to derive separate chromium payouts for days 0-18 (102 mg Cr/d) and days 18-35 (70.5 mg Cr/d). The CV between capsules in release rate was c.14%.

No further information had been published describing the application of chromium CRC when the current research project commenced in November 1985. At this time there were still varying views concerning optimum matrix composition (50 vs 65% Cr<sub>2</sub>O<sub>3</sub>), method of matrix formation (cast core vs compressed tablet), amount of matrix (3.0 cm vs 6.0 cm) and orifice diameter (7.00 mm vs 9.00 mm) for sheep capsules. Except for the indoor trial reported by Laby et al. (1984), no validation of the accuracy with which CRC could be used to derive faecal output in grazing animals had been published. Similarly, data describing the effects of feeding level, of changes in the level of feed, and of different types of pasture, on CRC release rate characteristics or pattern of chromium in the faeces were incomplete.

Despite these gaps the CRC appeared to offer major advantages compared to existing animal-based techniques for measuring herbage intake of grazing animals. These included reduced labour, less animal handling, and the possibility of more flexible and less frequent faceal sampling, all factors which could reduce costs or increase the number of animals on experiment.

## Commercial development of intraruminal CRC

The licencing rights to commercially develop and sell world-wide CSIRO intraruminal CRC technology for the release of a variety of substances were purchased by Captec Pty Ltd. of Melbourne in 1984. Previously, similar technology for monensin delivery had been sold to Elanco, who developed the rumensin anti-bloat capsule for cattle (Elanco Products Co., Australia). A Captec chromium CRC for sheep with a 30 day delivery period became available commercially in 1987 (Ellis and Rodden 1987). The specifications of the capsule included a 65% Cr<sub>2</sub>O<sub>3</sub> matrix, 9.00 mm orifice and daily delivery of 123-150 mg Cr. A cattle chromium capsule with a multi-orifice endplate, 65% Cr<sub>2</sub>O<sub>3</sub> matrix, and a 20-22 day delivery of about 1150 mg Cr/d was marketed by

Captec in late 1988.

## Estimating Herbage Digestibility

Accurate determination of the digestibility of the herbage consumed by the animal is important for deriving reliable estimates of herbage intake because the divisor of faecal output is (100-digestibility; see Equation 1.1). For example a standard error of 1.5 units of digestibility is only 2% of a mean digestibility of 75%, but is 6% of the mean indigestibility of 25% (Hodgson and Rodriguez 1971). Feed digestibility can be estimated directly (<u>in vivo</u>) by measuring feed intake and faecal output in indoor feeding trials using herbage from the outdoor experimental area. A measurement period of 10 days following a preliminary adaption period of similar length is usually adopted (Dulphy and Demarquilly 1983). However, these estimates may not be applicable to field grazing because feed is mechanically harvested, opportunity for animals to select herbage is modified, and the pattern and level of herbage consumption is changed (Meijs 1981). High costs for labour and equipment are also disadvantages of this method. Indirect methods of estimating digestibility have therefore been developed.

Marker-ratio techniques are based on the concentration of a completely indigestible indicator in both feed and faeces (Barnicoat 1945; Kobt and Luckey 1972). The apparent digestiblity of the feed (Da) is equal to;

Da = 1-(marker in feed/marker in faeces)

Indicators which occur naturally in the diet include lignin, chromogens and acid insoluble ash (Meijs 1981; Fahey and Jung 1983). External markers are the same as those which are used for estimating faecal output. The ratio technique has not been widely adopted, mainly because of problems associated with the collection of a representative sample from the grazed sward, and incomplete recovery of the indicator (Langlands 1975).

Faecal-index techniques involve the prediction of digestibility from the composition of the faeces. A regression equation describing the relationship between digestibility and the faecal indicator, developed from a series of indoor trials, is used to predict the digestibility of herbage consumed at grazing (Langlands 1975). Accuracy can be improved by including more than one indicator in the regression (Bird et al. 1984b). Substances used as faecal indices include chromogens, methoxyl, macerate crude fibre and normal acid fibre but the most widely used has been nitrogen (Langlands 1975; Meijs 1981). More recent interest has centred on the use of rare earths (Krysl et al. 1988). The faecal-index technique is subject to error because the regression relationships may not be directly applicable to outdoor grazing, are dependent on representative sampling of the faeces, and may change between seasons, level of feed intake, herbage allowance and animal species (Coop and Hill 1962; Langlands 1975; Meijs 1981). Labour requirements and costs to complete the <u>in vivo</u> trials are high and there may be difficulties in harvesting herbage similar to that selected by the animal if herbage availability on the grazed area is low.

Sward characteristics, such as the availability of pasture and the proportion of green material, were used by Bird et al. (1984b) to predict digestibility. This information can be readily collected in grazing trials but appropriate relationships between sward components must first be defined by <u>in vitro</u> or <u>in vivo</u> analysis of sward samples.

Animals fistulated in the oesophagus or rumen have become widely used for collecting samples (extrusa) from grazed swards which are then tested in an in vitro digestibility procedure or with the ratio technique (Holcheck et al. 1982). Surgical preparation of animals is relatively straight-forward and fatalities are uncommon (Torrell 1954; McManus et al. 1962; Godwin and Chaffey 1985). The use of rumen-fistulated sheep is less popular because rumen evacuation is labour intensive and unpleasant, there is considerable potential for sample contamination and large animals are required (Van Dyne and Torrell 1964). On the other hand, samples from trained oesophagealfistulated sheep can be readily collected in the field over a period of 5 to 30 minutes and from a wide range of sward conditions (Hodgson and Rodriguez 1971). Contamination of oesophageal extrusa with rumen contents can be avoided by plugging the oesophagus, avoiding collections at times when animals are usually ruminating, or minimising the duration of the collection period (Holcheck et al. 1982). Australian evidence that grazing behaviour and therefore extrusa composition was dependent on prior experience on a sward (Langlands 1967) was not confirmed in Britain (Hodgson and Rodriguez 1971), but this may be dependent on the level of sward variability. Coates et al. (1987) suggested that extrusa collected per os from cattle was not representative of the herbage grazed by resident animals as assessed by the faecal carbon isotope ratio technique. However, collections were made after an overnight fast and sward samples for resident animal intake were based on hand-plucked samples. Age, breed or sex differences within a species are not an important source of variation in extrusa composition (Langlands 1967; Le Du and Baker 1981). Langlands (1975) recommended that saliva contamination of extrusa, which is related to the DM content of the herbage consumed and the rate of herbage intake, be corrected for by separating the solid and liquid fractions. However, Armstrong et al. (1989) found insufficient evidence to advocate correction for saliva, although the presence of saliva did increase digestibility values slightly in a non-linear manner. It is therefore prudent to sample at times during

the day when saliva production is low (Armstrong et al. 1989). Sampling times should be adjusted to ensure that changes in sward conditions through time, which are greater under rotational grazing, are accounted for (Hodgson and Rodriguez 1971; Hamilton et al. 1973). This is also important under conditions of continuous grazing if the plant communities grazed during the day change (e.g. grazing up a slope on hill pastures). Between-animal variation, potentially the largest source of error in collecting representative extrusa samples, can be reduced by increasing the number of fistulated animals or collecting samples from the same fistulated animals over a series of treatments (Hodgson and Rodriguez 1971). An added advantage of oesophagealfistulated animals is that extrusa can be subjected to botanical composition (Bircham 1981) and further chemical analysis.

Hand-plucking herbage to obtain samples comparable to that observed to be consumed by the trial animals is a simple method of obtaining herbage for in vitro digestibility analysis. If rotational grazing is practiced herbage can be plucked from an exclosure cage, fitted prior to grazing, to represent post-grazing sward conditions. An appreciation of the composition of the herbage consumed can be obtained by this approach (Walters and Evans 1979). Manual collection of samples, which can provide similar estimates of herbage digestibility to those obtained from fistulated animals (Le Du and Baker 1981), is most reliable when there is a high proportion of green material in the sward and moderate to high grazing pressure is being exerted. The technique may be more accurate with cattle because they are less selective grazers than sheep (Bird et al. 1984b).

In vitro digestibility laboratory analyses are based mainly on rumen-fluid (Tilley and Terry 1963), pepsin-cellulase (Jones and Hayward 1973) or NDF (neutral detergent fibre)-cellulase assays (Roughan and Holland 1977). However, because unknown samples are calibrated against standards of 'known' <u>in vivo</u> digestibility, precision is dependent on how accurately the standards have been established (McLeod and Minson 1976; Minson and Butler 1983). An <u>in vitro</u> digestibility assay is acceptable if the residual standard deviation of the estimate of <u>in vivo</u> digestibility is no greater than  $\pm 2.5$  units (Roughan and Holland 1977). Comparisons of the techniques indicate that each method yields similar results (e.g. Omed et al. 1989), although Givens et al. (1989) suggested that <u>in vivo</u> digestibility could be predicted more accurately by using a combination of <u>in vitro</u> methods. Other methods of predicting digestibility are ruminal incubation in nylon bags (i.e. <u>in situ;</u> Playne et al. 1978) and infrared spectroscopy (Holcheck et al. 1982).

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Langlands (1967) claimed that the precision of the 'fistula technique' for estimating herbage digestibility was low, with standard deviations of  $\pm 1.6$  digestibility units between sheep,  $\pm 1.3$  units between days and  $\pm 2.7$  units for the combined sheep x day interaction and error term. Subsequent research suggested most of this error could be attributed to processing of the samples and the <u>in vitro</u> assay (Langlands 1975). However, Hodgson and Rodriguez (1971) demonstrated that the <u>in vitro</u> digestibility of extrusa was almost identical to the <u>in vivo</u> digestibility of the diet determined on the same animals. The <u>in vitro</u> assay was again identified as the largest potential source of error.

## Summary:Accuracy of Measurement Techniques for Estimating Faecal Output and Herbage Intake

The suitability of a technique for estimating herbage intake can be evaluated in terms of four criteria: accuracy, risk of bias, convenience of application and the range of conditions to which the technique can be applied (Hodgson 1986). The sward difference approach is simple to apply under a wide range of sward conditions but is subject to considerable errors associated with estimating pre- and post-grazing herbage mass. Geenty and Sykes (1986) found that group intakes estimated by the disappearance of DM from the sward over grazing intervals of 3 to 4 days were 28-40% lower than the values obtained by chromic oxide dilution. Similarly, Ulyatt et al. (1974) found that intakes were 40% lower with the difference technique than estimates of intake made from the total collection of faeces and herbage in vitro digestibility. Sward tissueturnover studies are expensive and only provide group mean estimates of doubtful accuracy under a limited range of conditions. Water-turnover estimates of herbage intake are very difficult to obtain under outdoor grazing conditions because respiratory losses of water are highly dependent on air temperature and humidity, which can fluctuate widely over a 24 hour period. Feed intakes based on liveweight changes and grazing behaviour are more accurate and easier to obtain with modern sensitive electronic monitors (Penning et al. 1984; Penning and Hooper 1985), but are prone to error because animal grazing behaviour is highly variable between days and between animals. Nevertheless, the quantitative data describing grazing behaviour and production responses are important for understanding the pasture-animal interface and for designing improved grazing management systems. Total collection of faeces is impractical for many experiments and, because of the effects of the collection equipment on animal behaviour, yields results of questionable accuracy, particularly if the animals have received inadequate prior training with the collection harnesses and bags (Hutchinson 1959). At present the most readily applied, adaptable and accurate technique for measuring herbage intakes is the indirect estimation of faecal output using an indigestible marker and an associated in vitro assessment of feed digestibility.

Chromic oxide is the most extensively validated and widely used faecal marker. Procedures to minimise potential sources of bias associated with its use and factors contributing to reduced accuracy are well documented, as discussed previously.

Hodgson and Rodriguez (1971) recorded coefficients of variation of estimates of faecal output and feed intake in grazing animals that ranged from 5 to 13% using oesophageal-fistulated animals and twice-daily drenching with  $Cr_2O_3$ . Comparable values for feed intake ranged from 2.5 to 81% (mean 16.4%) for a large number of indoor feeding trials reviewed by Heaney et al. (1968). Values for indoor trials at the Hurley Grassland Research Institute were 3-16% for faecal output and 9-22% for feed intake (Hodgson and Rodriguez 1971). Random errors associated with twice-daily drenching of chromic oxide for predicting faecal output can be reduced to a CV of about 7% if sward ring sampling is adopted (Meijs 1981), but are 3-5 percentage units higher with grab sampling (Coop and Hill 1962; Meijs 1981). Corbett et al. (1978) and Bird et al.(1984b) estimated that feed intake in grazing animals could be estimated with a CV of 15-20% by indirect measurement using  $Cr_2O_3$ .

In the indoor feeding trial of Laby et al. (1984) the faecal output of sheep fitted with CRC was estimated with a CV of 4%, although sampling errors were probably minimised by sub-sampling from pooled total collections of faeces for individual animals. Errors associated with determining herbage indigestibility (CV >5%) are independent if oesophageal-fistulated animals are used. The potential minimum CV of estimating feed intake using CRC is therefore c. 7%. Under these conditions the minimum number of animals per group required to detect a 10% differences in feed intake at P < 0.1 is 5 (Cruickshank et al. 1987).

In conclusion, the most appropriate technique for determining herbage intakes in pregnant and lactating ewes under continuous grazing is estimation of faecal output from the concentration of faecal chromic oxide delivered by intraruminal CRC and in <u>vitro</u> estimates of the digestibility of extrusa collected from oesophageal-fistulated animals grazed with the ewes. However, while the potential of CRC is apparent from preliminary trials in Australia, validation studies are incomplete and no evaluations have been conducted under New Zealand pastoral conditions. A series of validation experiments are therefore necessary before applied field studies can commence.

## PURPOSE AND SCOPE OF THE INVESTIGATION

The initial objectives of this study were threefold:

- 1. To validate the use of CRC for measuring feed intakes of sheep under New Zealand pastoral conditions.
- 2. To use CRC to examine the relationships between pasture availability, herbage intake and productivity in ewes of different pregnancy status and rearing rank.
- To design, using models incorporating the relationships defined by studies under (2) above, improved grazing management systems for the ewe during late pregnancy and lactation.

At the time the project commenced it appeared from the published literature and nonpublished reports of further CRC validation studies funded by Captec (Australia) Ltd that the intraruminal capsule technology was near to commercial release. It was therefore anticipated that objective (1) could be achieved relatively quickly. However, this proved not to be the case and considerably more effort was devoted to developing appropriate methods for CRC use in sheep. Consequently, fewer applied studies were conducted and the third objective received less attention.

An early aspect of the research programme was the validation of an assay to provide for the consistent and preferably complete recovery of chromium from the faeces. This is an essential prerequisite to the indirect estimation of faecal output when Cr<sub>2</sub>O<sub>3</sub> is used. The experiments conducted to validate the assay are described in Chapter Two. At the same time the initial indoor feeding trials investigating the effect of feed type and level of feeding on CRC containing a 6.0 cm of 50%  $Cr_2O_3$  pressed tablet matrix commenced. These experiments are reported in Chapter Three. Although the specifications of the CRC used in the first two experiments were later to be substantially changed, the results provided important information for the application of capsules in the first grazing experiments with CRC in sheep (Chapter Four). Ram lambs were fitted with faecal collection bags and, with the exception of one group, each sheep was dosed with a single 6.0 cm 65% Cr<sub>2</sub>O<sub>3</sub> pressed tablet matrix CRC. The rams were exposed to three contrasting grazing regimens which represented the range of conditions under which CRC were likely to be eventually used in New Zealand. The grazing treatments were compared in terms of their effect on CRC plunger travel rate, chromium recovery and diurnal variation of chromium in the faeces. One finding of this study was that the intraruminal capsules appeared to depress voluntary herbage intake. Two further experiments, one conducted indoors using a relatively homogeneous feed (lucerne chaff), and the other outdoors on spring pastures, were undertaken to further assess the effect of CRC with different wing configurations on voluntary feed intake. These

experiments, reported in Chapter Five, demonstrated that the stiffer wing configuration of the capsules used in the first three experiments was probably responsible for depressing voluntary herbage intakes, as this response was not measured in sheep fitted with CRC having a more flexible and less pointed wing structure.

A second finding from the initial grazing trial was that recovery of chromium from the faeces was influenced by the level of feed intake. The effects of large changes in feed intake of two contrasting feeds on the recovery of faecal chromium and the rate of chromium release from CRC was therefore investigated using rumen-fistulated wethers housed indoors (Chapter Sever.). The capsules used in this experiment, with the exception of their wing structure, were identical to those later released commercially (i.e. a 3.0 cm pressed tablet matrix of 65% Cr<sub>2</sub>O<sub>3</sub> extruded through a 9.00 mm orifice).

The accurate determination of the rate at which  $Cr_2O_3$  is released from CRC is critical to their successful application in field trials. A series of three serial slaughter experiments, using ewes fitted with different types of CRC (i.e. with 3.0 or 6.0 cm of 65%  $Cr_2O_3$  matrix and 9.00 mm orifices), were therefore conducted to ascertain whether the rate of plunger travel in intact sheep was the same as that which was more readily recorded in rumen-fistulated animals. These experiments, reported in Chapters Six and Eight, indicated that the rate of matrix release was 8-12% slower in the rumenfistulated animals. An alternative means of estimating  $Cr_2O_3$  release rate, by determining the endpoint of chromium release from the pattern of chromium disappearance from the faeces, was therefore used in later experiments to supplement data from rumen-fistulated sheep. Obviously this was not necessary for trials where plunger travel could be measured directly from CRC recovered by slaughter. The slaughter experiments also enabled the effect of changes in feeding level during pastoral grazing on CRC chromium release rates to be quantified.

Ultimately, the adoption of CRC for ruminant nutrition studies is dependent on the accuracy with which feed intake can be predicted. The validation experiments described above provided some indication of this aspect of CRC usage but, because capsule technology was being updated during this period and some of the experimental designs were subsequently found to compromise the pattern of chromium appearance in the faeces, a separate field study was conducted to compare faecal outputs predicted from faecal chromium concentration with those measured by total collection. This experiment included an evaluation of the accuracy of faecal sampling methods, either directly from animals per rectum or indirectly from marked sites on the sward, for predicting the average faecal output of groups of animals. The details of this experiment are presented in Chapter Nine.

On the basis of the almost linear release of  $Cr_2O_3$  from CRC in the first three validation experiments and results obtained by Australian researchers, a pilot study of the application of CRC in ewes from mid-pregnancy to weaning was conducted in the first year of the project. This study, reported in Chapter Ten, provided measurements of the relative herbage intakes of single- and twin-bearing/rearing ewes when grazed together during pregnancy and lactation. Although the subsequent validation experiments indicated that a more efficient faecal and pasture sampling regimen could have been implemented, the results later provided guidelines for a detailed nine-week study of the effect of continuous grazing at different but fixed sward heights on herbage intake and productive performance of single- and twin-bearing ewes during lactation. The results of this experiment, and their practical application to commercial sheep farms, are discussed in Chapter Eleven.

The experiments conducted during this research programme provided information on how CRC can be managed to ensure reliable estimates of feed intakes of sheep at pasture, and identified some of the limitations of controlled release technology for chromic oxide. These aspects, and areas which require further research, are outlined in the concluding chapter.

## **CHAPTER TWO**

# VALIDATION OF AN ASSAY FOR THE ANALYSIS OF FAECAL CHROMIUM CONCENTRATION

### INTRODUCTION

Indirect estimation of faecal output, using chromium as the indigestible marker, is dependent on consistent accuracy in the measurement of chromium concentration in samples of faeces (Kobt and Luckey 1972). The use of chromium controlled release capsules (CRC) in ruminants poses two new problems with respect to the assay of chromium. First, the concentration of chromium in the faeces is lower than for other methods of  $Cr_2O_3$  administration. Second, reduced animal-handling and labour requirements allow the number of experimental animals and the number of samples to be increased. An assay which provides for consistently high recoveries (100±2%) of chromium at relatively low concentrations (0.1-0.5 mg Cr/g DM) in large numbers of faecal samples is therefore required.

Insolubility of  $Cr_2O_3$ , which makes it an effective marker in feed intake and digestibility studies, also makes its recovery from facees difficult. Faceal samples generally need to be subjected to a digestion process to convert insoluble  $Cr_2O_3$  (Cr III) to a soluble form (Cr VI). In animal nutrition studies the digestion reagents used generally fall into three categories: perchloric acid (e.g. Fenton and Fenton 1979); phosphoric acid with potassium bromate (e.g. Christian and Coup 1954; Williams et al. 1962); and fusion of the sample with sodium hydroxide and sulphuric acid (e.g. Fisher et al. 1972). Nitric acid has also been used as a digestion reagent (Robinson et al. 1986), but recoveries of chromium by this method can be low (Mir et al. 1989). The alternative to chemical digestion methods is X-ray fluorescence spectrometry (XRFS) determination of chromium, which gives chromium concentration directly from raw faecal material (Lazar and Manson 1965; Evans et al. 1977).

Digestion with perchloric acid is not favoured when preparing large numbers of samples because of the danger of explosion (Fisher et al. 1972). In addition, Williams et al. (1962) found that potassium perchlorate solutions did not spray satisfactorily for atomic absorption analysis. The sodium hydroxide-sulphuric acid fusion method described by Fisher et al. (1972) is slow, relatively expensive and may result in interfering ions from the crucible being brought into solution (Stevenson and de Langen 1960; Lee et al. 1986). For these reasons modified processes of digestion, originally described by Christian and Coup (1954), using a phosphoric-manganese acid mix with potassium bromate have generally been favoured when atomic absorption spectrophotometry is used for chromium determination.

Some authors indicate that the phosphoric-manganese-potassium bromate oxidation of chromic

oxide is sensitive to temperature. At low hot plate temperatures (<170°C) oxidation is incomplete and at high temperatures (>270°C) the oxidising power of the solution is diminished and the reaction reverses (Christian and Coup 1954; Aggett and O'Brien 1981; Costigan and Ellis 1987). However, Williams et al. (1962) specified no temperatures in the description of their method, and Le Du and Penning (1982) recommended hot plate temperatures of 280-290°C. Similarly, heating times during digestion range from 5-7 minutes for the entire digestion (Williams et al. 1962) to several hours (Costigan and Ellis 1987).

Methods of determination of chromium in solution include titration (Christian and Coup 1954; Fisher et al. 1972), colourimetry (Stevenson and Clare 1963; Mathieson 1970; Fenton and Fenton 1979), atomic absorption spectrophotometry (AA) (Williams et al. 1961; Costigan and Ellis 1987) and inductively coupled plasma emission spectrometry (ICPES) (Lee et al. 1986). Titration and colourimetry are less suited to the low faecal chromium concentrations (4.0-20.00  $\mu$ g/ml) associated with the use of chromium capsules (Feldman et al. 1967). These are 10-25% of the concentrations achieved by administering chromium in the form of Cr<sub>2</sub>O<sub>3</sub>-impregnated paper (Raymond and Minson 1955), gelatin capsules (Pigden and Brisson 1956) or mordanted feed (Lobato et al. 1980). AA and ICPES both offer high sensitivity to low chromium concentration (<1.0  $\mu$ g/ml) and ease of sample preparation (Williams et al. 1962; Fisher and Lee 1982).

The main disadvantage of AA is inter-element interference. These effects may be enhancing (Cu, Al, Mg and Ca), depressive (Na, K, Zn and Sr) or selective (Fe) (Yanagisawa et al. 1970; Thompson 1978; Aggett and O'Brien 1981; Lee et al. 1986). They are significantly greater in air-acetylene than in nitrous oxide-acetylene flames, primarily because of the higher temperature of the latter. Thompson (1978) and Lee et al. (1986) demonstrated that changes in chromium-oxidation state during emission into the flame could lead to incorrect determinations because the relationship between absorbance and chromium concentration was non-linear. This was less noticeable when a nitrous-oxide flame was used. The effects of interference, particularly with air-acetylene flames, can be suppressed by adding buffering solutions to the digest (Williams et al. 1962; Thompson 1978), and by using lean flame conditions, although this reduces sensitivity (Aggett and O'Brien 1981a,b). Alternatively, the standards can be made up by using blank faecal material obtained from the same source as the analyte (Stevenson and de Langen 1960; Costigan and Ellis 1987)

AA chromium determination is sensitive to instrument settings, particularly burner height. For nitrous oxide a red feather of 10 mm in a non-luminous flame, with the light path centred between 3.5 and 8.0 mm above the burner slot (depending on the instrument type) is recommended (Yanagisawa et al. 1970; Thompson 1978; Lee et al. 1986). The difficulty of reproducing flame conditions between batches is therefore a potential source of error (Lee et al. 1986). This may be minimised by preparing common between-batch samples for analysis, and by not altering instrument settings between runs.

ICPES, which does not require oxidation of Cr (III) to Cr (VI), offers relative freedom from chemical interferences, high sensitivity, minimum sample preparation, high between-batch repcatability, and the opportunity to take multi-element measurements (Lec et al. 1986). However it is considerably more expensive than other techniques and therefore not well suited to the analysis of very large numbers of samples. The same comments apply to XRFS chromium determination. An additional factor is that AA equipment is readily available in most nutrition laboratories whereas ICPES and XRFS, because of their greater capital cost, are usually less accessible.

Lee et al. (1986), after comparing four methods of chromium determination, concluded that obtaining good precision in  $Cr_2O_3$  recovery in biological samples was difficult because of the potential sources of error discussed above. For the purposes of this study it was considered that a phosphoric-manganese mix with potassium bromate as a digest and AA determination provided the best means of measuring faceal chromium, particularly given the large number of samples to be analysed. This chapter describes the establishment and validation of such an assay.

### DESCRIPTION OF THE ASSAY METHOD

The procedures described by Williams et al. (1962), Le Du and Penning (1982) and Costigan and Ellis (1987) for measuring faecal chromium were used as a basis to develop a chromium assay suitable for the equipment and facilities available at Massey University. After carrying out the validation experiments described in this Chapter a modified assay, described in detail below, was developed.

- Approximately 1.0 g of previously oven-dried faeces was weighed into 30 ml pyrex low form beakers and oven dried at 100°C until a constant weight was achieved (3-4 hours). The faeces were then reweighed before being placed in a furnace (Eurotherm, McGregor Ltd, Auckland) for overnight (12 hours) ashing at 550°C. If the ash percentage of the sample was required, the ash weight was recorded after cooling in a desiccating chamber.
- 2. An aluminium block (600 mm long x 300 mm wide x 140 mm deep), drilled to a depth of 12.5 mm for individual placement of 60 beakers, was preheated to a temperature of 145-150°C at the centre. Temperatures were maintained at ± 2% of the set reading using an Omron control unit (Tateisi Electronics Co, Osaka). The temperature gradient from the centre of the block to the outside beakers was 7°C. Six (6) ml of the phosphoric acid-manganese mixture (30 ml of 10% (w/v) MnSO<sub>4</sub>.4H<sub>2</sub>0 solution in 1 l of 85% phosphoric acid) was added from a dispenser (Witeg, West Germany) to the faecal ash, gently

swirled and the beakers placed in the aluminium block with a heat proof glass cover unti the digest temperature reached 135-145°C (c. 20 minutes). This ensured good dispersion of the ash material, which was usually still in pellet form after ashing (see below). The beakers were then removed and the digest cooled to less than  $100^{\circ}$ C before adding 3.5 m of potassium bromate (4.5% w/v) and reheating under the glass cover until the diges temperature was 195-210°C. It was subsequently found that the ratio of digestion agent: recommended by Williams et al. (1962) - 4ml of acid mix and 6 ml of potassium bromate gave higher recoveries and better agreement between duplicates (Kassano 1989). The digest was then cooled and 10-15 ml of hot (60-70°C) distilled water was added to prevent gel formation (Stevenson and de Langen 1961). The beaker contents were quantitatively transferred to a 50 ml volumetric flask and made up to volume with distilled water. This was mixed thoroughly by inversion and allowed to stand for at leas 6 hours before 10 ml aliquots were poured off into 25 ml polystyrene storage pots Alternatively, the final digest could be filtered directly through one sheet of Whatmar No. 541 filter paper.

3. Aliquots were generally analysed by AA within 48 hours of preparation, although the digest was found to store well for several weeks. Five standards (0, 2.5, 5.0, 10.0 and 20.0 µg/ml) were prepared for the 357.9 nm wavelength measurements and five (0, 10.0, 20 50.0 and 100 µg/ml) for the 428 nm wavelength measurements) from the blank faeca material collected from sheep grazing the same site as the test animals. The blank material was treated in an identical manner until the completion of digestion wher appropriate volumes of 1 mg/ml Cr (2.8285 g K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> per litre of distilled water) were added to the digest prior to making up to volume in 50 ml volumetric flasks Alternatively, where no faecal ash material was available, multi-element standard: including chromium (III) nitrate (BDH Chemicals Ltd) were used.

Pool samples of faecal material, collected from animals fitted with chromium CRC, were assayed in duplicate with each batch to monitor between-batch variation.

Samples were read on a 1982 1L 457 AA (Instrumentation Laboratory Inc., Massachussetts U.S.A.) with a single slot burner head. The lamp current was 6 mA, the bandpass 0.5 nm and the flame produced was luminous with a 10 mm red core. Machine settings between runs were checked by a common working standard and within-run machine drift was monitored by aspirating a working standard every 10-20 readings. Samples with a RSD > 5% were reread.

Initially, determinations during validation were made using an air-acetylene flame Subsequently, a  $N_2O$ -acetylene flame was used, primarily because of the reduced potential fo interference by other ions in the digest solution.

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The recovery of added chromium was calculated by the formula:

=

Recovery (%) = <u>Chromium recovered  $(mg/g) \times 100$ </u>

Chromium expected (mg/g) 1

where chromium recovered was calculated as the product of concentration of Cr in the faces  $(\mu g/g DM)$  and dry matter output per sheep (g DM/d)

For spiked chromium samples, chromium expected (mg/g) was calculated as:

(<u>Cr<sub>2</sub>O<sub>3</sub>added (mg) x 0.6843</u>) Faccal sample DM (g)

For CRC-released chromium, chromium expected (y:mg Cr/d) was calculated by the formula:

y = <b>n</b>	r <sup>2</sup> . d.i	.t.j.
where	r	= radius of the chromium tablets (0.7 cm)
	δ	= lineal density of chromium tablet (mg/mm)
	i	= proportion of $Cr_2O_3$ in matrix
	t	= rate of plunger travel (cm/d)
	j	= proportion of Cr in $Cr_2O_3$ powder (0.6843)

## **RECOVERY OF CHROMIUM FROM FAECAL SAMPLES**

Estimates of chromium recovery can be obtained by preparing samples with known amounts of pure  $Cr_2O_3$  and following these through the ashing and digestion process. Potassium dichromate is not a suitable source of chromium for this purpose (Costigan and Ellis 1987).

Finely ground industrial  $Cr_2O_3$  used for the manufacture of chromium CRC matrix tablets was added to oven-dried and ground (2 mm sieve) 20.0 g samples of facees obtained from sheep fed pasture or lucerne chaff to yield the Cr equivalents ranging from 10 to 80 µg/ml. The  $Cr_2O_3$ was mixed with the facees in a food processor for 3-4 minutes by adding distilled water and working into a slurry. Samples were then oven-dried to a constant weight and 1.000 g duplicates were ashed, digested, and measured by AA using faceal ash standards and chromium (Cr III) nitrate standards as described previously. Pure samples of  $Cr_2O_3$  and  $Cr_2O_3$  plus 1.000 g of facees, representing a range (10-80 µg/ml) of chromium concentrations were assayed by the same method.

The effect of sample weight on recovery of chromium was investigated by assaying a serial dilution (4.00, 2.00, 1.00, 0.50 and 0.25 g) of faecal DM with a Cr concentration of  $5.00 \ \mu g/ml$ . A serial dilution of a  $60 \ \mu g/ml$  faecal DM from 1.00 to 0.50, 0.25 and 0.125 g was also assayed.

Percentage recoveries of chromium from spiked faecal samples are summarised in Table 2.1.

The overall mean ( $\pm$  sem) recovery from the 30 samples was 94.44  $\pm$  1.78%. Recoveries tended to be poorer at chromium concentrations of 80 µg/ml. This is partly a function of the slightly flexed nature of the AA 0-100 µg/ml standard curve at 429 nm (Lee et al. 1986), but may also indicate that there were insufficient digest reagents to bring all of the added Cr<sub>2</sub>O<sub>3</sub> into solution (Christian and Coup 1954). Although not measured in this experiment, impurities in the added Cr<sub>2</sub>O<sub>3</sub> were found by Costigan and Ellis (1987) to reduce recovery rates. Low recoveries were also apparent with some of the samples spiked to 10 µg Cr/ml. This may reflect incomplete mixing of the small amounts of Cr<sub>2</sub>O<sub>3</sub> added to the faeces (Lee et al. 1986).

Expected Batch 1 Batch 2 Batch 3 Cr ( $\mu$ g/ml) Recovery CV<sup>a</sup> Recovery CV Recovery CV 10 98.85 2.22 14.58 0.0091.65 93.00 20 94.25 9.38 101.60 0.7089.25 10.38 40 95.30 5.79 99.35 4.48 100.30 0.07 60 99.70 0.71 104.60 97.00 3.35 0.2080 82.20 81.45 5.30 \$8.00 2.73 8.95 Mcan 93.91 97.10 4.53 92.36 4.55 4.68

Table 2.1 Recovery (%) of added Cr from separate batches of duplicate spiked faecal samples from sheep fed pasture.

<sup>a</sup>Coefficient of variation between duplicates (%).

In the second evaluation, with faecal samples derived from animals fed lucerne or pasture (n=20 in each casc), chromium recoveries of  $99.49 \pm 1.42\%$  and  $97.47 \pm 2.60\%$  respectively were achieved. The CV between duplicates ranged from 0.61 to 12.53% and averaged 3.50, 4.50, 4.67 and 5.26% respectively for the four batches tested. Poorer recoveries (85 to 94\%) were again achieved at the highest chromium concentration ( $80 \mu g \text{ Cr/ml}$ ). The recovery of chromium was not affected by the type of feed that faeces were derived from.

In the third evaluation, recovery of Cr from single samples of  $Cr_2O_3$ , with (n=20) and without (n=10) facces added, averaged  $103.14 \pm 4.20\%$  and  $105.34 \pm 1.18\%$  respectively, after two outliers (>3.5 SD) were removed from each group. There was no apparent reason for the poor recoveries (17 to 43%) of the outliers.

Recovery of chromium was linear (r = 1.00 and r = 0.98, respectively) across a four-fold range in sample weight on either side of the 1.000 g sample size which would normally be used for the assay. This indicates that the very small samples sometimes obtained by grab sampling from the rectum can be assayed reliably for chromium. Costigan and Ellis (1987) routinely use a 0.1- 0.2 g DM faecal sample in their chromium assay. Similarly, samples of faeces can be bulked across days of a 5-day measurement period (0.5 - 1.0 g DM/day) prior to digestion if required.

The levels of chromium recovery achieved compare favourably with those reported by other researchers using the same digestion procedure. For example, Stevenson and De Langen (1960) achieved recoveries of 97.5 to 99.2%, while Costigan and Ellis (1987) recorded a mean recovery of  $Cr_2O_3$  of 98.6%.

## EFFECT OF FAECAL ASH CONTENT ON RECOVERY OF CHROMIUM

The ash content of faecal material is dependent primarily on the level of soil ingestion and will vary with grazing height, weather conditions and between individual animals (Coop and Abrahamson 1973; McGrath et al. 1982). The ash content of faeces could lie within the range 10-60% for individual sheep within a grazing group (Scoffield 1970; Clarke et al. 1986). This may be significant where AA determination of chromium is used because the ash, which has a high concentration of a range of elements (Healey et al. 1974), is a potential cause of interelement interference during flame emission. The effect of faecal ash content on recovery of added chromium was therefore investigated.

Single samples with a range of faecal ash contents were prepared by mixing different proportions of high ash faeces (obtained from sheep which ingested soil while grazing to low residual dry matter levels during wet winter conditions) with low ash faeces (obtained from sheep fed hay indoors). Each sample was spiked with 10  $\mu$ g/ml Cr using K<sub>2</sub>Cr<sub>2</sub>0<sub>7</sub> solution after digestion. AA absorbance and chromium readings for a N<sub>2</sub>0-acetylene flame at 357.9 nm were recorded. The latter were read from a standard curve (0-20  $\mu$ g/ml) derived from chromium standards containing no ash.

Samples representing a wide range of background material and with different ash contents were spiked by adding 10  $\mu$ g/ml Cr, using K<sub>2</sub>Cr<sub>2</sub>0<sub>7</sub> solution, after ashing but prior to digestion. The effect of ash on Cr recovery was also estimated by cross reading standards with different faecal ash backgrounds against faeces-free standards.

No significant effect of ash content on chromium absorbance was found in the spiked composite faecal samples (Table 2.2). The correlation between ash contents ranging from 18.04 to 54.75% and chromium readings of r = -0.13 indicates that the hotter flame conditions of N<sub>2</sub>0-acetylene override potential interference problems from elements in the faecal ash material. Similarly, chromium concentrations showed a slight trend (r = 0.58) to decrease as ash contents decreased from 89.95 to 0.01% for the samples from sheep faeces, soil, herbage and no ash material. Chromium readings of spiked samples also did not differ if the samples used to derive the AA standard curve originated from faecal material which had different ash contents and feed backgrounds, or if they were made up without faecal material.

Sample	Batcl	h 1	Batch	2
	Ash Content (%)	AA reading (μg Cr/ml)	Ash Content (%)	AA reading (μg Cr/ml)
1	18.04	10.42	0.01	9.65
2	22.29	10.16	8.81	9.49
3	25.87	10.34	11.38	9.62
4	29.75	10.14	12.58	9.62
5	33.17	10.29	14.23	9.77
6	36.97	10.38	21.59	9.80
7	40.17	10.23	53.00	9.95
8	43.84	10.05	83.93	10.10
9	46.88	10.15	89.95	9.91
10	50.86	10.29		
11	54.75	10.37		
Mean		10.26		9.76
SD		0.12		0.20
r <sup>a</sup>		-0.13		0.58

Table 2.2 Effects of ash content on recovery of added chromium (Cr (VI)). Chromiun concentrations are the mean instrument readings for a standard curve derived from non-as standards.

<sup>a</sup>Correlation between ash contents and chromium readings.

# RECOVERY OF CHROMIUM FROM GROUND FAECAL SAMPLES AND FAECAL PELLETS

In previously published assay methods faecal material has commonly been ground through a 1-: mm sieve and then sub-sampled prior to analysis to ensure uniformity of chromium in the faeces. Where pulse doses of chromium are administered and there is, as a consequence significant diurnal variation in faecal chromium concentration (Raymond and Minson 1955 Pigden et al. 1956), mixing samples obtained at different times of the day or across days a different intervals is appropriate. However, the continuous slow release of chromium from CRC reduces diurnal variation of faecal chromium (Ellis et al. 1981). Furthermore, chromiun should be evenly dispersed through the contents of the rumen and so be uniformly distributed through the faeces (Laby et al. 1984).

Grinding facces has potential disadvantages of sample weight loss (Roofayel et al. 1983) and where stainless steel mills are used. loss of chromium through adherence to hammer (Stevenson 1962; Costigan and Ellis 1987). In addition the fine dust material associated witl grinding is a potential hazard because chromium is potentially carcinogenic (Vincent 1986).

Faecal grab samples, the primary source of faeces from sheep treated with chromium CRC, are also small (c. 0.3-4.0 g DM/sample) and provide sufficient material in most cases for a single set of duplicate samples (i.e. 2.0 g DM each). A comparison of AA readings (and chromiun recovery) for faccal pellets vs ground faccal samples was therefore made. Duplicate samples (c. 1.0 g DM) of facces, either ground or in their intact form (pellets), from 10 wethers each fitted with a single 65% chromium CRC and fed lucerne chaff indoors were assayed as described previously. The facces were obtained by sub-sampling from the total collection of each sheep on the same day. Ground faeces were prepared by milling through a 2.00 mm sieve (Creston, Stanmore, England). Four separate faecal pellets from rumen-fistulated wethers fed lucerne chaff, pasture (ryegrass and white clover) or white clover indoors were also assayed separately to measure the variation in chromium concentration between pellets. The effects of faecal form on chromium readings were assessed using variance components analysis by equating mean squares to their expectations according to the procedure described by Scarle (1971).

Mean AA readings for the pellet and ground facees were not significantly different. Variation between duplicates was not significant, although it was higher for the pellet samples than for the ground samples (CV = 3.77 vs 2.69%, Table 2.3). In both cases 80% of the duplicates yielded RSD <5%. For ground samples the variance between observations on the same sheep was 0.073 and the variance between sheep was 2.219. The corresponding variance components for the pellet samples were 0.095 and 1.893, respectively.

Ѕһсср	Ground facces			Pellet facces		
	AA rdg	CV	Recovery	AA rdg	CV	Rccovcry
	(µg/ml)	(%)	(%)	(µg/ml)	(%)	(%)
1	6.20	9.52	79.97	8.15	4.65	105.18
3	3.41	1.17	82.59	3.36	1.27	81.32
5	4.68	1.07	96.14	4.79	1.79	98.23
7	5.11	0.32	96.77	5.19	4.26	98.23
9	8.79	6.10	122.92	7.51	8.47	104.92
11	5.44	0.28	86.07	5.50	3.82	87.06
13	5.33	1.79	102.67	5.48	2.03	105.52
15	6.91	1.92	98.76	6.69	7.66	95.60
17	7.21	1.45	108.51	6.66	2.04	100.19
20	6.44	3.32	88.40	6.22	1.72	85.29
Mean	5.95	2.69	96.28	5.95	3.77	96.16
SD	1.47	2.07	12.95	1.37	2.177	9.13

Table 2.3 Mean atomic absorption (AA) readings, coefficient of variation (CV) between duplicates within sheep and estimated chromium recoveries from ground and pellet faecal samples for a single day's collection from sheep fed lucerne chaff (10 days after CRC administration).

The mean recoveries of Cr, based on actual capsule plunger travel for sheep 1, 7, 9 and 20 and average plunger travel for the remainder  $(1.03 \pm 0.06 \text{ mm/day})$ , were similar for both groups. While the overall recovery of Cr of c.96% is acceptable, the variation between sheep (CV of 13.45 and 9.49% respectively for the ground and pellet samples) is high. Some of this variation (5.45%) can be attributed to between-animal differences in capsule plunger travel and to the recoveries being based on the faceal output of a single day. Subsequent experiments using the chromium assay also indicate that a higher proportion of potassium bromate (6 ml) to acid (4 ml) in the digest would have reduced the variation between duplicates.

The dry weights of the faecal pellets assayed separately ranged between 0.11 and 0.26 g. These sample sizes yielded concentrations of chromium in the digest of between 0.59 and 1.06  $\mu$ g Cr/ml, which are near the lower limit of detection by AA (Williams et al. 1962). The mean (±sd) concentration of chromium in the four faecal pellets of sheep fed lucerne chaff, pasture and clover were 210±10, 173±8 and 240±13 mg Cr/g DM, respectively.

These results indicate that grinding of faecal material collected from sheep fitted with CRC is not an essential prerequisite to the recovery of faecal chromium for within-day estimates. This represents a significant saving in labour and allows faecal grab samples, which are frequently small, to be analysed intact. The slightly higher variability between pellet samples can be countered by preparing duplicates for analysis. Where samples are bulked across several days, mixing equal parts of intact faeces (or grinding) will still be necessary to ensure uniformity of chromium in the material analysed.

#### VARIATION IN BACKGROUND CHROMIUM LEVELS

High variable background levels of chromium in plant and soil ash material could pose serious problems in obtaining accurate recoveries of CRC chromium in faeces. Natural levels of Cr are about 10  $\mu$ g/kg in fresh plant material but may be as high as 100  $\mu$ g/kg in some soil types, particularly those with a serpentine rock background (Brooks 1983). Natural levels of Cr in plants are usually below 5  $\mu$ g/kg, but may be as high as 100  $\mu$ g/kg in plants growing on Cr-rich soils such as those derived from serpentinites. It is not clear to what extent this clevation is due to small particles of soil not removed from the plant prior to analysis as opposed to the incorporation of Cr into plant tissues (Reeves 1986). Problems with background chromium contamination are most likely to occur where the surfaces of pasture plants have a high soil content, such as under rotational grazing in wet conditions and on small research plots where chromium has been used intensively for intake studies over a number of years (Corbett 1981). A reliable estimate of the soil material ingested by sheep can be obtained from the ash content of pasture and faecal samples (Scoffield 1970; Nes 1975). This may be as high as 60% under high grazing pressures during winter (Clarke et al. 1986). Background levels of chromium in herbage and soils of proposed experimental areas should therefore be quantified prior to trials with chromium CRC commencing. The following section describes the determination of chromium in pasture and soil samples from a number of sites near Massey University.

Pasture and soil samples were collected from 6 sites within 2.0 km of the University Campus. The sites, two of which were likely to be used for subsequent intake study trials, represented different soil types and pasture species. Pasture samples were plucked by hand and thoroughly washed to remove any soil material. Soil samples were collected from the top 1.0 cm of the ground surface, as material from this region was most likely to contaminate ingested herbage. Both soil and plant samples were oven dried at 100°C to a constant weight. Duplicates (c.1.0 g) of materials from each site were then ashed overnight (12 hours) at 550°C and digested as described previously. Digest samples were aspirated into a N<sub>2</sub>0-acetylene flame and chromium concentrations read from a non-ash standard curve (0.5, 1.0, 2.5, 5.0, 10.0  $\mu$ g/ml) using the Cr line at 357.9 nm.

Plant and soil concentrations of Cr averaged 0.15 and 0.54  $\mu$ g/ml (8 and 27  $\mu$ g/g DM) respectively for the 6 sites (Table 2.4). These values, which are at the lower limit of chromium detection by AA, compare favourably with the levels of 0.01 mg/g DM obtained by Williams et al. (1962) for facees from sheep not dosed with chromium.

Sitc <sup>a</sup>	Ash	(%)	Chromium (µg Cr/g DM)		Sward Type
	Herbage	Soil	Hcrbagc	Soil	
1	8.22	88.71	5	23	Prairic grass
2	11.37	88.76	9	39	Rycgrass
3	9.71	88.95	6	22	Ryegrass/white clover
4	9.33	88.43	8	32	Ryegrass/white clover
5	11.78	91.99	11	22	Clover
6	10.91	87.41	6	24	Lawn turf
Mcan	10.22	89.04	8	27	
SD	1.36	1.54	2	7	

Table 2.4Background concentrations of chromium in plant and soil samples collected fromdifferent sites at Massey University.

<sup>a</sup>Site locations were:1,2=Dairy Cattle Research Unit; 3,4=Shecp and Becf Cattle Research Unit (SBCRU) top terrace; 5=DSIR pasture cultivar plots SBCRU top terrace; 6=Massey University campus.

Faecal chromium concentrations in adult sheep fitted with 65%  $Cr_2O_3$  CRC typically range from 150 to 950  $\mu$ g/g DM. Background error in herbage heavily contaminated with soil could therefore range from less than 1% to as high as 10% of these values. Largest errors will occur with sheep of mature body size on <u>ad libitum</u> intakes of soiled pastures. The size of the background error can be reduced by running blank faecal samples to provide a correction value or incorporating blank faecal material as a zero value in the set of standards. These steps will reduce background "noise" to 1-3% for chromium readings less than 250  $\mu$ g/g DM.

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The mean ash content of the herbage samples of 10.22% is higher than the value of 8.38% obtained by Nes (1975) for ryegrass/white clover pastures and reflects the different species composition of the sward as well as the possibility of low levels of soil contamination. The mean soil ash content of 88.73% was approximately 7% lower than those obtained by Nes (1975) for mainly recent soil types, and indicates why soil contamination can rapidly increase the ash content of faecal material.

The results indicate that chromium background levels on the proposed experimental areas were low. This reflects the very low or nil usage of chromium on these sites, as well as the low Cr in the soil parent material (Reeves 1986).

The problem of background contamination, where it occurs, can be reduced by increasing the release rate of chromium to generate high administered chromium concentrations relative to background levels. There are two difficulties with this approach. First, the problem is exacerbated by a more rapid build-up of background chromium. Second, it is technically difficult to develop matrix carriers which can deliver high daily release rates of chromium for periods which are useful experimentally (Laby 1986). Alternatively, a second marker element which is highly or consistently correlated with chromium levels in the background material can be assayed concurrently. Titanium, which is used as an indicator of soil contamination in faeces (Scoffield 1970; Nes 1975), may be suitable for this purpose, although there is no published information about its relationship with soil chromium.

The most difficult situation occurs where the levels of background chromium are highly variable. For this reason, soil and pasture samples from potential research areas should be investigated for chromium contamination prior to the commencement of trials using chromium CRC. Sites with the lowest level of contamination should be selected and if possible sheep without CRC should be run concurrently with trial sheep to provide a correction measure for background Cr during the trial.

## **INTRA- AND INTER-ASSAY VARIATION**

Variation in the assay procedure for samples within- and between-batches is a potential source of error in determining Cr recovery (Lee et al. 1986). Including pool samples with each batch allows this source of variation to be monitored.

The intra- and inter-assay variation was assessed with three pool samples of low, medium and high Cr concentrations (30,50 and 80  $\mu$ g/ml, respectively). The pool samples were assayed in four independent batches. All samples from each batch were subsequently read by AA on four separate occassions. A common set of Cr standards was used at each AA reading. AA readings were tested in an ANOVA model which included terms for variation between batches, between machine readings and between duplicates within batches. Between-batch variation was significant (P < 0.001), but only accounted for 5.28% of the total sum of squares (TSS) in the model. Variation in machine readings of samples (0.2% of TSS) and between duplicates (2.9% of TSS) were not significant. Between-batch variation was mainly attributable to variation in the readings of the highest concentration pool samples.

The stability of analyte and variation between AA readings was also tested by recording the Cr concentration in aliquots 1, 7 and 14 days after digestion. The CV between readings was 1.2%, confirming that analyte could be stored in plastic vials for at least two weeks before AA determination if necessary (Costigan and Ellis 1987). This result and the low variation attributable to machine readings in the previous test show that machine settings and flame conditions are repeatable.

## SUMMARY

A phosphoric acid-manganese-potassium bromate digestion of ashed faeces was used to solubilise Cr (III) to Cr (VI) for determination by AA. Mean Cr recoveries of 94.4-103.8% were obtained from  $Cr_2O_3$ -spiked samples made up with faeces from a range of background materials. Chromium recovery was independent of sample size (over the range 0.25-4.00 g DM), ash content of the faeces, and form of the faecal sub-sample (pellet form vs ground). Recoveries of chromium tended to be lower and more variable at higher concentrations of chromium (>4 mg Cr/g DM). It may be necessary, at these concentrations, to add additional volumes of the digest reagents in the same ratios as recommended by Williams et al. (1962), to bring all of the Cr into solution (Christian and Coup 1954). However, faeces from sheep fitted with CRC are unlikely to exceed 1.5 mg Cr/g DM. Chromium concentrations as low as  $5 \mu g/g$  DM were measured in herbage samples but the reliability of AA readings is reduced at concentrations below  $50 \mu g/g$  DM (Williams et al. 1962).

Determination of background chromium concentrations in herbage and soil samples from potential trial sites indicated that these were low and unlikely to contribute significantly to error when determining chromium added by CRC. However, the occasional inexplicably low recovery of chromium and high variation between some duplicates suggests that results should be carefully screened to identify outlying values and duplicates with a CV greater than 5% so that the analysis can be repeated for these samples.

Analyte for AA determination stored well for several weeks and coefficients of variation within and between batches were generally less than 5%. However, pool samples representing the range of chromium concentrations assayed should be included within each batch to monitor these sources of variation through time. In addition, variation due to the AA can be monitored by reading appropriate chromium pool samples that have not been run through the ashing and digestion phases of the assay with each run of unknown samples.

## **CHAPTER THREE**

# EFFECT OF HERBAGE TYPE AND LEVEL OF INTAKE ON THE RELEASE OF CHROMIC OXIDE FROM INTRARUMINAI CONTROLLED RELEASE CAPSULES IN SHEEP

## INTRODUCTION

Controlled release capsule (CRC) technology for the continuous and uniform release of the indigestible marker chromic oxide (Cr2O3) into the rumen over periods of 20-30 days may provide an improved method of estimating voluntary herbage intake in grazing ruminants (Harrison et al. 1981, 1982; Laby et al. 1984). Preliminary studies with CRC in sheep (Ellis et al. 1981; Laby et al. 1984) and cattle (Ellis et al. 1982) show that intraruminal capsule technology has the potential to surpass existing methods of intake measurement because the uniform release of Cr2O3 into the rumen decreases diurnal variation of the marker in the faeces and because the single application of the CRC reduces both animal disturbance and labour requirements. However, these studies do not cover the range of environments CRC may encompass. There is thus a need to define the performance characteristics of sheep CRC when they are exposed to different types of herbage, levels of herbage intake and management systems before they can be routinely applied to studies of voluntary herbage intake. This chapter presents the results of two experiments in which rumen-fistulated and intact sheep, fitted with chromium CRC, were fed five different herbages at three daily allowances to determine the effects of herbage type and level of intake on chromium release rates through time.

#### MATERIALS AND METHODS

#### Experiment 1

The objective of this experiment was to determine within- and between- sheep variation in the rate of chromium release from CRC. Five 14-month old Romney wethers (weighing  $43.3 \pm 2.04$  kg, mean  $\pm$  sem) were rumen-fistulated by the method of Komarek (1981) and housed in metabolism crates. Animals were offered lucerne chaff, twice daily at 0830 and 1600 h, to provide intakes equivalent to 1.1 maintenance (M) requirements (Rattray 1986) for 10 days. Maintenance was calculated as 0.7 times the daily energy requirements for a wether of equivalent liveweight grazing outdoors (Rattray 1986). Lucerne chaff was assumed to contain 9.6 MJ ME/kg DM at 85% DM (Coop 1986). During this period each sheep was treated with a selenium-fortified anthelmintic for internal parasite control. Lucerne chaff was supplemented with 1.0 g of a mineral mix (0.05 g NaMoO<sub>4</sub>, 0.5 g Na<sub>2</sub>SO<sub>4</sub> and 0.8 g NaCl/kg DM feed) each

ground through a 1.00 mm sieve (Cranston, England) for <u>in vitro</u> digestibility analysis by the method of Roughan and Holland (1977).

On the eleventh day after animals entered the metabolism crates (d 0 of measurement), three chromium CRC (50%  $Cr_20_3$  matrix, 7.0 mm orifice, 6.0 cm core; Mark I design, CAPTEC (NZ) Ltd Auckland) were attached by nylon string to the cannula plug (Harrison et al. 1982) of each wether. CRC plunger travel was measured on d 3 and thereafter at 7-day intervals, by removing the capsules from the rumen and measuring the position of the matrix on both sides of the barrel using vernier callipers (Mitutoyo, Tokyo). Capsules were quickly doused in warm water (c.25°C) to remove rumen material prior to measurement and returned to the rumen within two minutes to minimise temperature fluctuations. On d 8, each wether was fitted with a harness and bag for the total collection of faeces during a 10 day determination of feed digestibility. Harnesses and bags were removed on d 19. Indoor feeding at 1.1 M continued until d 43, when the animals were moved to outdoor grazing on mixed ryegrass - white clover pastures. Monitoring of plunger travel continued at weekly intervals until d 100 when the  $Cr_20_3$  matrix first expired in one of the CRC.

## Experiment 2

This experiment was run concurrently with experiment 1 with the objective of quantifying the pattern of  $Cr_20_3$  release from CRC in sheep offered four different types of herbage at three allowances.

Sixteen 14-month old Romney wethers, eight of which were rumen-fistulated, were housed in metabolism crates and divided into four groups, each comprising two intact and two rumen-fistulated sheep. Harnesses and faecal collection bags were fitted on the same day. The groups were assigned to receive one of the following feeds - white clover (Trifolium repens, ev. Grasslands 'Huia'), ryegrass (Lolium perenne, ev. Grasslands 'Ruanui'), ryegrass and white clover mixed, or meadow hay, for the duration of the indoor feeding period. Ryegrass and clover were harvested at 1500 h each day from pure swards (Ulyatt 1971; Purchas and Keogh 1984) using a sickle-bar mower. The ryegrass-clover feed was made up by mixing material from the two pure swards to achieve a clover DM content typical of an improved pasture sward during late spring (Smetham 1973). The average composition of the fresh pasture feeds is summarised in Table 3.1. The meadow hay had been made from a mixed pasture sward, of predominantly ryegrass and clover composition, during the previous summer.

	Swa	rd Component (% Di	y matter, Mean ± se	<u>m)</u>
Feed type	Grass	Clover	Weed	Dead
Clover	0	81.7±1.8	9.0±1.8	9.2±1.8
Ryegrass	85.1±3.0	0	7.5±1.9	7.3±1.5
Ryegrass/clover	58.6±6.1	25.8±4.0	7.1±1.6	8.5±1.1

Table 3.1	Dry matter com	position of pasture	feeds, experiment 2.

Each of the groups was offered their respective feed at approximately maintenance allowances for a 10-day period. At this stage (d 0) a single chromium CRC (from the same manufacturing batch as experiment 1) was attached to the cannula plug of the fistulated animals by nylon string, or orally administered to the intact wethers using a lubricated flexible tubing to release the CRC at the thoracic inlet. Following CRC insertion, feed allowances were reduced to the equivalent of 0.8 M from d 8. Maintenance requirements were based on assumed energy concentrations of 7.5, 10.2 and 11.7 MJME/kg DM and DM contents of 85, 15 and 16% respectively for hay, clover and ryegrass (Ulyatt et al. 1980). Allowances were then changed in the following sequence for each group: d 15-21, 1.1 M; d 22-28, 1.4 M; d 29-35, 0.8 M and d 36-42, 1.1 M. Fresh feed allowances for individual sheep, calculated each day on the basis of the mean DM content of duplicate samples taken from the feed the previous day, were offered once daily at 1600 h. Refusals were weighed at the same time, prior to being bulked within feeding groups, thoroughly mixed and sub-sampled in duplicate (c. 100 g fresh weight) for DM determination. The fresh feed and refusal DM samples were oven dried for 24 hours at 80°C to a constant weight. Daily DM intakes (DMI, g/d), calculated as the difference between DM offered and refused, are summarised with feed DM digestibilities for each feeding level in Table 3.2. Samples of feed offered (1 g DM/d) were bulked within feeding levels and ground for in vitro digestibility analysis (Roughan and Holland 1977).

Table 3.2Group mean dry matter intakes and digestibilities of feeds at each feeding level,experiment 2.In vitro digestible organic matter contents of the dry matter of samples of feedstaken at each feeding level are also shown.

			Fee	ding level ( x	Maintenan	ce)		
		1.0	0.8	1.1	1.4	0.8	1.1	
Feed type	Parameter	0-7 <sup>a</sup>	8-14	15-21	22-28	29-35	36-42	Lsd <sub>0.05</sub>
Ryegrass/clover	DMI <sup>b</sup>	522	474	606	796	529	702	36 <sup>c</sup>
	DMD <sup>c</sup>	72.1	67.7	63.S	71.2	71.2	72.3	1.7
	DOMDd	69.3	64.5	59.3	65.2	66.2	69.2	
Clover	DMI	467	441	603	825	490	632	45
	DMD	82.4	79.5	75.5	76.6	75.2	75.9	1.8
	DOMD	73.2	71.7	72.2	71.3	73.1	74.0	
Ryegrass	DMI	534	438	509	614	523	652	72
	DMD	71.5	63.8	54.6	62.8	65.2	64.7	3.9
	DOMD	69.8	59.1	59.7	65.0	67.9	59.2	
Hay	DMI	817	676	878	1029	735	940	50
	DMD	58.6	59.8	55.5	57.6	58.6	58.1	1.7
	DOMD	55.0	55.1	55.4	54.2	52.6	53.0	

<sup>a</sup>Day of experiment.

<sup>b</sup>Dry matter intake (g/d).

<sup>c</sup>Dry matter digestibility (%).

<sup>d</sup>In vitro digestible organic matter in the dry matter (DOMD, %).

<sup>e</sup>Lsd values are for comparison only between means in the same row (Gill 1988). DOMD values are point estimates only for each feeding level and have no within-feeding level variance.

CRC plunger travel in the fistulated sheep was measured at 7 d intervals after the first measurement on d 3. Liveweights were recorded at d 0 and d 43. On d 43 the wethers were transferred to outdoor grazing with the animals from experiment 1. Monitoring of plunger travel in the fistulated animals continued at weekly intervals until d 100.

Total bagged collections of faeces were obtained from sheep from d 0 to d 42. The fresh weight was recorded, faeces thoroughly mixed and two sub-samples of c.100 g taken for DM determination. These were oven dried at  $80^{\circ}$ C to constant weight for at least 72 hours. A 30-50 g sample of the dried faeces was retained for chromium analysis. Four-hourly rectum grab samples were obtained over a 24 hour period from sheep (n=8) in the hay and clover groups on d 18 and d 32, when the animals were on 1.1 and 1.4 M levels of feeding, respectively. A further series of four-hourly samples were obtained from the same animals on d 53 while they were at grazing.

#### Chromium analysis

Faeces were assayed for chromium by atomic absorption spectrophotometry using the method described in Chapter Two. Samples from individual animals were assayed on a daily basis for the 0.8, 1.1 and 1.4 M feeding levels and bulked over the last 4 days of the 0.8 and 1.1 M feeding levels. Analyses for chromium were repeated for samples where the coefficient of variation (CV) between duplicates exceeded 5%. The intra-assay CV averaged 2.9% and the inter-assay CV 3.7%.

#### Statistical analysis

CRC plunger travel measurements within- and between-sheep in experiment 1 were tested for linearity using a split-split-plot model (Gill and Hafs 1971; Gill 1988). The model included main effects for sheep, date of plunger measurement (specified as time in linear and quadratic terms) and the interactions between time and sheep, CRC between sheep and CRC within sheep (see Appendix I). Plunger travel data from experiment 2 were analysed in the same manner, except the double split-plot model included feed type rather than CRC's within sheep. Univariate linear regressions were calculated to determine the average rate of chromium release through time for individual CRC. Faecal chromium concentrations from the diurnal variation study were subjected to repeated measures analysis. Analyses were undertaken using the 'REG' (Gilmour 1985) and 'SPSSX' (SPSSX 1983) statistical packages.

Chromium recovery was calculated as the percentage of the expected daily release of chromium from individual CRC recovered in the faeces, using the formulae outlined in Chapter Two. Two sheep were excluded from the chromium analyses, one because recovery of chromium was affected by rumen spillage due to cannula loss and the other because of CRC failure.

#### RESULTS

#### Experiment 1

#### Feed intake

A consistent group mean dry matter intake (DMI) of lucerne chaff of  $771\pm7$  g/d was achieved from d 0-42. The <u>in vivo</u> DM digestibility of lucerne chaff was  $63.5\pm0.6\%$ , with an assumed energy concentration of 9.6 MJ ME/kgDM (<u>in vitro</u> DOMD = 63.0%). The estimated average daily energy intake of 7.4 MJ ME was therefore equivalent to slightly more than the maintenance requirements for a 39 kg yearling ram housed indoors (Townsley 1985; Rattray 1986) and close to the 1.1 M objective. As a result liveweights were only 0.6 kg greater than the 39.1±1.3 kg initial liveweight by the end of the indoor feeding period.

### CRC plunger travel

Total plunger displacement from d 0-42 in the fistulated sheep, with the exception of one CRC which failed between d 7 and d 14, averaged 25.0±0.4 mm. The full split-split-plot model, including interaction terms, indicated that 90.4% of the total sums of squares could be accounted for by fitting a straight line and an intercept for each sheep (P<0.001). Exclusion of the single outlier increased this to 98.1%. The proportion of the total sums of squares due to variation between CRC within sheep was therefore small and not significant, indicating that CRC exposed to a common environment performed similarly. However, there was significant (P<0.05) variation about the line which increased with time. Fitting a quadratic term indicated that this lack of fit could be explained by the slightly curved nature of plunger displacement with time (Figure 3.1), rather than by systematic variation. Linearity of chromium release was maintained until d 51. From this time until d 100, CRC plunger travel was less uniform. In four cases this was associated with the temporary loss of the rumen cannula and the consequent exposure of the CRC to air temperatures lower than that of the rumen. Interestingly, plunger travel generally recovered over a period of several days to the previous rate once CRC were returned to the rumen. Release rates increased near the end of CRC life because of the more rapid dissolution of the last of the nine tablets that comprised the initial matrix core, and were lower during the first measurement interval (3 d) because of the time required to soften the matrix at the orifice and to initiate the extrusion process. Average plunger travel and chromium release rates were therefore calculated from d 3. The pooled regression of plunger travel (y) on time (x) for the 14 CRC with uninterrupted plunger travel between d 3-42 was:

 $y (mm/d) = 1.129 (\pm 0.294) + 0.593 (\pm 0.012)x, r = 0.999, P < 0.001.$ 

Chromium release from d 3-42 averaged  $62\pm 1 \text{ mg Cr/d}$ , with a maximum and minimum of 65 and 56 mg Cr/d, respectively.

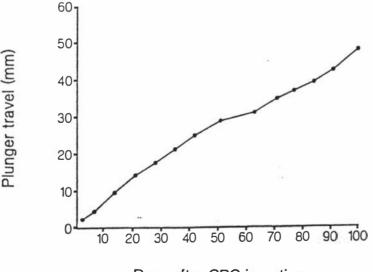




Figure 3.1 Controlled release capsule (CRC) plunger travel from insertion until expiration of the first matrix core in fistulated wethers, experiment 1. All standard errors are < 1.6 mm.

# CRC plunger travel

Details of plunger travel and the associated linear regression parameters for CRC in fistulated wethers on the different feed types are presented in Table 3.3. Chromium was released at the same average rate ( $62\pm 2 \text{ mg Cr/d}$ ) as in the sheep fed lucerne chaff, but variation between CRC was greater (CV = 9.6% vs 5.7%).

Table 3.3 Total CRC plunger displacement from day 0-42, linear regression (a + bx) of plunger travel (y) on time (x) and average daily chromium release rates in fistulated wethers on different feed types, experiment 2.

		Total	Regr	ession para	meters <sup>C</sup>	Chromium
Feed type	Sheep	travel (mm)	а	Ь	r	rclease (mg Cr/d)
Ryegrass/clover	1	24.50	1.108	0.586	0.993	63
	4d	22.00	2.258	0.498	0.988	56
Clover	6	23.50	1.746	0.545	0.992	60
	11 <sup>e</sup>	20.50	1.192	0.485	0.992	52
Ryegrass	10	25.00	0.361	0.610	0.997	63
	15	26.50	1.120	0.616	0.998	66
Hay	8	27.50	0.154	0.684	0.995	70
	14	27.00	0.832	0.637	0.997	67

<sup>c</sup>Pooled regression:  $y (mm/d) = 1.206 (\pm 0.397) + 0.579 (\pm 0.016) x$ , r = 0.981, P < 0.001.

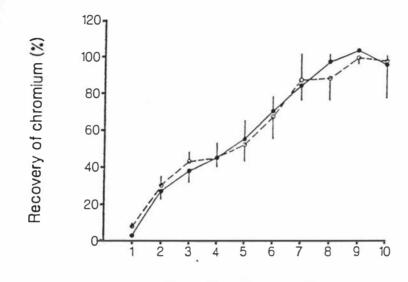
<sup>d</sup>CRC performance may have been affected by two rumen spills due to loss of cannula.

<sup>e</sup>Plunger travel decreased from d 22-42 in this sheep.

Initial examination of the average rates of chromium release suggested that plunger travel tended to increase with decreasing DMD (Table 3.2), the highest and lowest release rates being achieved on the hay and clover feeds, respectively. In all cases plunger travel was highly correlated with time ( $r \ge 0.988$ ). This was confirmed by the split-plot analysis which indicated that 96.5% of the total sums of squares could be explained by fitting plunger travel with time as a straight line and an intercept (P<0.001). The intercepts of the lines for each of the feeds were not significantly different. The quadratic term for time, included after the linear term to account for non-linear variation, was significant (P<0.001), but remaining systematic variation about the lines for feeds was not. This shows, as for the CRC in wethers fed lucerne chaff (experiment 1), that plunger travel decreased marginally with time. The interaction between feed type and rate of plunger travel was significant (P<0.001). However, for practical purposes these differences were small and for feeds with similar characteristics (e.g. fresh pastures) a single linear regression line could be used to determine the average rate of chromium release.

# Appearance of chromium in the faeces

Chromium from the CRC appeared in the faeces on the day following insertion into the rumen but recovery of chromium did not exceed 90% of the average daily release from the CRC during the linear phase until days 6 to 9. There was considerable variation between sheep on the same feed type in the pattern of chromium appearance in the faeces (Figure 3.2). Equilibrium conditions were achieved more rapidly in the intact (a mean of 7.1 days) than in the fistulated (a mean of 8.5 days) wethers.



Days after CRC insertion

Figure 3.2 Recovery of chromium (mean ± sem) from the faeces of fistulated (\_\_\_\_) and intact (----) wethers, experiment 2.

#### Recovery of chromium

Recovery of CRC chromium from the fistulated sheep averaged  $97.4\pm2.6$ ,  $100.2\pm2.8$  and  $103.8\pm3.6\%$  of the expected daily output for the 0.8, 1.1 and 1.4 M feeding levels, respectively. The minimum and maximum recovery for the three feeding levels was 83.5 and 111.2%, respectively. Recovery of chromium did not differ significantly between feeds, between feeding levels or between sheep within feeds. However, the tests of significance had relatively low power because of the small number of animals involved.

The CV in daily recovery of chromium within feeding levels for individual sheep (n = 7 d/level) was approximately 20%, indicating that a low level of reliability can be placed on an intake estimate based on a single day's faecal sample. Variability in faecal chromium recovery may,

however, have been increased because the animals' feed allowances were changed every 7 days. This particularly applies to the first 3-4 days of each feeding level. Chromium recoveries were therefore obtained for a bulked sample for the final four days of the 0.8 and 1.1 M feeding levels for both the intact and fistulated wethers (Table 3.4). This indicated that when CRC release rates within the range measured in the fistulated sheep on the same feed type were applied, recoveries of chromium in the intact wethers were similar (P > 0.1) to those obtained in the fistulated animals.

Table 3.4 Chromium recoveries (recovered/expected) from faeces of fistulated (F) and intact (I) wethers bulked across the last four days of each feeding level, experiment 2.

		Fccding lev	cl	
Feed	Sheep	0.8 M	1.1 M	
	type			
Ryegrass/clover	F	95.8±0.4 <sup>a</sup>	94.5±10.5	
	I	97.9±1.2	107.8±2.6	
Clover	F	105.5 <sup>b</sup>	96.2±4.2	
	I	88.9±3.4	$100.0 \pm 3.3$	
Ryegrass	F	88.1±12.3	105.5±6.5	
	Ι	106.6±15.4	97.8±11.3	
Hay	F	96.4±2.7	105.1±2.0	
	Ι	96.7 <sup>c</sup>	105.7	
Overall Mean <sup>d</sup>		96.4±2.7	98.8±3.2	

 $^{a}\%$  recovery, mean ± sem, n = 2.

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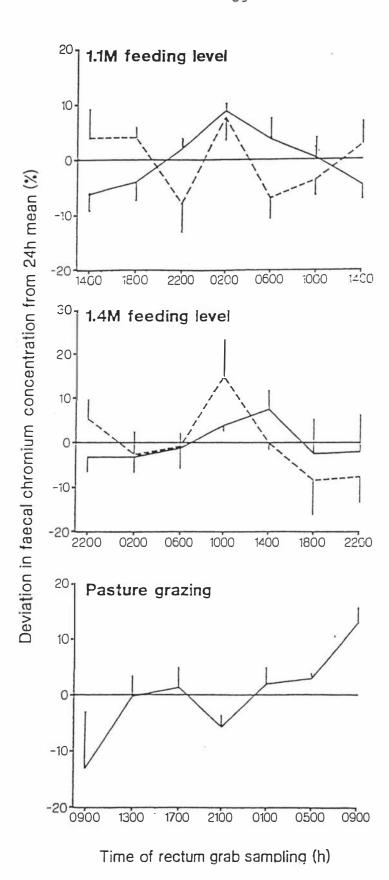
<sup>b</sup>One sheep excluded because of rumen spillage due to loss of cannula. <sup>c</sup>One sheep excluded because of CRC failure.

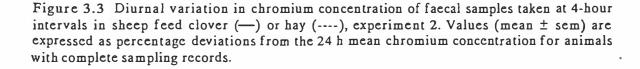
<sup>d</sup>Differences between sheep type and feed types were not significant.

#### Diurnal variation in faecal chromium concentration

The CV between four-hourly faecal chromium concentrations for individual animals ranged from 4.0% to 17.9% (overall mean  $8.3\pm0.8\%$ ), during the diurnal variation sampling periods. Variation was elevated in one of the sheep fed hay because faecal chromium concentrations were low (<80 µg Cr/g DM) relative to natural background chromium levels and the lower detection limit of 15-30 µg Cr/g DM for chromium by atomic absorption spectrophotometry (Williams et al. 1962). If this animal was excluded the average CV between four-hourly samples was reduced to 7.2±0.5%. The four-hourly faecal chromium concentrations at the 1.1 M feeding level were not significantly different (Figure 3.3). The difference between the mean four-hourly chromium concentration and an independent measure for the 24-hour bagged collection was less than 2%. At the 1.4 M feeding level (d 32) the effects of sampling time on faecal chromium concentration were also non-significant. At both feeding levels the pattern of variation in faecal chromium concentration was not consistent across feed types, suggesting that variation was a random effect rather than an inherent animal circadian rythmn.

During the period when the wethers were grazing at pasture (in vitro DMD = 72.4%) rectum grab samples were obtained at 48 of the 49 potential sampling times. Faccal chromium concentration generally increased with time (Figure 3.3). However, only the final 0900 h reading was significantly (P<0.10) different from the readings obtained for the remainder of the day. The increasing trend in chromium concentration may have been associated with the change in average pasture height, which declined from 6.6 cm to 3.0 cm over the 24 h period.





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DISCUSSION

These experiments have demonstrated that chromium release over a period of at least 100 days can be achieved in sheep CRC with a 6.00 cm core, 50%  $Cr_2O_3$  matrix and 7.00 mm orifice. Plunger travel, and hence chromium output, during this period decreased marginally with time but for practical purposes a simple linear regression equation for the first 42 days best explained the relationship between plunger travel and time. Although the sheep were transferred from indoor feeding to outdoor grazing after 42 days, the pattern of chromium release was disrupted in a noticeable manner only if cannula loss occurred and, as a consequence, CRC were exposed to an environment different to that of the rumen. The slight reduction in rate of chromium release over time may have been associated with a reduction in spring tension as the matrix was extruded.

Three CRC malfunctions (out of 31 tested in the two experiments) occurred. No apparent cause of failure in the CRC fitted to the fistulated wether was evident, but the two CRC failures in the intact animals may have resulted from damage sustained during drenching. Ellis et al. (1981) experienced a 1/6 failure rate with an earlier capsule design. In the present trial CRC were found not to be readily administered by the flexible rubber tubing while the sheep sat in an upright position. Subsequently, it was found that CRC were more easily administered when the sheep was restrained in its normal standing position, particularly if a custom-made CRC drenching gun (Captee Ltd, Laverton, Australia) was used to release the capsule behind the tongue. Capsule failures can be readily identified by an irregular pattern or low recovery of chromium from the faeces.

Variation in the rate of chromium release from CRC within sheep was not significant, indicating that CRC in a similar rumen environment will have comparable performance characteristics. However, the results from experiment 2 show that where there are large differences in the physical characteristics of feed offered, reduced uniformity of CRC between feed types can be expected. Thus, if a herbage intake trial is being conducted with treatment groups on widely different feed types (e.g. hay vs clover), independent chromium release rates for dissimilar feed types should be estimated where possible. The decision whether to use one or more rates of chromium release should be taken with respect to the purpose for which intake data are to be used, bearing in mind that a lower level of precision in determining CRC plunger travel will occur in intact animals because direct measurement of CRC plunger travel is not possible (Ellis et al. 1988).

Laby et al. (1984) reported no differences in the rate of chromium release between CRC fitted to intact vs fistulated sheep. This implies that the restricted CRC rumen movement due to its attachment to the cannula, and differences in the rumen environment due to fistulation <u>per se</u>, have no effect on chromium release rate. No direct comparison of plunger travel was possible

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in the experiments reported here but, based on extrapolation from faecal chromium recoveries, average daily release rates of chromium appeared to have been similar in the two groups.

Chromium can be expected to appear in the faeces within 24 hours of CRC insertion in intact sheep but steady state levels of chromium will not be achieved until five to eight days later. The variability in chromium appearance probably arose because daily faecal output does not correspond uniformly to daily herbage intake (Pigden and Brisson 1956). The more rapid attainment of steady state conditions in intact sheep may have been associated with the different methods of insertion into the rumen (CRC administered orally being potentially subjected to more vigorous treatment) and the effects of periodic removal of CRC from fistulated animals for measurement of plunger travel. For practical purposes it would be prudent to wait until at least eight days after CRC administration before taking the first faecal samples for intake estimation. A more rapid attainment of a steady state of  $Cr_20_3$  in the faeces could be anticipated with feeds which have a lower rumen mean retention time (RMT) (Faichney 1983). Although the data for experiment 2 were restricted to 24-hour precision, the trend in intact sheep was for equilibrium to be achieved most rapidly in the sheep fed hay (low RMT) and least rapidly in the clover group (high RMT).

Chromium recoveries were generally within 90-110% of expected values for individual sheep within feeding levels, but some unacceptable levels of recovery occurred. These would result in inaccurate estimates of herbage intake. Re-assay of samples from these animals established that the assay was not at fault. Poor recoveries may therefore have been attributable to: (a) changes in the rate of chromium release between feeding levels (but this would be small as indicated by the linearity of plunger travel); (b) feeding level effects on the passage of chromium to the anus; or (c) faecal sampling errors (Moran et al. 1987). With respect to the effect of feeding level, Lambourne and Reardon (1963) suggested that a period of 2-4 days was necessary to allow chromium to reach a new steady state after a change in the level of feed intake. This transition effect would have contributed to the c.20% CV in chromium concentrations within feeding levels and may also have influenced the level of chromium recovery.

Diurnal variation in faecal chromium concentration was not significant, unlike that recorded for other methods of  $Cr_2O_3$  administration (Raymond and Minson 1955; Lambourne 1957a, b; Langlands et al. 1963). This low variation can be attributed to the continuous release of  $Cr_2O_3$ into the rumen (Pigden and Brisson 1956; Ellis et al. 1981). However, the variation observed in faecal chromium concentration (c. 8% CV) at both the two indoor feeding levels and under free grazing conditions is unlikely to be completely eliminated (Lambourne and Reardon 1963). It compares favourably with the 6.2% CV in faecal chromium concentration recorded by Ellis et al. (1981) in samples taken at two-hourly intervals, over a period of eight hours, from wethers fed lucerne pellets. This negligible within-day variation in marker chromium concentration means that flexible sampling routines can be adopted. That feature, combined with the elimination of daily dosing of animals, will substantially reduce both animal disturbance and labour requirements.

The problem of low marker chromium concentrations relative to environmental levels of chromium (experienced during the grazing diurnal variation study) is less likely to occur with the commercially available CRC which have a 50-60% higher daily release of chromium because of the 65% Cr<sub>2</sub>0<sub>3</sub> matrix and larger (9.00 mm) orifice (Laby et al. 1984).

These experiments have demonstrated that, in sheep, CRC plunger travel is consistent across a range of feeding levels and herbage types under indoor feeding conditions. In the next Chapter the effect of different patterns of pasture intake, representing alternative grazing management practices, on plunger travel is investigated.

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# **CHAPTER FOUR**

# EFFECT OF DIFFERENT GRAZING SYSTEMS ON THE RELEASE OF CHROMIC OXIDE FROM INTRARUMINAL CONTROLLED RELEASE CAPSULES IN SHEEP

#### INTRODUCTION

The release rate of Cr<sub>2</sub>O<sub>3</sub> from intraruminal controlled release capsules (CRC) in sheep is little affected by different herbage types and feeding levels under indoor feeding conditions (Laby et al. 1984; Chapter Three). However, the main application of CRC will be in animals that are grazing at pasture or range browsing. In these environments animals are physically more active and are exposed to a more variable climate than when housed indoors (Rattray 1986). Daily feed intakes may be 10 to 70% higher (Coop and Drew 1963; Joyce 1968) and also more variable between days than under indoor conditions. For example, in situations where rotational grazing is being practised, ewes may attain ad libitum intakes on the first day of a new pasture break but by the end of the planned grazing period intakes may be well below those required for maintenance (Sheath 1982). Such extremes in feed intake could affect the pattern of Cr2O3 excretion in the facees (Raymond and Minson 1955; Lambourne 1957b) and influence the rate of Cr<sub>2</sub>O<sub>3</sub> release through changes in the interface between the capsule orifice and rumen contents. The purpose of the experiment described in this Chapter was to evaluate the performance of chromium CRC in sheep subjected to a range of pasture conditions under different grazing management systems. The experiment also provided the opportunity to determine whether the rumen presence of CRC affected voluntary herbage intake.

#### MATERIALS AND METHODS

Experiment 3 Pastures

Three 0.25 ha plots of predominantly ryegrass - white clover pastures at Massey University's Sheep and Beef Cattle Research Unit were prepared over a 6 week period prior to the commencement of the experiment in May 1986. These were designed to reflect the range in pasture conditions under which CRC were likely to be used in New Zealand. The three pastures were classified by allowance and grazing management as:

- H = High allowance (>7.5 cm pasture height), continuously grazed.
- L = Low allowance (<3.0 cm pasture height), continuously grazed.
- R = On-off grazing a high daily allowance, with sheep restricted to 4 hours grazing per day on pastures exceeding 7.5 cm in height.

Pastures on the trial area were mowed to a height of 5.0 cm in late February and the surplus stem material was removed by a forage harvester. Plots were subsequently grazed by ewes to create the required sward conditions.

# Animals

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Twenty 8-month-old Romney rams were eartagged, fitted with faecal collection harnesses and allocated to one of four groups of 5 animals (balanced for liveweight) before their introduction to pastures adjacent to the experimental plots. Two days later (d 0) a single chromium CRC (65% chromium matrix, 6.1 cm core, 9.0 mm orifice, Mark 11 wing design) was administered to each sheep in three of the groups using a lubricated flexible rubber tube to release the CRC at the thoracic inlet.

Grazing of the experimental pastures commenced on d 3 when groups of rams were allocated to plots as follows; group I to L, group II to H and group III to R. Rams in the fourth (control) group (IV), with no CRC fitted, were grazed with group III. The groups grazed each pasture treatment for a period of 7 days, with total faecal collections by bag being made at 0900 h on days 5-7. On day 7, rectum grab simples were also taken at 0830, 1230 and 1630 h. Thereafter the groups were rotated around each of the plots over a period of 21 days in a 3x 3 Latin Square design. Rams were weighed at 0900 h (unfasted) at the commencement and end of each grazing period. Animals were drenched with Valbazen (MSD Agvet, Auckland) for internal parasite control as part of the normal three-weekly drenching programme on d 10. Faecal bags were fitted and adjusted over a 24 hour familiarisation period prior to the first three day faecal collection. The first recorded daily faecal output therefore commenced 7 days after administration of the CRC by which stage  $Cr_2O_3$  was expected to have reached a steady state concentration in the facces (Ellis et al. 1981). The total daily fresh weight of bagged facces was recorded for each sheep before duplicate samples of c. 100 g were taken for DM determination. These were oven dried at 80°C for 72 hours to a constant weight. A 20g subsample of the dried faeces was retained for chromium analysis.

At the end of the 21 day grazing experiment faecal collection harnesses were removed and the rams were grazed as a single group for 5 days on a high pasture allowance (pasture height > 5.0 cm) prior to their slaughter at a local meatworks. CRC were recovered from the 15 treated animals within 10 minutes of slaughter and plunger displacement since insertion was measured to 1.0 mm by dial callipers (Mitutoyo, Tokyo).

Companion measurements of CRC plunger travel were made twice-weekly on CRC from the same manufacturing batch fitted in 5 rumen-fistulated wethers. CRC were suspended into the rumen by nylon string attached to the cannula plug and were removed from the rumen for only

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1-2 minutes at each measurement date. The fistulated animals were grazed separately from the rams on a nearby sward which was 4.0-5.0 cm in height and of similar botanical composition to the main trial area.

# Pasture measurements

Pasture mass and sward height were measured on the first and last day of each 7 day grazing period. Herbage from six 0.25 m<sup>2</sup> quadrats, cut to ground level with an electric handpiece in each plot, was washed to remove soil contamination and oven dried at 80°C for 24 hours to determine pasture dry matter. Height measurements, taken on the same day as the herbage cuts, were based on 25 Ellinbank Pasture Meter Readings (EPM; Earle and McGowan 1979). The botanical composition of the sward was assessed at the commencement of the trial by segregating hand-plucked samples from each plot into grasses, clovers, weeds and dead material, and determining their relative DM contents. Hand-plucked herbage samples corresponding to material consumed by the rams were collected from each plot at the commencement of grazing periods for determination of <u>in vitro</u> digestibility by the method of Roughan and Holland (1977). These samples were oven dried at 80°C for 24 hours and ground through a 1.00 mm mesh prior to being were assayed in duplicate against standards consisting of six herbage samples of known <u>in vivo</u> digestibility collected from wether sheep fed indoors.

#### Chromium analysis

The chromium concentration in both the daily sub-sample of facees collected by bags and the 4hourly rectum grab samples on day 7 of each grazing period was determined using the method described in Chapter Two. Facees collected from group IV were used to make up standards to correct for environmental chromium. The intra-assay coefficient of variation (CV) was 1.2% and the inter-assay CV 3.9% The recovery of chromium from the facees was calculated as outlined in Chapter Three.

#### Statistical analysis

Analysis of variance was used to compare treatment effects on capsule rate of plunger travel in the capsules. Coefficients of variation and simple correlations (r) were calculated for withinday estimates of faecal chromium concentration and between alternative faecal sampling routines, respectively. Duncan's multiple range test was used to compare mean estimates of faecal output for different times of rectum grab sampling. Faecal outputs of group III and group IV rams were analysed by a split-plot model which included main effects for group, sheep within group, treatment and interaction terms (Gill 1988). The effect of liveweight was removed by covariance prior to testing group differences using sheep within groups as the error term.

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#### RESULTS

# Sward Conditions

Changes in sward characteristics and herbage digestibility during the trial are summarised in Table 4.1. Pasture height and mass declined over the trial on each of the plots. The amount of dead material in the L sward, as assessed visually, decreased during the trial. This probably explains both the decline in herbage dry matter content and the increase in <u>in vitro</u> OMD values. In addition the clover content of the H and R swards was substantially higher than that of the L treatment.

Table 4.1Sward conditions during experiment 3. Measurements made on days 3, 11 and 18correspond to the commencement of periods 1, 2 and 3 respectively. Samples collected on d 25correspond to the end of period 3.

					Day of	neasure	ment					
		d 3			d 11			d 18			d 25	
Parameter	L	н	R	L	н	R	L	н	R	L	н	R
Pasture mass											_	
(kg DM/ha) <sup>8</sup>	1034	3046	3164	557	2533	2558	575	1877	2477	412	1587	1822
Pasture height (cm) <sup>b</sup>	5.0	13.0	14.0	3.8	12.0	12.1	3.0	9.0	11.0	3.0	9.2	11.0
Dry matter content												
(%)	41.7	15.7	17.6	41.3	17.6	18.1	20.0	17.5	20.0	20.0	16.0	16.0
Pasture composition												
(% DH)												
- grasses	60.7	70.9	74.7									
- clovers	2.8	20.7	15.8									
- veeds	1.3	2.3	1.9									
- dead	35.1	6.1	7.7									
<u>In vitro</u> digestibility	(*)											
OND	58.39	72.12	70.69	63.38	68.37	70.37	66.97	65.66	69,14	67.95	65.30	68.19
DOMD	49.42	62.96	62.04	53.91	59.82	61.65	57.86	57.38	60.29	58.58	56.68	59.89
Ash	21.75	12.58	11.92	19.13	12.62	12.00	15.25	13.53	13.26	15.34	14.83	12.21

<sup>a</sup>Mean of 6 quadrats per plot.

<sup>b</sup>Mean of 25 EPM readings.

# Plunger Travel and Chromium Release Rate

All the CRC were recovered at slaughter and, except for one sheep in group 1 in which total plunger travel was only 8.00 mm, plunger displacement ranged between 17.5 and 23.3 mm (Table 4.2). Chromium release rates were on average higher (P<0.05) in group I animals which commenced on the L sward, but within-group CV in plunger travel was greatest (8.84%) in group II animals.

		Group	
	Ι	II	III
Treatment sequence	LRH	HLR	RHL
Initial Liveweight (kg)	39.1±0.3	$39.2 \pm 0.5$	38.9±0.1
Plunger travel (mm)	$22.3 \pm 0.3^{a}$	$19.9 \pm 0.8$	$20.0\pm0.7$
Average travel (mm/d)	$0.78 \pm 0.01$	$0.70 \pm 0.03$	$0.70 \pm 0.02$
Chromium release (mg Cr/d)	111(3.16) <sup>b</sup>	99(8.84) <sup>c</sup>	99(7.50) <sup>c</sup>

Table 4.2 Sheep liveweights, CRC plunger travel and average daily chromium release rates, experiment 3. Figures in brackets are CV (%).

<sup>a</sup>One outlying CRC excluded

<sup>b,c</sup>Mcans with different superscripts are significantly different at P<0.05.

CRC plunger travel in the fistulated wethers (c. 65 kg liveweight) over the same 29 d period was 11% slower ( $0.65 \pm 0.03 \text{ vs} 0.72 \pm 0.02 \text{ mm/d}$ , P<0.05) than in the intact ram hoggets. Variability in plunger travel was slightly greater (CV = 9.4%) in the rumen-fistulated wethers.

# Recovery of Faecal Chromium

Chromium recovery, based on the average rate of CRC chromium release up to the time of slaughter, was significantly (P < 0.001) influenced by grazing treatment, being lowest when the rams were continuously grazed at the low herbage allowance and highest when the H treatment was grazed (Table 4.3).

Table 4.3Actual faecal output (FO), faecal ash content, estimated OMI and recovery of<br/>chromium (mean ± sem) for different grazing treatments, experiment 3.

	Grazing	FO	Ash	OMI <sup>b</sup>	Intake <sup>C</sup>	Chromium
Group	trcatment	(gDM/d)	(%)	(g/d)	(% Maint)	recovery (%)
Ia	L (P1)	205 ± 14	37	320±16	0.25	87±3
	R (P2)	151 ± 15	21	$392 \pm 40$	0.46	95±6
	II (P3)	293 ± 15	24	$639 \pm 21$	0.71	113±5
II	L (P2)	136 ± 25	33	284 ± 39	0.29	53±4
	R (P3)	149±15	25	$338 \pm 51$	0.38	89±3
	H (P1)	305 ± 10	16	$859 \pm 28$	1.02	113±4
III	L (P3)	134 ± 15	52	376±31	0.33	60±8
	R (P1)	215 ± 52	21	$365 \pm 42$	0.43	96±5
	H (P2)	289±11	19	717 ± 17	0.80	121±9
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<sup>a</sup>Five animals per group, except group I where data for one ram lamb with a faulty CRC were excluded.

<sup>b</sup>Estimated from mean actual faecal output per period and herbage <u>in vitro</u> digestibility values for each treatment period.

<sup>c</sup>Energy intake (DMI x DOMD x 16.3) expressed as a proportion of recommended maintenance energy intake of 9.5 MJ ME/d for a 35-40 kg hogget (Rattray 1986).

Group mean OMI, estimated from the actual faecal outputs and hand-plucked herbage in vitro digestibility values for treatment periods, were much lower than originally planned for the trial - particularly on the L and H treatments. Only group II rams on the H sward apparently consumed sufficient pasture to maintain liveweight. However, this result is not compatible with the liveweight records which indicated that liveweights at the end of the 21 d grazing trial were similar to the weights shown in Table 4.2. Organic matter intake was similar for different groups on the same treatment, except for the L sward during period 3 when very wet ground conditions were experienced. The effect of these conditions is evident in the 52% faecal ash content of group III hoggets during period 3. High levels of soil contamination clevated faecal DM output on the L sward relative to the R treatment despite higher feed intakes occuring on the latter.

A comparison of the ash content of the hand-plucked herbage samples (Table 4.1) and actual faecal ash content (Table 4.3) suggests that visual selection of herbage thought to have been consumed by the rams was different to actual intake. The lower ash content of the <u>in vitro</u> digestibility samples implies that the quality of feed consumed may have been overestimated. If this was the case the feed intakes shown in Table 4.3 would be underestimated.

# Effect of CRC on Herbage Intake

Actual faccal outputs in the control hoggets (group IV) were consistently higher (P < 0.01) than those of their grazing companions fitted with CRC (group III) on each of the grazing treatments (Table 4.4). The largest difference of 40% occurred during the final grazing period on the L sward. Faccal outputs for both groups differed significantly (P < 0.001) between grazing treatments. Mean liveweights of the groups at the beginning of each period, fitted as a covariate in the split-plot model, did not differ significantly between the two groups.

Tablc 4.4	Faccal outputs (g/DM; mean±sem) of ram hoggets (n=5/group) with (+CRC)
or without (-	CRC) intraruminal chromium CRC, experiment 3.

Grazing	Faccal o	utput	
treatment	+ CRC	- CRC	Difference (%) <sup>a</sup>
R (P1)	141±17	179± 7	27
H (P2)	290±12	348± 8	20
L (P3)	$215 \pm 52$	$302 \pm 48$	<u>40</u>
Mean	215±24	246±25	28

<sup>a</sup>Treatment differences (P < 0.001) and differences between groups on each treatment (P < 0.01) were significant.

# Within-Day Variation in Faecal Chromium Concentration

Faecal outputs derived from the concentration of Cr in rectum grab samples collected at 0830, 1200 and 1630 h on the final day of each grazing period are shown in Table 4.5. These values are compared with the faecal outputs estimated from a sub-sample of faeces taken from the 24 h total collection and actual faecal outputs on the day of sampling. Actual faecal outputs were generally poorly predicted because of the inconsistent recovery of chromium at the different feeding levels (Table 4.3). The mean within-day CV in chromium concentration in the grab samples obtained at 0830, 1200 and 1630 h was 8.5%. No circadium rhythm in chromium concentration was evident. Variation tended to be higher when the rams were grazing on the L sward. It was also more difficult to obtain a rectal sample from animals while on this treatment and, in 33% of the cases, only one or two of the planned three samples per animal were collected. The irregular pattern of intake on the R treatment, relative to 24 h continuous grazing, was not reflected in increased diurnal variation in chromium concentration. The overall correlation between the mean grab sample and the 24 h estimates of faecal output was 0.95. This value includes between-assay error because 24 h samples were assayed separately.

Table 4.5 Faecal output (g DM/d; mean $\pm$ sem) estimated from the concentration of chromium in rectum grab samples at 0830,1200 and 1630 h and a sub-sample of faeces (24 h) taken by the total collection of faeces (actual) on the final day of each grazing period, experiment 3.

					Faccaloutp	ut	_
Sampling tir	πc		0830 h	1230 h	1630 h	24 h	Actual
Group 1	Period	1(I.)	245 ± 29	189±5 <sup>a</sup>	168 ± 7 <sup>a</sup>	222 ± 17	174 ± 34
(n = 4)		2(R)	119±6	127±6	119±6	b	126±9
		3(H)	228 ± 11	307 ± 54	213±7	276±16	302±14
Group II	Period	1(11)	271 ± 10	267 ± 7	$273 \pm 18$	257±12	272 ± 33
(n=5)		2(L)	$182 \pm 9^{a}$	216±12	$219 \pm 15^{a}$	_b	122±11
		3(R)	$154 \pm 11^{a}$	116 ± 12	159±10	156 ± 11	176±12
Group III	Period	1(R)	121±11	115 ± 10	114±12	123 ± 11	103±24
(n=5)		2(L)	$266 \pm 35$	272 ± 29	265 ± 28	265±42	313±20
		3(H)	352±32	357 ± 35	$399 \pm 11^{ac}$	298 ± 22	$204 \pm 48$

<sup>a</sup>Samples were not collected from all animals at these times.

<sup>b</sup>Samples were not assayed.

<sup>c</sup>The difference between 1630 h and 24 h mean estimates was significant at the 5% level. All other differences between 0830, 1200, 1630 and 24 h estimates were non-significant.

The reliability of a single daily rectum grab sample (0830 h), collected over a three day period, for estimating average daily faecal chromium concentration was also assessed. The mean threeday 0830 h reading (x) was highly correlated (r=0.97) with, and not significantly different from, the corresponding 24-h (y) chromium determinations (y = -0.33 + 0.95x). The correlation between the chromium concentration for individual animals on a single day and the three-day mean value ranged from 0.87 to 0.96. Estimates of faecal chromium levels are therefore more reliable if grab sampling is maintained over several days.

# DISCUSSION

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This trial, the first with chromium CRC at Massey University under pastoral grazing conditions, has identified several aspects of capsule performance which require further investigation. The most serious concern is the apparent depressing effect of CRC presence on herbage intake. Although there were only five animals in each group and the variation in faceal output between animals was high on the L treatment, the consistently higher outputs in the control rams on each of the treatments suggest that this difference is real. Depression of faceal output could have occurred either because of physical discomfort to the animal caused by the CRC or because some component of the chromium carrier matrix modified rumen activity. These factors were investigated in trials described in the next Chapter.

A second aspect of concern is that recovery of faecal chromium was related to herbage allowance. Validation of the chromium assay (Chapter Two) has shown that differences in faecal ash content are unlikely to have contributed to this difference. The remaining possible causes are therefore non-uniformity of plunger displacement or changes in the rate of rumen turnover and hence rate of passage of faeces and chromium to the anus (Kameoka et al. 1955; Lambourne 1957; Moran et al. 1987). Both of these could result from marked changes in feed intake. In this respect the trial design of seven day treatments and a 4 day adjustment period before collection of faeces at the new feeding level probably provided insufficient time for chromium to adjust to a new steady state in the faeces, especially when the animals were shifted to the L sward (Corbett et al. 1960). This suggests that if different feeding levels are to be used within the life of a CRC, an adequate adjustment period, corresponding to the magnitude of change in feed intake, should be allowed for before faecal sampling at each new feeding level. Lambourne and Reardon (1963) suggested 2-4 days was necessary to allow Cr to reach a new steady state for twice daily drenching with chromic oxide

The very low feed intakes for the L sward (0.19-0.25 M), in particular, may have modified the rumen environment (Faichney 1980) and reduced the rate of matrix extrusion. It is notable that average plunger travel was faster and less variable in group I animals which were exposed to less nutritional stress while on the L sward. The pattern of chromium recovery relative to the pasture mass grazed and the 11% lower rate of plunger displacement in the rumen-fistulated

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animals also support the view that rumen conditions could modify CRC performance.

The presence of harnesses and faecal collection bags, as well as the CRC, is likely to have contributed to the lower than planned OMI (Hutchinson 1959). Although the seven day adjustment period for harnesses was probably adequate, the 24-hour familiarisation period for bags prior to collection may have been insufficient. It may also have been more effective to have had the animals fitted with collection bags throughout the three grazing periods, rather than using the "three days off - four days on" sequence. Emptying bags once- rather than twice-daily may have contributed to the depressed intakes, particularly on the high-allowance set - stock treatment. No estimate of the loss of faecal material from collection bags could be made, although visual checks of the swards indicated that this was minor. These results therefore support the assertions of previous researchers (Meijs 1981) that harnesses and faecal collection bags are not a satisfactory method for estimating herbage intakes with the possible exception of studies which are of a short duration.

Despite the possibility that CRC performance may have been compromised by the low levels of feed intake, within-day variation in faecal chromium concentration was low (CV = 8.5%) and without an apparent circadian rhythm. This compares favourably with results reported by Ellis et al. (1981) for chromium CRC and is similar to the levels of diurnal variation achieved when  $Cr_2O_3$ -impregnated paper is drenched twice daily (Langlands et al. 1963a). This low level of diurnal variation facilitates a flexible once-daily faccal sampling routine for the estimation of faecal chromium concentration.

Initially it was thought that CRC chromium release rates for intake studies could be taken directly from the measurements obtained from CRC in fistulated animals. Although the rumenfistulated wethers in this experiment did not graze the same swards as, and were both older and heavier than, the rams lambs the 11% difference in rate of plunger travel between the rumenfistulated and intact animals is sufficiently large to question the reliability of this method of estimating CRC chromium output. The slower and more variable rate of chromium release in the fistulated wethers could be explained by the combined effects of removing the CRC from the rumen environment for measurement and the different gaseous conditions of a fistulated rumen (Laby 1986).

In conclusion this trial has identified several possible deficiencies of CRC under pastoral grazing. However, the experimental design is likely to have either caused or exaggerated some of the responses observed. Further trials to quantify the effects of CRC on feed intake (Chapter Five) and of feed intake on chromium release rates (Chapters Six, Seven and Eight) were therefore carried out.

# **CHAPTER FIVE**

# EFFECT OF ADMINISTRATION AND RUMEN PRESENCE OF CHROMIUM CONTROLLED RELEASE CAPSULES (CRC) ON HERBAGE INTAKE

# INTRODUCTION

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Estimates of the mean intake of a flock of sheep in a pastoral grazing situation could be obtained indirectly by taking a sequence of faecal grab samples from a representative sub-group of the flock fitted with chromium CRC (Ellis et al. 1981; Laby et al. 1984). An assumption implicit in this approach is that the capsule-treated animals continue to behave in an identical manner, with respect to intakes, to the remainder of the flock. However, results from experiment 3 (see Chapter Four), where the mean faecal dry matter output of 8-month ram lambs dosed with chromium CRC was significantly lower than that of a comparable group without CRC, suggests that the rumen presence of CRC may depress intake. Further evidence that CRC may modify sheep performance was found in a Northland trial where lower growth rates were recorded in CRC-treated lambs than in untreated animals (20-35 kg liveweight) in the initial period after capsule administration (Venning 1986). In a more serious case a proportion of lambs (<25 kg liveweight) in a Wairarapa anthelmintic trial died as a result of CRC damage to the reticulum (Anon. 1986).

Depression in voluntary intake could occur as a result of physical injury to the throat and oesophagus during CRC administration, from CRC irritation to the rumen and reticulum wall linings, or because a component of the CRC matrix acts to modify rumen digestion. Some of these responses may be dependent on the liveweight of the treated animal. The effect of CRC on feed intake, feed digestibility and faecal output in sheep of a range of liveweights was therefore investigated in two experiments described in this Chapter. The first experiment was conducted indoors using a relatively homogeneous feed, while the second was carried out under pastoral grazing conditions. The indoor experiment also provided the opportunity to assess the accuracy with which voluntary herbage intake could be predicted from the concentration of marker chromium in the faeces because both feed intake and faecal output were recorded. A comparison of the effect of alternative methods of preparing faecal samples for assay on the recovery of chromium is also reported.

# MATERIALS AND METHODS Experiment 4

Twenty eight-month-old Romney rams, with 24-hour fasted liveweights ranging from 26-47 kg  $(37.7\pm1.6 \text{ kg}; \text{mean}\pm\text{sem})$  were introduced to indoor feeding conditions in metabolism crates. Lucerne chaff was offered <u>ad libitum</u> for a preliminary adjustment period of 7 days until consistent daily intakes were achieved. Feeding, and weighing of refusals and facees, occurred once-daily at approximately 0900 h.

After the adjustment period the rams were reweighed and divided into two groups of 10, each with a similar mean and range of liveweight. Daily feed intakes and faecal outputs for <u>ad libitum</u> allowances (10-15% of initial allowance remaining as refusals) were then recorded for a period of 8 days (period 1, d 0 to d 8). One chromium capsule (65% Cr<sub>2</sub>0<sub>3</sub>, 3.0 cm pressed tablet matrix, 9.00 mm orifice, Mark II design) was administered to each animal in one group of 10 rams on day 9, and feed intakes and faecal outputs for both groups were measured for a further 10 days (period 2, d 9 to d 18).

Four rams covering a range of liveweights (29.5-49.5 kg) from the capsule-treated group were slaughtered at the end of period 2. The ocsophagus and rumen wall linings were inspected for evidence of physical damage and the capsules recovered to measure the magnitude of plunger travel since insertion. Duplicate samples (c.100 g) of the feed offered were oven dried at  $80^{\circ}$  for a 24 h period to determine the feed dry matter content each day. The dry matter content of feed refusals was determined from a single sample (c.100 g) collected from each sheep daily and dried in the same manner as described above. Daily feed intakes (DM1, g/day) were calculated as the difference between DM offered and DM refused.

The total fresh weight of facces was recorded each day. Two sub-samples (c.100 g) were taken after thorough mixing and oven dried at 80°C for a period of 72 h to determine DM content. Faccal DM output per day was calculated as the product of the wet weight of the faeces and their DM content. The digestibility of the feed DM was then determined from the equation:

Digestibility (%) = Intake (g/d) - Faecal output (g/d) Intake (g/d) Three methods of preparing faeces for estimating mean chromium concentration (mg/g DM) over a 5-day period (d 14-18) for individual animals were compared. These were:

- Daily-M Duplicate intact faecal samples (1 g DM/d) were analysed for each day of facces collection. The predicted daily faecal outputs were then summed across days to derive a mean 5-day faecal output. The daily predicted faecal outputs for individual animals were also used to estimate intakes within days.
- Bulk-G Dried faccal samples (2 g DM/d) were bulked over the 5-day period and ground through a 1.00 mm sieve (Cranston, England). After thorough mixing, duplicate samples (1.0 g) were assayed for chromium content and a mean faecal output was derived.
- Bulk-I Dried facees (1 g DM/d) were bulked across the 5-days and ashed intact. The entire ash residue for the 5.0 g faceal sample was analysed for chromium and an average chromium concentration was derived for each animal. The same procedure was used to form a composite sample for a 3day period (days 16, 17 and 18) to test the effect of a reduced sampling period.

The recovery of chromium (calculated as described in Chapter Three) was expressed as a percentage of the average daily release rate of chromium from the capsules recovered by slaughter. Daily faceal outputs were predicted from faceal chromium concentrations and compared with actual outputs for the last 5 days of the treatment period. Predicted daily intakes were derived by dividing faecal DM output by the average DM digestibility of the lucerne chaff over the same 5 day period.

# Experiment 5

Experiment 5 was conducted as a result of the findings in experiment 4. Thirty-two rams (12 months old) with fasted (24 h) liveweights ranging from 26 to 46 kg ( $39.5\pm1.1$  kg) were shorn, drenched with Nilverm (Coopers, Wellington) and fitted with faecal collection bag harnesses on August 14, 1987. They were then introduced to <u>ad libitum</u> feeding conditions on predominantly ryegrass-white clover pastures on the trial grazing area for a period of 8 days to allow rumen adaptation to the feeding conditions and to familiarise the animals with the harness equipment. Faecal collection bags with plastic bag liners were fitted for the last 2 days of this period.

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75 0900 h at

The rams were weighed off pasture at 0900 h at the commencement of the pre-treatment collection period (period 1) and approximately paired for liveweight to form two groups of 16 animals. Total faecal collections then continued once daily at 0900 h for 8 d. At the end of this period the sheep were reweighed off pasture (0900 h) and a single QS chrome CRC was administered to each animal of one group (capsule-treated, CRC+) to commence the treatment period of 20 d (period 2). Paired animals not treated with CRC formed the control group (CRC-).

The capsules contained 12 mm (2 tablets) of 65%  $Cr_2O_3$  matrix and 18 mm (3 pellets) of sucrose, but had the same specific gravity as the commercially produced 30 mm core 65%  $Cr_2O_3$  capsule. The use of this custom-made capsule meant that any effect of the  $Cr_2O_3$  matrix per sc on animal performance could be monitored from about day 12 of period 2, when the  $Cr_2O_3$  - containing tablets would have expired.

Twice-daily collections of facees (c. 0900 and 1600 h) commenced on d 3 of period 2 when harness sores became apparent on some animals. These were treated with zinc cream and, with additional foam rubber padding fitted under the harnesses, cleared up within 2 to 3 days. Periodic treatment of animals for this condition was required for the remainder of the trial but had no apparent effect on daily faecal output.

The rams were also weighed at 0900 h off pasture on d 8, 15 and 20 of period 2. Pasture height on the grazing area was measured at 2-4 d intervals using an Ellenbank Pasture Meter (50 readings/paddock; Earle and McGowan 1979). The pastures grazed were maintained within the height range 5.4 to 12.1 cm (1100 to 2400 kg DM/ha), to ensure that the rams could maintain maximum intakes (During et al. 1980).

Five animals were slaughtered, two on d 12 of period 2 following a sharp decline in faecal output and liveweight, and three at the completion of the trial. The two sheep initially slaughtered, and one in the latter group, were found to have physical injury to the oesophagus because of a faulty CRC application procedure, but no capsule damage to the reticulum or the rumen was found in any of the slaughtered sheep. However, the initial slaughter results prompted the running of a second trial to investigate whether the method of capsule administration affected faecal output. The control group (n = 16) was therefore paired for liveweight at the end of period 2 to form two groups of 8 animals. One group (D +) was dosed in the normal manner, but without releasing the capsule into the rumen. This mock insertion involved placing the capsule down the oesophagus to the thoracic inlet, while the animal was in a standing position. The rubber tubing was lubricated prior to each administration. The second group (D -) was untreated. Total faecal outputs of the D+ and D- groups were measured for a further period of 7 d. The animals were weighed at 0900 h at the commencement and end of this period.

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Fresh facces (bulked across twice-daily collections) were thoroughly mixed, weighed and then sub-sampled (c. 100 g) in duplicate. The sub-samples were oven dried at 80°C for a period of 72 hours to measure faceal dry matter (DM) content. Faecal DM output per day was calculated as described in experiment 4.

# Statistical Analysis

Data arising from daily measurements of feed intake, faecal output and digestibility in period 2 of experiment 4 were subjected to repeated measures analysis to test differences between treatment groups and the interaction between group and day of sampling. Mean intakes or outputs from period 1 were fitted as covariates. Heterogeneity of intra-group regressions of intake or output in period 2, on the corresponding variables in period 1, was tested according to the procedure of Searle (1971). Statistical analyses were conducted using the 'REG' statistical package (Gilmour 1985). Data from two sheep, which exhibited severe scouring during period 1, were eliminated as outliers according to the procedure of Snedecor and Cochran (1967). Removal of these individuals had the effect of making the test of group differences more conservative. Data from the chromium analyses were subjected to regression analysis (SPSSX 1983). Outlier values were identified by their Mahalanobis and Cook's distance values (Norusis 1985).

Daily faecal outputs in period 2 of experiment 5 were subjected to repeated measures analysis as described for experiment 4. Three sheep from the capsule-treated group with damage to the oesophagus were eliminated as outliers according to the procedure of Snedecor and Cochran (1967). One sheep, introduced as a replacement for a death in the CRC+ group, was excluded from the repeated measures analysis because of missing observations during the first 5 days of the pre-treatment period (Cole and Grizzle 1966). The repeated measures analysis was therefore performed with 16 and 12 animals in the CRC- and CRC+ groups respectively.

The same statistical analysis was applied to the "method of capsule application" trial (i.e. D + vs D-), but with faccal outputs from the last 5 days of period 2 used as a pre-treatment covariate. Liveweights on d 15 of period 2 were also fitted as a covariate.

#### RESULTS

# Experiment 4 Faccal output and feed intake

Mean intakes, digestibilities and faecal outputs for the 8- and 10-d treatment periods are shown in Table 5.1. Relative to mean feed intakes in period 1, the intakes of the sheep in the contro group increased more than in the capsule-treated group during period 2 (101 vs 35 g/day). Thi difference was significant (P < 0.10) after correcting for period 1 mean intakes and liveweight Sheep with high intakes in period 1 retained their high intakes in period 2 irrespective of group However, the mean intakes of animals in the capsule-treated group were 71 g lower in period 2 (P < 0.10) than those in the control group. Treatment with capsules therefore reduced intakes ir period 2 by an absolute amount across the intake (liveweight) range, rather than having ar effect proportional to period 1 intake (i.e. proportional to liveweight).

Table 5.1Effect of rumen presence of chromium controlled release capsules on liveweight,feed intake, feed digestibility and faceal output in capsule-treated and control groups,experiment 4.

	Control (CRC-)	Capsule (CRC+)	Sign.
Number of animals <sup>a</sup>	9	9	
Liveweight (kg)	$34.8 \pm 2.2$	38.3 ± 2.7	
Feed intake (g/d)			
- Period 1	1188 ± 53	1183 ± 95	NS
- Period 2	1289 ± 59	1218 ± 95	+
aecal output (g/d)			
- Period 1	518 ± 25	511 ± 43	NS
- Period 2	532 ± 26	503 ± 41	*
igestibility (%)			
- Period 1	$56.4 \pm 0.5$	56.4 ± 0.5	NS
- Period 2	$58.5 \pm 0.4$	$58.3 \pm 0.4$	NS

<sup>a</sup>One outlier from each group excluded, see Materials and Methods.

Daily DM intakes for each group during the trial (Figure 5.1) indicate that the mean intakes of the groups followed a similar trend but diverged with time during period 2. However, the group x day interaction was non-significant.

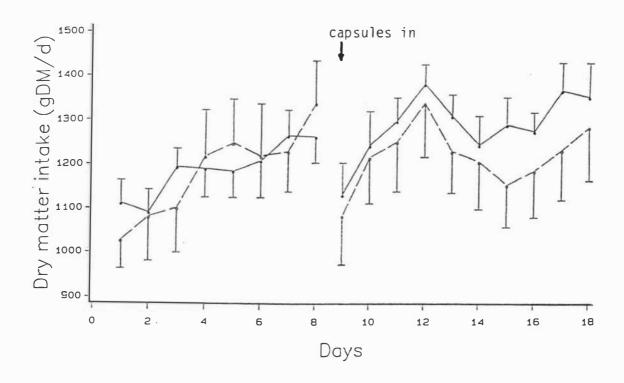


Figure 5.1 Mean daily voluntary intakes of control (----) and capsule-treated (----) groups, experiment 4. Bars indicate sem.

The mean digestibility of the feed increased significantly (P < 0.05) from 56% to 58% in both groups between period 1 and period 2, reflecting a change in feed quality. Digestibility was not significantly different between the two groups after capsule administration (P > 0.10).

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Mean faecal DM outputs during period 2 increased by 14 g/d in the control group but decreased by 8 g/d in the capsule group, reflecting both the smaller increase in feed intakes of capsuletreated animals and the higher digestibility of feed in period 2. Faecal DM output in period 2 corrected for liveweight and period 1 output, was significantly (P<0.05) lower in capsuletreated than in control sheep. The group x day interaction was non-significant indicating that capsule effects were similar on each day of period 2.

#### Appearance of chromium in the faeces

Approximately steady-state levels of chromium in the faeces were not achieved until 6 days after CRC insertion (Figure 5.2). Added chromium was evident in the faeces 24 h after CRC insertion and increased in a non-uniform manner between sheep up to more than 80% of the expected concentration in the faeces over the next 5 days.

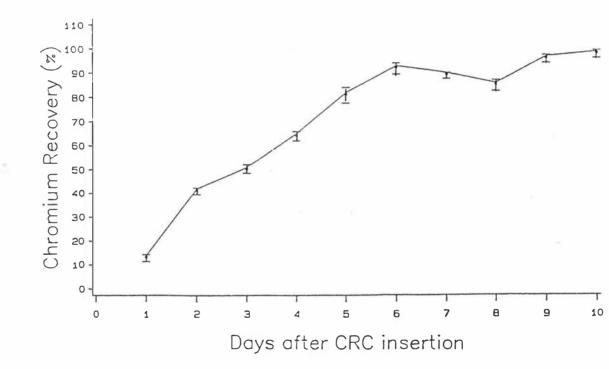


Figure 5.2 Pattern of recovery of chromium (Cr) in the faeces after insertion of CRC in yearling sheep, experiment 4. Bars indicate sem.

### Chromium release rates

Plunger travel in capsules recovered from the slaughtered animals averaged  $10.3 \pm 0.3$  mm. This represents a release rate of 146 mg Cr/day. Plunger travel was slowest for a sheep in which the CRC was found lodged in the reticulum. The reduced rate of matrix extrusion in this. animal may have been due to the orifice being blocked by tissue. The CV between sheep of Cr release was 5.96%.

#### Recovery of chromium

Chromium recoveries for individual animals (Daily-M) from days 6 to 10 ranged from 85.8 to 99.4% (overall 5 day mean  $\pm$  sem, 93.1  $\pm$  1.3%). Low recoveries of faecal chromium in two animals (65.9% and 72.5%) depressed the group mean recovery on day 8 (Figure 5.2). Repeating the assay for these animals did not improve the recovery of chromium, indicating that the faeces sample was not representative of daily output, that chromium excretion varied between days or that chromium had not fully reached steady state levels in the faeces of these sheep.

Chromium recoveries for the bulked ground and intact samples averaged 97.2  $\pm$  2.6% and 101.3  $\pm$  2.2% respectively for the 5 day period (Table 5.2). None of the Bulk-1 samples required reassay, but assays of 15% of the Bulk-G, and 20% of the Daily-M samples, were repeated to recheck recoveries or because of a high CV between duplicates. In this respect, indoor trials with chromium provide an advantage because samples with unexpectedly low or high chromium concentrations can be checked against actual output and rejected or retained accordingly. This recourse is normally not available in field trials unless some animals are fitted with faecal collection bags (Langlands 1975).

Parameter	Slaughtered sheep (n = 4)	Other sheep (n = 6)	All Shecp (n = 10)
Actual rate	$146.0 \pm 2.3$	146.0 <sup>a</sup>	146 <sup>a</sup>
Predicted rate			
Daily-M	137.5 ± 5.4	$135.4 \pm 2.3$	$137 \pm 2.6$
-	$(93.8 \pm 1.1)$	$(92.8 \pm 1.6)$	$(93.1 \pm 1.3)$
Bulk-G	$133.0 \pm 0.3$	$147.7 \pm 5.8$	141.8 ± 3.6
	$(91.5\pm2.9)$	$(101.2 \pm 3.1)$	(97.2±2.6)
Bulk-I	$144.9 \pm 7.9$	$150.6 \pm 5.6$	$148.3 \pm 4.0$
	$(98.8 \pm 2.8)$	$(103.0\pm 3.2)$	$(101.3\pm2.2)$

Table 5.2 Actual and predicted rates of CRC chromium excretion (mg Cr/d), and recoveries of chromium for alternative faecal sampling methods in slaughtered and other sheep, experiment 4. Figures in brackets are the percentage ratio of predicted to actual values.

<sup>a</sup>Assumed to be the average release rate measured in CRC recovered by slaughter.

The use of actual chromium release rates improved the overall mean chromium recovery by only 0.8% (P>0.10) in the three animals in which plunger travel differed from the mean, indicating a normal distribution of plunger travel variation. This suggests that average chromium release rates will provide satisfactory mean estimates of chromium recoveries across several days, providing the between-day CV of plunger travel is relatively low, as in this trial. Estimates for individual animals within days may, however, be misleading.

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# Prediction of faccal output and feed intake

Predicted faecal outputs and feed intakes, derived from the chromium concentrations in faeces prepared by the three sampling methods (and assuming 100% recovery of chromium), are shown with measured faecal outputs in Table 5.3. The ratios of group mean predicted: actual intakes were 107%, 103% and 98% respectively for the Daily-M, Bulk-G and Bulk-I samples.

The respective regression equations for predicted (y) versus actual (x) faceal output (g DM/d) for the three sampling regimes are summarised below:

Daily-M:	$y = 6.2 + 0.92 (\pm 0.05) x$	r = 0.99 ***
Bulk-G:	$y = 24.98 + 1.02 (\pm 0.15) x$	r = 0.92 ***
Bulk-I:	y = 41.53 + 1.11 (+ 0.11) x	r = 0.96 ***

The corresponding relationships between estimated and actual (y, g DM/d) feed intake, using the predicted faecal output values and an average digestibility of 58.8% were:

<sup>y</sup> intake = 67.37 + 0.88 (± 0.08) Daily-M	r = 0.97 ***
<sup>y</sup> intake = 5.37 + 0.97 (± 0.17) Bulk-G	r = 0.90 ***
<sup>y</sup> intake = -38.22 + 1.06 (± 0.14) Bulk-I	r = 0.93 ***

Using individual animal 5-day mean digestibilities rather than the overall group mean value explained a further 2.6 to 4.3% of the variation in predicted intakes.

**Table 5.3** Predicted and actual faecal dry matter output (g DM/d) and feed intakes (g DM/d) of yearling sheep for alternative faecal sampling methods over a 5-day collection period, experiment 4. Figures in brackets are the ratio of predicted to actual values expressed as a percentage.

Parameter	Slaughtered sheep (n = 4)	Other sheep (n=6)	All sheep (n = 10)
Actual faccal output	424 ± 50	532 ± 46	489 ± 37
Predicted faccal output			
Daily-M	457 ± 50	574 ± 52	527 ± 40
	$(108\pm2)$	$(108\pm 2)$	$(108 \pm 1)$
Bulk-G	$470 \pm 61$	$526 \pm 40$	$503 \pm 33$
	$(110\pm 4)$	$(99 \pm 3)$	$(103 \pm 3)$
Bulk-I	425 ± 41	517 ± 42	480 ± 32
	(101± 3)	(98 ± 3)	(99 ± 2)
Actual feed intake	$1039 \pm 113$	1299 ± 113	1195 ± 88
Predicted feed intake <sup>a</sup>	`		
Daily-M	1110 ± 77	$1395 \pm 125$	1281 ± 97
	$(107\pm2)$	$(108 \pm 3)$	$(107\pm 2)$
Bulk-G	$1140 \pm 148$	1277 ± 97	$1221 \pm 81$
	$(109 \pm 3)$	$(99 \pm 4)$	$(103 \pm 3)$
Bulk-I	$1031 \pm 100$	$1255 \pm 101$	$1165 \pm 78$
	(100±4)	(98±4)	(98±3)

<sup>a</sup>Assuming a feed DMD of 58.8% across all sheep.

Bulking intact facces over a 3-day period provided estimates of faccal output and feed intake of similar precision to the 5-day samples. Group mean faccal output was estimated to be 491 g/d for the 3 days (actual, 482 g/d), while feed intake, using an average feed digestibility of 58.8%, was predicted from these values to be 1192 g/d (actual, 1172 g/d). The correlations (r) between predicted and actual values for faccal output and feed intake were 0.97 and 0.89, respectively.

Mean daily feed intakes could be predicted from Daily-M samples to within c.10% of the actual values under the controlled conditions of this experiment (Table 5.4), if the actual recovery of 93% was applied.

Faccal output (g/d		ut (g/d)	Digestibility (%)		Feed intake (g/d)	
Day	Predicted	Actual	Predicted	Actual	Predicted	Actual
6	501	495	58.8	58.1	1215	1185
7	481	462	58.8	58.9	1167	1127
8	534	490	58.8	58.1	1296	1177
9	476	492	58.8	58.9	1155	1214
10	475	503	58.8	59.9	1153	1271
Mean	493	489	58.8	58.8	1197	1195
scm	18	17	0.0	0.1	43	41

Table 5.4 Daily group mean predicted and actual faecal dry matter outputs, feed digestibility and dry matter intakes, experiment 4.

Prediction of faccal outputs and feed intakes for individual animals within days was less accurate (r = 0.94 and 0.81 respectively for 50 cases). Removal of three outlier values improved the prediction of faccal output by a further 2%. However, if actual digestibility values for individual animals within days were used the correlation between predicted and actual daily DM intakes improved to 0.94. This clearly demonstrates that the major problem in predicting daily intakes is variation in feed digestibility between days. The CV between animals for digestibility of 8.83% reflects the preference shown by some animals for the more digestible leaf component of the lucerne chaff and to some extent the overlap of facces exerction between 24 hour collection periods. The latter situation means that daily faccal output may represent the feed intake of more or less than one day depending on the level of feed intake and its rate of passage through the digestive tract.

# Experiment 5

# Effect of CRC on faccal output

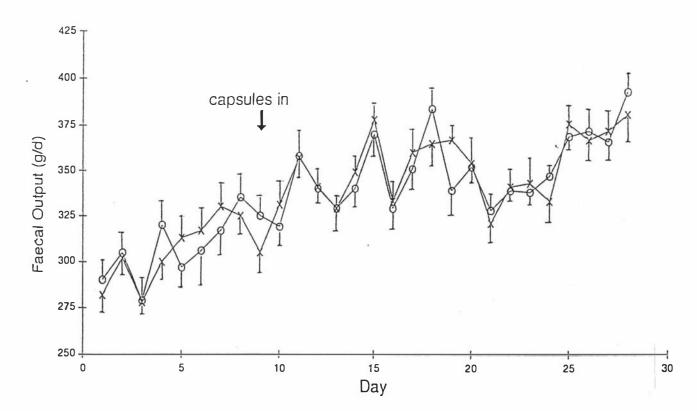
Mean faccal outputs of the CRC+ and CRC- animals during the pre-treatment and treatment periods of 8 and 20 d respectively are shown in Table 5.5

Table 5.5	Effect of rumen presence of controlled release capsules on faecal output of control
and capsule	e-treated hoggets during pre-treatment and treatment periods, experiment 5.

	Control (CRC-)	Capsulc-treated (CRC+)
Number per group	16	12 <sup>a</sup>
Liveweight (kg)		
- pre-treatment (d 0)	$38.9 \pm 1.6$	$39.0 \pm 1.7^{b}$
- post-treatment (d 20)	$45.8 \pm 1.5$	$45.3 \pm 1.3$
Faecal output (g DM/d)		
- pre-treatment (period 1)	$305 \pm 10.6$	306 ± 8.2
- treatment (period 2)	$349 \pm 7.1$	$350 \pm 6.9$

<sup>a</sup>Three outliers, and one sheep with incomplete records are excluded, see Materials and Methods. <sup>b</sup>All differences between groups were non-significant.

Mean faecal outputs for both groups during each treatment period were similar (Table 5.5) and increased by an average of 44 g/d between period 1 and period 2. This increase was significant (P<0.01) for both groups. Differences between the groups in average daily faecal outputs were not significant after correcting for period 1 mean outputs and liveweight. The test of heterogeneity of the intra-class regressions showed that the slope of the relationship between period 1 and period 2 outputs was similar in the two groups (P>.10). Thus sheep with high outputs in period 1 retained their high outputs in period 2.



Mean daily faecal outputs of control (0-0) and capsulc-treated (x-x) ram Figure 5.3 hoggets, experiment 5. Bars indicate sem.

Lighter animals at the commencement of the trial were in poor condition and their larger liveweight gains during the trial probably included some compensatory growth (Drew et al. 1973, Table 5.4). Treatment groups were not significantly different in liveweight during the trial.

#### Effect of method of capsule administration on faecal output

Mean faecal outputs of the mock capsule insertion (D+) and no mock insertion (D-) animals during the pre-treatment (5 d) and treatment (7 d) periods are shown in Table 5.6. Liveweights on the day of administration and on the last day of faecal collection are also presented.

Table 5.6 Effect of mock insertion of CRC on mean  $(\pm \text{ sem})$  faccal outputs during the pretreatment and treatment periods, experiment 5. Liveweights at insertion and on the last day of faccal collection are also shown.

Mock insertion (D+)	No mock insertion (D-)
$369 \pm 15.2$	$369 \pm 12.4^{a}$
$422 \pm 16.0$	$410 \pm 13.3$
$45.8 \pm 2.2$	$45.7 \pm 2.2$
$46.8 \pm 2.1$	$46.9 \pm 2.1$
	$369 \pm 15.2$ $422 \pm 16.0$ $45.8 \pm 2.2$

<sup>a</sup>All differences between groups were non-significant.

Mean faccal output in the D+ group increased by 12 g/d more than in the non-treated group during the treatment period relative to levels in the pre-treatment period. This was not significantly different and neither was the pattern of daily faccal excretion. The faecal output of one sheep in the D+ group declined after mock insertion but inspection of the daily outputs indicates that these declined slowly over the treatment period and did not exhibit the sharp depression after capsule administration that is characteristic of a physical injury.

#### DISCUSSION

# Effect of CRC on Faecal Output and Feed Intake

The results of the indoor feeding trial confirm the findings of experiment 3 (Chapter Four), that faecal dry matter outputs differed significantly between groups with and without capsules, although the percentage difference was substantially smaller in this study than in experiment 3 (7.8% vs 20-40\%). The intake data indicated that capsule insertion reduced voluntary feed intake (P<0.10), but did not influence the digestibility of feed consumed. No evidence of physical irritation of the rumen wall or of damage to the reticulum or oesophagus was evident in the ram lambs that were slaughtered. However, the reticulum of a sheep contracts very

strongly to approximately one third of its normal size on average once every minute (Waghorn and Reid 1983; Waghorn et al. 1986). Regular forceful contractions against the extended and relatively rigid wings of the Mark II capsule type could therefore cause considerable discomfor to the animal. In older animals, where the linings of the reticulum and rumen are thicker and darker, sites of irritation may not be readily evident. It would also seem likely that physical discomfort due to the capsule would be greatest where the animals were able to move about freely. This may explain why the differences in faccal output were larger in experiment 3 than in these experiments. There is also a possibility that a chemical from the matrix carrier had a depressing effect on intakes, or that the presence of the capsule reduces rumen volume, albeit to a small extent. The subsequent outdoor grazing trial, (experiment 5), addressed some of these issues.

The results from experiment 5 indicate that the rumen presence of QS chrome capsules (compared with the Mark II design used in the experiments reported in Chapters Two and Three and in experiment 4) had no effect on daily faecal output. QS capsules had idential plastic moulding dimensions to the Mark II type capsule, except that the wing structure was more flexible with a bulbous tip. It can be inferred that the modified wing structure eliminated differences in faecal output between CRC+ and CRC- animals. Unfortunately, a faulty CRC applicator made up for the purposes of the trial resulted in perforation of the oesophagus in three animals. In two of the cases this was fatal. The subsequent investigation of dosing technique showed that releasing the capsule at the thoractic inlet while the animal is in a standing position had no effect on faecal output if correct equipment is used. It would be preferable, however, to use the simpler procedure of releasing the capsule at the back of the tongue using the CRC application gun developed for Captee capsules (Captee Ltd, Laverton, Australia) and allowing the animal's swallowing motions to transport the capsule into the rumen. X-ray studies show that this occurs readily in yearling sheep (Venning 1986).

## Prediction of Faccal Output and Feed Intakc

Results from experiment 4 indicate that faecal grab sampling for predicting faecal output should not commence until at least 6 days after capsule insertion. This corresponds to the general recommendation for other methods of chromium administration that there be a 5 to 7 day treatment period before faecal sampling commences (e.g. Wanyoike and Holmes 1981), but is less than the time taken for chromium to reach a steady state in the faeces in experiment 2 (Chapter Three). This can probably be attributed to the c. 100% higher daily release rate of the capsules used in the current experiment because of their 2.00 mm larger orifice size and greater  $Cr_2O_3$  concentration in the matrix (65% vs 50%). The attainment of steady state levels of chromium in the faeces after 6 days was similar to the pattern observed by Harrison et al. (1982) and Laby et al. (1984) in sheep fitted with CRC. However, the chromium release rate was 12% higher than that recorded in **rumen-fistulated sheep with capsules** of similar specifications under Australian pastoral conditions (Ellis and Rodden 1987). Variation in the rate of chromium release (CV = 6%) was lower than the 8% reported by Ellis and Rodden (1987), but similar to that recorded in an earlier Australian study (Ellis et al. 1981).

The different faecal sampling procedures used in this trial indicate that bulking faeces across 5 days, and assaying the combined sample intact, enables group mean faecal output to be predicted to within 2% of the actual mean value. Bulking intact faeces reduce costs for labour and chemicals. However, extra care needs to be exercised to ensure that intact faeces are bulked on an equal weight basis each day. This is more easily completed with ground faeces but grinding has potential disadvantages of loss of faecal material and chromium, as well as requiring additional labour (Roofayel et al. 1984; Costigan and Ellis 1987). There is also no advantage to be obtained by bulking and grinding the small faecal samples (0.5-1.0 g) often obtained by grab sampling, since the entire sample will be required for analysis. The similar accuracy in predicting faecal output from the 3- and 5-day bulking periods indicates that it would be possible to reduce the bulking (sampling) period to 3 days in situations where intakes and feed quality are relatively uniform between days, such as in fixed sward height experiments (Bircham 1981).

The accuracy of predicted faecal outputs and feed intakes compares favourably with the results of other researchers. Laby et al. (1984), for example, achieved an estimated:actual faecal output ratio of  $102 \pm 1\%$  for chromium CRC in penned sheep fed a range of diets. However, the estimates for individual sheep in this trial were based on samples drawn from total faecal production bulked over the prediction period. Ellis and Rodden (1987) achieved estimates of faecal output in three sheep fitted with CRC which were  $95 \pm 4\%$  of the actual output over a period of three days when sub-samples of total faecal output were collected off the pasture rather than from faecal grab samples. In the current experiment, prediction of faecal output was improved for the Daily-I samples because the true recovery rate of chromium was used. Normally 100% recovery of chromium is assumed for outdoor grazing studies (Le Du and Penning 1982) because it is only possible to adjust for the recovery rate of chromium in these circumstances if total collections are made from some animals fitted with collection bags as described by Walters and Evans (1979), and Geenty and Sykes (1986). For the Bulked-I samples the biasw as lower because an average of 98% of the chromium was recovered.

## CONCLUSIONS

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It can be concluded that the wing design of the QS capsules, which are available commercially, causes less physical discomfort to treated animals. As a result no difference in faecal output (and hence presumably in voluntary feed intake) was evident in treated animals during experiment 5. It therefore seems likely that the depression in intakes and faecal outputs observed in the earlier experiments was associated with the the less flexible wing configuration of the capsules used. Inflexible or sharp-ended wings could cause considerable discomfort if contraction occurred while the capsule was lodged in the reticulum. If several hundred such

contractions occurred with the capsule in the same orientation, physical injury and in some cases reticulitis would result. No effect of material released from the capsule while in the rumen was measured on digestibility in the indoor trial, or on faceal output while the sheep were at grazing.

The loss of some lambs due to injuries sustained during the administration of CRC indicates that care must be taken to avoid damage occurring to the throat and oesophagus during dosing. Animals must be firmly restrained and the dosing equipment should be carefully checked to eliminate sharp edges. For full throat drenching the releasing rod should be clearly marked to ensure that the rod does not pass beyond the end of the flexible rubber tubing when the capsule is applied.

Under the controlled conditions of experiment 4, within-day chromium concentrations in the facces of individual animals explained 88% of the variation in faccal output but only 65% of the variation in individual daily intakes because of large between-day differences in digestibility. Pooling the results of the daily samples across 5-days enabled 94% of the variation in average feed intake between animals to be explained. Although daily sampling has high labour and chemical costs compared to facees bulking methods, the pattern of chromium excretion through time can be monitored and possible problems with CRC performance or faceal sampling can be identified. Overall, these results show that chromium CRC have considerable potential for obtaining indirect estimates of herbage intake in sheep. However, this experiment has also demonstrated that the measurement of rate of release of  $Cr_2O_3$  from CRC is critical to the successful application of this technology in field studies. Factors which may affect the rate of  $Cr_2O_3$  release from CRC are investigated in the next Chapter.

# **CHAPTER SIX**

# EFFECTS OF HERBAGE ALLOWANCE AND FEED TYPE ON CHROMIUM RELEASE RATES IN TWO TYPES OF INTRARUMINAL CRC

#### INTRODUCTION

A knowledge of the characteristics of chromium release from intraruminal CRC is an essential prerequisite to their use in experiments measuring herbage intakes. Preliminary investigations with rumen-fistulated wethers have shown that chromium is released from CRC with a 3.0 cm core of Cr<sub>2</sub>O<sub>3</sub> matrix over a period of 29 to 33 days (Vincent 1985). Chromium release is linear between days 4 and 25. Thus, if a 7 to 8 day period after insertion is required for Cr<sub>2</sub>O<sub>3</sub> to reach steady state concentrations in the facees (experiment 2, Chapter Three and experiment 4, Chapter Five), faecal sampling for the purposes of estimating herbage intake could be carried out over a period of approximately 18 days. This period is more than adequate for the 5 to 10 day interval normally used to obtain an estimate of intake at one level of herbage allowance. It is inadequate, however, if measurements from the same animals are required for three or more pasture treatments. This is because a period of 2 to 4 days, depending on factors such as feed type and the level of intake, is necessary for Cr<sub>2</sub>O<sub>3</sub> to achieve a new steady state in the facees after a change in the level of herbage intake (Lambourne and Reardon 1963; Faichney 1983). Furthermore, many animal nutrition studies are conducted over periods of more than 20 days often corresponding to a phase in the production cycle (e.g. pregnancy or lactation) - and intake measurements at intervals during this time are required. To achieve this with 3.0 cm core CRC, more than one application of the capsules to treatment animals would be necessary.

An alternative is to use CRC which have a longer linear release phase. This can be achieved by increasing the length of the matrix core, changing the matrix composition or reducing the diameter of the orifice (Laby et al. 1984). The latter option is not viable if the resultant faecal chromium concentrations are low relative to the background levels of environmental chromium (see Chapter Thrce). The most simple method of lengthening CRC life is to increase the length of pressed tablet matrix within the same plastic barrel.

A 100-day period of  $Cr_2O_3$  release has been achieved in sheep CRC with a 6.0 cm core, 50%  $Cr_2O_3$  matrix, and 7.00 mm diameter orifice in fistulated wethers (Chapter Three). No comparable data on the performance of 6.0 cm core CRC with a 65%  $Cr_2O_3$  matrix and 9.00 mm orifice have been published. Nor are data available for 3.0 cm core CRC of the same specifications in intact sheep grazing at pasture. This Chapter presents the results of serial slaughter studies in which the pattern of  $Cr_2O_3$  release from 3.0 and 6.0 cm core CRC was examined in ewes offered a range of herbage allowances. These release patterns are compared with those obtained from fistulated wethers.

# MATERIALS AND METHODS

Experiment 6

A single 3.0 cm core, 65%  $Cr_2O_3$  matrix, 9.00 mm orifice CRC (Captec, Mark II design) was administered to each of 16 mixed age (MA) Romney ewes in June 1986. CRC were inserted to the thoracic inlet by a lubricated flexible tubing. The individually identified ewes were then weighed and clearly spray-marked before being returned to a larger flock of 400 ewes. Mean ( $\pm$  sem) weight of the treated ewes at this time was  $45.0\pm2.3$  kg. The flock was subjected to an on-off grazing system during pregnancy, with 3-4 hours grazing each morning on pastures of 3-10 cm in length. During the remainder of the day the ewes were restricted to a holding area with pastures of 400-500 kg DM/ha. Sample weighings of the flock at regular intervals indicated that daily intakes were equivalent to about 0.9 maintenance under this grazing regime. CRCtreated ewes were divided into one group of 4 ewes and four groups of 3 ewes (balanced for liveweight). Groups were slaughtered at 0830 h on days 5 (4 ewes), 12, 20, 24 and 28 after CRC insertion. CRC were recovered from the rumen within five minutes of slaughter and plunger displacement since insertion (d 0) was measured on both sides of the CRC barrel by vernier callipers (Mitutoyo, Tokyo).

CRC from the same manufacturing batch were attached by nylon string to the cannula plug of five rumen-fistulated wethers on d 0. The wethers were set stocked separately on a mixed ryegrass - white clover pasture similar in composition to that grazed by the ewes. Herbage intakes of the wethers were estimated from the height of pasture grazed and were judged to be sufficient to meet maintenance requirements. CRC were removed from the rumen twice-weekly, for approximately two minutes on each occasion, to measure plunger displacement. The characteristics of matrix wear at the orifice and any unusual aspects of CRC performance were noted for both groups of sheep in which CRC were tested.

#### Experiment 7

Two 65%  $Cr_2O_3$ , 9.00 mm orifice chromium CRC (Captec, Mark II design), with 3.0 and 6.0 cm matrix cores respectively, were inserted into each of 21 MA Romney ewes at Massey University's Sheep and Beef Cattle Research Unit in September 1986 (d 0). The ewes, which had been shorn three days prior to CRC insertion, were then allocated to a mixed rygrass-white clover pasture sward. Ewes were subsequently slaughtered in groups of three on days 4, 12, 20, 28, 39, 49 and 63 after CRC insertion. Except for the first slaughter group, which monitored the initial matrix wetting and extrusion process, ewes were slaughtered on the final day of the feeding regimes shown in Table 6.1.

		Feed parameters				
Location	Days <sup>a</sup>	Туре	Allowance	Maintenance		
			(kgDM/ewe/d)	( <b>x</b> M)		
outdoors	0-4	pasture	2.5	1.4		
	5-12	pasture	2.5	1.4		
	13-20	pasture	0.8	0.6		
	21-28	pasture	1.8	1.0		
Indoors	29-40	cut pasture	0.8	1.0		
	40-49	lucerne chaff	1.0	1.4		
	50-63	hay	0.6	0.6		

Table 6.1 Trial location and associated feeding conditions of ewes, experiment 7.

 $^{a}$ day 0 = day of CRC insertion.

During the first 28 days ewes grazed on three 0.25 ha plots prepared to represent high ( $\geq$  1250 kg DM/ha), low ( $\leq$  400 kg DM/ha) and medium (800-1000 kg DM/ha) herbage allowances (Table 6.1). Pasture conditions at the commencement and end of each feeding period were assessed by recording 50 Ellinbank Pasture Meter readings (Earle and McGowan 1979) per plot. Pasture height (cm) was calibrated against pasture mass (kg DM/ha) using five 0.22 m<sup>2</sup> quadrats cut to ground level from each plot by an electric shearing handpiece. Each pasture cut was washed to remove soil contamination before being oven dried at 80°C for 24 h to determine DM yield.

From d 29 the ewes were individually housed in metabolism cages at the University's Animal Physiology Unit. Rations, calculated from energy requirement data of Rattray (1986), were offered once daily at 1600 h. To determine feed DM contents, duplicate samples of the feed offered were oven-dried at 80°C for 24 hours. Fresh feed allowances were adjusted according to the DM contents recorded for the previous 24-hour period. After their fresh weight had been recorded for each sheep, refusals were bulked within sheep, thoroughly mixed and sub-sampled in duplicate for DM determination. Dry matter intake (DMI, g/d) was calculated as the difference between DM offered and DM refused.

Total faecal outputs were not collected while the ewes were indoors but a sample of faeces (20-30 g DM) was retained from each ewe's daily excretion for chromium analysis. <u>In vivo</u> herbage DM digestibility values (DMD %) were obtained from wethers fed the same feeds at the same daily allowance in experiment 8 (see Chapter Seven). Faecal output was calculated from intake and digestibility. Ewes were weighed on d 0 (24 h fasted weight), and immediately before each change in feeding level while indoors (to enable group mean feed allowances to be calculated). CRC were recovered from the rumen within five minutes of slaughter. Plunger displacement was measured as described for experiment 6.

The design of experiment 7 was based on results of a preliminary test of 6.0 cm core CRC (from the same manufacturing batch) in rumen-fistulated wethers. In the preliminary test, a single CRC had been inserted into each of six rumen-fistulated wethers and plunger travel measured twice-weekly over 66 days. During this period the wethers grazed a mixed ryegrass-white clover pasture with a herbage mass of 800 to 1000 kg DM/ha. For comparative purposes, plunger travel for the five rumen-fistulated sheep from which complete records were obtained (rumen cannula loss over two separate 24 h periods having interrupted plunger travel in one rumen-fistulated wether) are reported with those obtained by serial slaughter of ewes in experiment 7.

# Statistical Analysis

CRC plunger travel data for both experiments were initially analysed by fitting a linear regression of plunger travel (y) on time (x), where plunger travel was the difference between initial plunger position (P<sub>0</sub>) and plunger position on the day of measurement (P<sub>1</sub>). For this model the variance (travel) associated with the measurement of plunger position was assumed to be constant (i.e. var (P<sub>1</sub>-P<sub>0</sub>) = var<sub>measurement</sub> = constant). In fact, this variance component proportionately decreases as the distance of plunger travel increases. A weighted regression model, of the same form as the simple linear model, was therefore also fitted with weights proportional to the inverse of the variance of travel (i.e. var(travel) = var((P<sub>1</sub>-P<sub>0</sub>)/t) =  $(1/t^2)var_{measurement}$ ; Snedecor and Cochran 1967). The effect of feeding level and CRC type on the rate of plunger travel in experiment 7 was tested by a split-plot model (Gill and Hafs 1971). The model included terms for feeding level, sheep within feeding level. The latter were tested against the residual (CRC x sheep) mean square. The nature of plunger travel through time was tested by dividing feeding level into linear, quadratic and remaining ("lack of fit") effects.

#### RESULTS

#### Recovery of CRCfrom Slaughtered Ewes

No losses of CRC through regurgitation occurred between insertion and slaughter in the intact ewes, but two of the 47 CRC (4.3%) tested in these ewesfailed. Leakage of rumenfluid into the barrel of one 3.0 cm core CRC occurred prior to the first slaughter (d 0-4) in experiment 6. This appeared to be related to a manufacturing fault, rather than to damage sustained during insertion or within the rumen. The second failure occurred in a 6.0 cm core CRC in experiment 7, between d 29 and d 39 after insertion. The time of failure was estimated from changes in

faecal chromium concentrations and the amount of  $Cr_2O_3$  matrix remaining at slaughter. The withdrawal of the matrix within the CRC barrel indicated that the failure was probably precipitated by the change in intake which occurred while the ewe adapted to indoor feeding.

#### Plunger Travel and Chromium Release Rates

Plunger travel for the 3.0 cm and 6.0 cm core CRC in both the fistulated and intact sheep was related to time in an almost perfectly linear manner (r > 0.984, Table 6.2). Thus, linearity of Cr<sub>2</sub>O<sub>3</sub> release was achieved despite the irregular pattern of intake associated with the on-off grazing treatment in experiment 6 and the wide fluctuations in the amount and type of herbage offered in experiment 7.

Table 6.2 Linear regression (y = a + bx) parameters and correlation coefficients (r) for CRC plunger travel (y) on time (x) in intact (I) ewes and rumen-fistulated (F) wethers, experiments 6 and 7. Standard errors for the slope and intercept are shown. Weighted regression parameters are shown in brackets.

Expt	Number	CRC	Shcc	P <u>Regr</u>	ession parameters		
	of	core	type				Sign. of
	sheep	length		a	b	r	regression
	(n)	(cm)					
i	16	3.0	I	-0.301±0.452	0.974±0.025	0.995	
				(-0.507±0.919)	$(0.965 \pm 0.039)$	(0.977)	(***)
i i	5	3.0	F	$-1.591 \pm 0.479$	$0.872 \pm 0.025$	0.984	•••
				(-2.963±1.001)	$(0.919 \pm 0.039)$	(0.928)	(* ** )
,	12 <sup>c</sup>	3.0	I	-0.459 ± 0.815	$0.908 \pm 0.043$	0.990	
				(-2.231 ± 1.547)	(0.973±0.063)	(0.963)	(***)
,	5	6.0	F	1.324 ± 0.496	$0.604 \pm 0.012$	0.992	
				(0.913±1.112)	$(0.608 \pm 0.021)$	(0.957)	(***)
	21 <sup>d</sup>	6.0	I	$1.357 \pm 0.736$	$0.710 \pm 0.020$	0.992	
				(3.321±1.514)	$(0.661 \pm 0.029)$	(0.966)	(***)

<sup>c</sup>One CRC failure at first slaughter excluded.

<sup>d</sup>One CRC outlier at d 49 slaughter removed, see text "Recovery of CRC from Slaughtered Ewes".

Weighted regressions had an inconsistent effect on the prediction of average rate of plunger travel (b), but consistently increased the size of the regression intercept (a). For this reason the correlation between predicted and actual values was poorer. Regression weighting to account for the decreasing variance due to measurement error was counteracted in some cases (e.g. experiment 7 intact ewes) by an increased variance of  $Cr_2O_3$  release through time (assuming the CRC have a given mean release rate at manufacturing). Subsequent analysis of the recovery of chromium from the faeces suggests that simple linear regression provides the most reliable expression of plunger travel through time.

Although comparisons of the rate of plunger travel in the fistulated and intact sheep are restricted because the groups were not grazed under the same conditions the performance of CRC from the same manufacturing batch can be contrasted. Plunger travel was 11.6% faster in 3.0 cm core CRC (0.97 vs 0.87 mm/d) in intact compared with fistulated animals in experiment 6, and 17.5% faster in the 6.0 cm core CRC in the intact ewes compared with CRC of the same type in the rumen-fistulated sheep in experiment 7.

Although the 3.0 cm and 6.0 cm core CRC in experiment 7 were identical except for the length of  $Cr_2O_3$  matrix, plunger travel was 27.9% slower (0.71 vs 0.91 mm/d, P<0.001) in the 6.0 cm core CRC when the two types were exposed to a common rumen environment (Table 6.2). This equates to average chromium payouts of 112 and 136 mg/d (to the final slaughter) respectively for the 3.0 cm and 6.0 cm core CRC. Between days 4 and 28, plunger travel was linear with time (r>0.99, P<0.001) in both types of CRC. There was no depression in the rate of plunger travel with time, but the small amount of systematic ("lack of fit") variation across animals at the same slaughter date was significant (P<0.05). The interaction between type of CRC and slaughter date was not significant. This shows that the difference in the rate of plunger travel between the 3.0 cm and 6.0 cm core CRC was constant through time. The greater rate of plunger travel in the 3.0 cm core CRC was associated with a greater depth of matrix erosion at the orifice (3 to 4 mm vs 2 mm in the 6.0 cm CRC). This pattern of matrix erosion was consistent across slaughter dates.

At the final slaughter (d 63) approximately 16 mm of the  $Cr_2O_3$  matrix remained in the 6.0 cm core CRC. If the 0.71 mm/d plunger travel rate had continued, linear release of chromium into the rumen could probably have been maintained for a further 15 d given that rapid dissolution of the matrix occurs once 3 to 5 mm of matrix remains. This would have given a total capsule life of 75 to 80 d in intact ewes.

Chromium release was slower up to the first slaughter (d 0-4), particularly in the 3.0 cm core CRC, because of time taken for wetting and extrusion of the matrix to commence. For this reason it is preferable to calculate the average chromium release rate after continuous extrusion of the matrix has been established. The slaughter data for experiments 6 and 7

suggest that this occurred within the first 3 to 4 days.

Average chromium release rates decreased in both the 3.0 cm and 6.0 cm core CRC when pasture allowances were reduced to <0.6 M equivalence and in the longer life CRC when intakes of hay were reduced to a similar level (Table 6.3). Chromium payouts increased when pasture allowances were raised to 1.0 M, but no comparable data following the low level of hay feeding were obtained. Low levels of feed intake may therefore have influenced the release rate of chromium. Release rates in the 6.0 cm core were also slower on average during the indoor feeding period. Interpretation of this pattern of response is difficult because there may have been confounding effects due to the preceding outdoor treatments (particularly the low level of nutrition). However, given the feeding level effect observed outdoors, reduced levels of intakes while indoors (due to the correction for reduced energy requirements for exercise (30%) and to initial adaption to indoor feeding) are a more likely explanation for the reduction in  $Cr_2O_3$  release than the effect of activity per sc.

Table 6.3 Average daily release rates of chromium from 3.0 cm and 6.0 cm core CRC to the
end of each feeding level, experiment 7. Except for the first intake period (d 0-4) release rates
are for plunger travel from d 4 until slaughter as defined in Table 6.1.

Location	Fccd intake (xM)	Chromium release (mg Cr/d)		
		3.0 cm	6.0 cm	
Outdoors	1.4	115 <sup>a</sup>	112 <sup>a</sup>	
	1.4	159	149	
	< 0.6	127	111	
	1.0	144	129	
Indoors	1.0		116	
	1.4		115	
	0.6		109	
Overall mean		143	122	
± sem		6	4	

<sup>a</sup>Low release rates associated with establishment of matrix wetting and extrusion (d 0-4).

#### Prediction of Faecal Output

Faecal outputs predicted from the mean faecal chromium concentration (FOcr) for the last three days of each indoor feeding level were derived in experiment 7. Daily chromium payout from CRC was estimated from the average rate of plunger travel (d 4-63) in the ewes slaughtered at the end of each feeding level. Predicted outputs were 91.1 to 104.6% of those estimated from the measured DMI and average in vivo digestibility of each feed (FO<sub>D</sub>; Table 6.4). Given that the proxy for true faecal output (FO<sub>D</sub>) includes errors due to variation between animals in feed digestibility, and that an average plunger travel was used to derive chromium payouts, the FO<sub>cr</sub> are surprisingly accurate.

Feed type	Sheep (n)	DMI (g/d)	DMD (%)	FO <sub>D</sub> (g/d)	FO <sub>cr</sub> (g/d)	FO <sub>cr/</sub> FO <sub>D</sub> (%)
Pasture	9	696±25 <sup>a</sup>	65.87	227±8 <sup>a</sup>	223±1 <sup>a</sup>	98.24
Lucerne	6	832±6	53.49	$303 \pm 3$	276±10	91.09
Hay	3	528±3	57.76	261±1	273±4	104.60

Table 6.4 Group mean DMI and faecal outputs predicted from DMD values (FO<sub>D</sub>) and faecal chromium concentrations (FO<sub>Cr</sub>), for three feed types offered indoors during experiment 7.

<sup>a</sup>sem refer to the variation between days for group means.

### DISCUSSION

The length of the 65%  $Cr_2O_3$  matrix in CRC with a 9.00 mm diameter orifices significantly (P < 0.001) influenced the rate of plunger travel and as a consequence the period of  $Cr_2O_3$  release per unit length of matrix core. CRC plunger travel was linear (P < 0.001) for a minimum of 21 days (d 4-25) in the 3.0 cm core capsules in intact ewes. In the 6.0 cm core CRC, plunger travel was linear (P < 0.001) until d 63 when the last group of ewes was slaughtered. By extrapolation it was estimated that the linear release phase could have continued until about d 75. This compares with the 100 d life measured in CRC with a 50%  $Cr_2O_3$  matrix and 7.00 mm orifice in experiments 1 and 2 (Chapter Three). These results are in agreement with those of Laby et al. (1984) who demonstrated that chromium release could be substantially modified by changing orifice diameter and matrix composition.

The minimum chromium payout from the 6.0 cm core CRC (>100 mg Cr/d) would enable herbage intakes of sheep producing up to 1.0 kg faecal DM/d to be estimated, providing environmental levels of chromium were low (<20  $\mu$ g/g DM; see p. 45). CRC of this design could therefore be used in longer term nutrition studies with sheep up to 70 kg in liveweight. Sequential estimates of feed intake in sheep, for example those coinciding with changes in physiological status, could therefore be made.

Plunger travel was consistently slower by 4.1 to 17.5% in the rumen-fistulated sheep than in the intact ewes. This contrasts with the study of Laby et al. (1984) who reported no difference between rumen-fistulated and intact wethers. Although differences may have been created by the separate grazing of the two groups, this seems unlikely because the results from experiment 7, and from experiments 2 and 3 previously (Chapters Three and Four respectively), suggest that the level of feed intake influences plunger travel only if intakes are very low (<0.6 M). Gender effects are also likely to have been negligible (Weston 1982). Modification of the capsule environment due to rumen fistulation and to the periodic removal of capsules from the rumen for measurement are therefore more plausible explanations of the depression in plunger travel.

The nil loss rate of CRC in ewes ranging from 26 to 60 kg in liveweight indicates that the flexible

wings provided a highly effective means of rumen retention. However, it is not known whether this also applies to sheep which are heavier than 60 kg.

CRC failed to perform to specification in c. 4% of the ewes slaughtered. In practice such malfunctions can be identified by low or irregular faecal chromium concentrations. The incidence of failure due to manufacturing faults, probably in the order of 10-20% in the earlier batches (Ellis et al. 1981), cannot be readily prevented but the results of experiment 7 suggest that CRC failure may be precipitated by sudden large reductions in feed intake. This occurred in some capsules when ewes were transferred without prior experience to indoor feeding conditions. A similar effect, under field conditions, could be created by the introduction of a new feed type. Exposure of sheep to very low levels of herbage intake (<0.6 M) may also result in inconsistent capsule performance or matrix dissolution because rumen contents become more liquid. Thus CRC are best suited to situations where sheep have been conditioned to the feeding regime to be tested and fluctuations in daily intakes are minimised.

The similarity between group mean faecal outputs calculated from marker chromium and those estimated from DMI and <u>in vivo</u> digestibility values for the cut pasture, lucerne and hay provides further evidence of the potential of CRC for estimating voluntary herbage intakes (Ellis and Rodden 1987). Although Laby et al. (1984) were able to estimate faecal outputs to within  $\pm 4\%$  of actual values in penned sheep, the results for the present experiment were achieved in ewes 36 days after CRC insertion and following a series of changes in feed intake (including a transition to indoor feeding). The final estimates for the hay feed were made between d 54 and 57 after CRC insertion. Thus 6.0 cm core CRC, with the same technology as 3.0 cm core CRC, are sufficiently robust to withstand the changes in feed allowance which researchers might wish to investigate and are capable of providing continuous linear release of chromium for at least 63 days.

The experiments reported here suggest that CRC plunger travel is slower in rumen-fistulated than intact sheep. However these results refer to situations where the groups were grazed separately, although on similar sward types. There is a need to investigate whether differences in plunger travel between rumen-fistulated and intact animals exist when they are grazed together, an issue which is addressed in Chapter Eight. A second feature of experiments 6 and 7 is that the average rate of chromium release was reduced during periods of low herbage intake. This may partly explain why lower levels of chromium recovery from the faeces were achieved when ram lambs were grazed at low herbage allowances in experiment 3 (see Chapter Four). An indoor experiment, described in the next Chapter, was therefore conducted to more accurately quantify the effects of large changes in feed intake on CRC plunger travel.

# **CHAPTER SEVEN**

# EFFECTS OF FEEDING LEVEL AND FEED TYPE ON THE PERFORMANCE OF COMMERCIAL CHROMIUM CRC UNDER CONTROLLED INDOOR FEEDING CONDITIONS

#### INTRODUCTION

Measurements made on CRC recovered from ewes by serial slaughter indicate that plunger travel may be reduced during periods of low feed intake (Chapter Six). A short-term reduction in plunger travel will compromise estimates of faecal output based on faecal chromium concentration and an average release rate for the capsule by changing the amount of  $Cr_2O_3$  excreted in the faeces each day. In addition, the interaction between changes in  $Cr_2O_3$  release rate and feed intake has important implications for the timing of faecal sampling, and the amount of chromium recovered in the faeces (Chapter Four). The experiment reported in this Chapter therefore tested the effects of large changes in the daily allowance of two types of herbage on the rate of CRC plunger travel and the pattern of chromium appearance in the faeces of rumen-fistulated wethers. This experiment also provided the opportunity to test the matrix and orifice technology of the type of chromium CRC which was to be commercially released. This type had a 3.0 cm core of a 65%  $Cr_2O_3$  pressed tablet matrix and an orifice diameter of 9.00 mm and was expected to generate a 25-day linear release of  $Cr_2O_3$ .

# MATERIALS AND METHODS Experiment 8

Two groups (n = 4) of rumen-fistulated 24-month old Romney wethers, balanced for liveweight, were housed in metabolism crates at Massey University's Animal Physiology Unit in October 1986. After being fitted with faecal collection harnesses one group (A), weighing 47.5  $\pm$  3.8 kg (mean  $\pm$  sem), was fed pasture cut each afternoon from a mixed ryegrass-white clover sward. The other group (B, 49.8  $\pm$  2.4 kg) received hay which had been made during the previous summer from pasture similar in composition to that offered to group A. These feeds were offered <u>ad libitum</u> once daily at 0900 h for a pre-treatment period of seven days.

At 0900 h the following day (d 0) a single chromium CRC (Captec, 3.0 cm core, 65% Cr<sub>2</sub>O<sub>3</sub> matrix, 9.00 mm orifice diameter, Mark II design) was suspended in the rumen by nylon string attached to the cannula. Each sheep was then weighed prior to refeeding and fitted with a faecal collection bag. Feed requirements for maintenance (M) were estimated to be 70% of the energy requirements specified by Rattray (1986) for sheep of the same liveweight grazing indoors. Maintenance feeding was adopted until d 4, when the wethers were assigned to one of

four feeding levels - 0.6 M, 1.0 M, 1.4 M, and 0.6/1.4 M on alternate days. Feeding levels were maintained for periods of six days duration and were arranged in a 4 x 4 Latin Square within feed types over a period of 24 days (d 4-28). Feed allowance, feed refusal and faccal output were measured daily at 0900 h for each sheep. Feed allowances were based on the DM content of duplicate samples of feed offered the previous day and assumed energy concentrations of 10.0 and 8.0 MJME/kg DM in the pasture and hay feeds respectively (Holmes et al. 1984). Refusals were weighed and duplicate subsamples taken for oven drying at 80°C for 24 h to determine DM %. Faeces, collected in plastic liners fitted inside the faecal bags, were weighed fresh, thoroughly mixed, subsampled in duplicate and oven dried at 80°C for a minimum of 72 h to determine the dry weight. A sample of c. 20 g of dried faeces from the daily output of each sheep was retained for chromium analysis (see Chapter Two for explanation of the method and calculations). Four-hourly rectum grab samples over a 24 h period were collected from each animal on d 12 to measure diurnal variation in faecal Cr<sub>2</sub>O<sub>3</sub> concentration.

Dry matter intake (DMI) and dry matter digestibility (DMD) were calculated as:

DMI (g/d) = feed DM offered - feed DM refused

DMD(%) = (DMI(g/d) - faccal output(g/d))/DMI(g/d)

The position of the CRC plunger was measured at insertion and thereafter at the commencement of each new feeding level using vernier callipers (Mitutoyo, Tokyo). Two readings, from opposite sides of the plastic barrel, were recorded and features such as matrix erosion at the orifice and leakage of rumen fluid into the barrel were noted. CRC were removed from the rumen at the end of d 28 and the wethers were reweighed before being returned to pasture.

# Statistical analysis

Plunger travel data were tested by split-plot analysis (Gill 1988). Mahalanobis and Cook distance values were used to test plunger travel data for outliers (Norusis 1985). Four-hourly faecal chromium concentrations were subjected to repeated measures analysis. Analyses were undertaken using the 'REG' (Gilmour 1985), SAS (SAS 1985) and SPSSX (SPSSX 1983) statistical packages.

Chromium recovery was calculated as the percentage of the expected average daily payout from individual CRC divided by the product of the concentration of the marker in the faeces (mg Cr/g DM) and total faecal output.

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#### RESULTS

#### Effect of Feed Type on CRC Plunger Travel

Total CRC plunger travel ranged from 24.5 to 29.5 mm during the 28 d trial and was highly correlated with time for both feed types once initiation of matrix extrusion had been completed by about d 3 (Table 7.1). With the exception of one outlier (Sheep 5, hay) average daily rates of travel and hence chromium release rates were similar for hay and pasture (135 vs 137 mg Cr/d).

Table 7.1CRC plunger travel (d 0-28), parameters for the regresson (y = a + bx) of plungertravel (y) on time (x) from d 4 to d 28 and average daily release rates of chromium, experiment8. Figures in brackets are standard errors of estimates for the pooled regressions.

			Regre		Chromiu	
Feed	Sheep	Total travel (mm)	a	b	r	release (mg Cr/d)
Pasture	1	25.50	-2.083	0.971	0.999	135
	2	25.25	-1.383	0.958	1.000	138
	3	25.50	-1.183	0.933	0.998	135
	4	24.25	-1.317	0.917	1.000	132
Pooled re	gression		-1.450 (0.213)	0.941 (0.012)	0.999	135
Hay	5	29.50	-1.657	1.127	1.000	162
	6	26.50	-1.400	0.975	0.999	140
	7	24.75	-0.883	0.908	0.999	133
	8	24.50	-0.750	0.925	0.997	137
Pooled reg	gression		-1.173 (0.653)	0.984 (0.038)	0.987	143
(without s	heep 5)		-1.011 (0.340)	0.936 (0.019)	0.997	137

# Effect of Feeding Level on Rate of Plunger Travel

The mean DMI, faecal output and associated DMD at each feeding level of pasture and hay are presented in Table 7.2. DMI at the 1.4 M feeding level was approximately twice that at 0.6 M, and for the daily alternation between 0.6 and 1.4 M feeding levels was equivalent to about 0.9 M. Feed digestibility values were affected by DMI and should be interpreted with caution since each 6 d feeding level includes an effect on faecal output from the preceding treatment.

Herbage	Parameter	0.6 <b>M</b>	1.0 M	1.4 M	0.6/1.4 M	Lsd <sub>0.05</sub>
Pasturc	DMI	452	731	807	640	107
	FO	161	248	277	218	16
	DMD	64.2	65.7	64.4	58.3	4.5
Hay	DMI	551	865	1045	794	62
-	FO	278	390	451	348	50
	DMD	49.6	54.8	56.5	49.9	5.2

Table 7.2 Mean dry matter intake (DMI, g/d), DM digestibility (DMD, %) and faecal output (FO, g DM/d) at each feeding level for pasture and hay, experiment 8.

<sup>a</sup>Lsd values are for comparison only between means in the same row (Gill 1988).

The split-plot analysis of plunger travel data indicated that 98.4% of the total sums of squares could be explained by a linear relationship between plunger travel and time (P < 0.001). There was no evidence of curvature in the pattern of plunger travel and systematic variation ("lack of fit") about the line was not significant. Differences in the rate of plunger travel between feed types explained only 0.9% of the total sums of squares (P > 0.10). However, feeding level (treatments) had some effect on the pattern of plunger travel (P < 0.10). Inclusion of one- and two- period carryover terms and their respective interactions with treatment (P > 0.10), indicated that the effect of DMI occurred within a feeding level, and not because of the feeding sequence.

## Recovery of Chromium from Facces

Although the primary objective of the experimental design was to test the effects of varying DMI on plunger travel (and hence chromium release rates), the pattern of chromium recovery from the faeces was also investigated since this would provide the information necessary to determine appropriate faecal sampling routines when animals are subjected to large changes in, and irregular patterns of, feed intake.

Chromium recovery was significantly (P < 0.01) affected by the level of DMI for both feed types (Table 7.3), and was not significantly different from 100% only for the 1.0 and 1.4 M feeding levels. This suggests that the passage of chromium through the sheep slowed when DMI was reduced. Chromium recoveries were reduced during the initial feeding treatment (d 4 to d 10) because there was insufficient time for a steady state of  $Cr_2O_3$  in the faeces to be established in all of the sheep. This is shown by the general increase in chromium recoveries where they were re-estimated from d 11 (Table 7.3). For the two sheep which commenced the feeding sequence at the 0.6 M feeding level, recoveries in excess of 90% were not reached until d 11. In contrast, this level of recovery was achieved between d 5 to d 9 at the higher levels of DMI. A minimum interval of 5 d was necessary for chromium to attain a new steady state at the 0.6 M feeding level

after a transition from a higher level of DMI. At the 1.0, 1.4 M and 0.6/1.4 M feeding levels, steady state levels were achieved within 3 to 4 days, irrespective of the preceding treatment. Chromium recoveries re-calculated for the final 3 days of each feeding level (Table 7.3), did not provide a consistent improvement in recovery rates and the effect of feeding level remained significant (P < 0.05). This was because equilibrium conditions had still to be reached in some animals, especially at the 0.6 M feeding level.

Table 7.3 Recovery of chromium (mean  $\pm$  sem), at each feeding level, from the faeces of wethers fed pasture and hay, experiment 8. Recovery of chromium from d 11 and for the final 3 days of each feeding level are also shown.

		Rco	covery of chromius	n (%)
Herbage	Fccding level	Entirc feeding pcriod	From d 11	Final 3 days
Pasture	0.6 M	71 ± 3	77	73
	1.0 M	94 ± 4	91	99
	1.4 M	92 ± 4	98	98
	0.6/1.4 M	78 ± 3	80	96
	Overall Mean	84 ± 2	87	87
Hay	0.6 M	78 ± 3	82	78
	1.0 M	91 ± 4	97	91
	1.4 M	99 ± 4	107	104
	0.6/1.4 M	87 ± 3	90	92
	Overall Mean	89 ± 2	94	91

# Diurnal Variation in Faecal Chromium Concentration

The percentage deviations of 4-hourly faecal chromium concentrations from the 24-hour mean for the pasture- and hay-fed sheep are presented in Figure 7.1. Variation was larger in the sheep fed hay than in those fed pasture (CV = 13.3% vs 8.1%), but the reason for this was not apparent. Diurnal variation could have been expected to be greater for the animals on the 0.6 M treatment because they consumed all their daily allowance within 2-3 hours of feeding in the morning, but no evidence of this was measured in the samples collected. Repeated measures analysis indicated that differences between time periods were not significant.

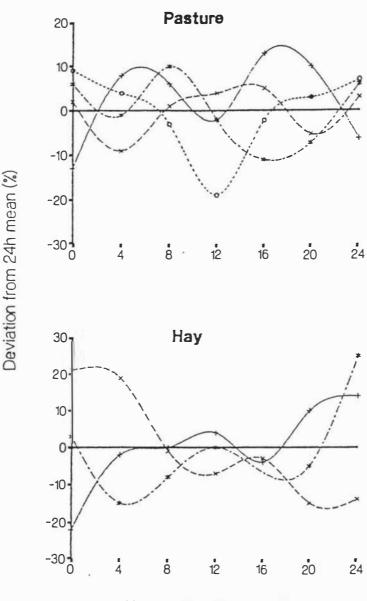




Figure 7.1 Diurnal variation in the chromium concentration of faecal grab samples (collected at four-hourly intervals from 1500 h) expressed as a percentage deviation from the 24 h mean, experiment 8 (-0.6 M; --1.0 M; --1.4 M; ....0.6/1.4 M feeding levels).

#### DISCUSSION

CRC plunger travel was largely unaffected by the level of DMI or changes in DMI between days. However, there was some indication that feeding level could affect plunger travel (P<0.1). This is likely to have been part of the reason for the pattern of chromium recovery observed in experiment 3 (Chapter Three) when rams lambs achieved very low (<0.6 M) levels of DMI. The average rate of plunger travel was 1.5% higher (P<0.1) in the sheep fed hay, confirming the results of experiment 2 where a similar effect was found with an earlier capsule design. This suggests that feeds such as hay increase the release rate of Cr<sub>2</sub>O<sub>3</sub> because rumen contents are more abrasive at the capsule orifice than rumen contents derived from fresh pasture.

The negative intercepts of the regressions of plunger travel on time (Table 7.1) indicate that release rates were slower during the first 3 days after CRC insertion, while the process of matrix extrusion was initiated. However, the average rates of plunger travel, once established from d 4, were consistent between animals across both the pasture and hay feeds, with the exception of one sheep. The reason for the higher rate of plunger travel in the outlier CRC was not established but may have been due to accelerated water uptake due to a matrix formulation fault, elevated gas pressure development caused by the animal generating almost no methane or the maintenance of the CRC in a region of the rumen where gas diffusion was optimised, (Laby 1986). One of the latter two are considered to have been the most likely causes. Average CRC plunger travel was comparable to those recorded in CRC of similar specifications in other environments (Table 7.4), given that there may have been some variation between the studies in matrix formulation.

Table 7.4	Comparison of average CRC performance in different environments (source: Laby
1986).	

Trial	Animal	Number of	Level of	Plunger	Variation
location	typc <sup>a</sup>	CRC	nutrition	travel	between CRC
				(mm/d)	(CV%)
Furnival (Aust)	Sheep	8	High	1.013	10.3
	Sheep	8	Very low	0.881	5.8
Clayton (Aust)	Cattle	10	-	0.925	5.2
Masscy	Sheep <sup>b</sup>	8 (7)	0.6 M-1.4 M	0.962 (0.936)	6.8 (2.8)

<sup>a</sup>Type of animal in which plunger travel was measured.

<sup>b</sup>Current experiment. Figures in brackets are with outlier excluded.

The analyses of chromium recoveries showed that, when faecal sampling routines are being designed, careful consideration should be given to the level of DMI and to the effect that transition between levels of DMI has on faecal output. For example, when DMI is low, the rate of passage of chromium marker is reduced and more time is required for steady state levels of chromium in the faeces to be reached. The period prior to the first sampling should be at least 10 days at a DMI equivalent to 0.6 M. Pigden and Brisson (1956) suggested that when twice daily drenching with  $Cr_2O_3$  gelatin capsules is employed, the preliminary pre-treatment period should be not less than 10 days. This procedure was commonly adopted thereafter when chromium was used in intake studies (e.g. Langlands et al. 1963a), and is twice the 5 d pre-treatment period recommended by Captec for chromium CRC. The results from this study indicate that, even when DMI are high, faecal sampling for intake estimation should not commence before d 8. This confirms the findings of experiment 2 (Chapter Three). Once an initial steady state of chromium in the faeces is established, an interval of 3 to more than 5 days, depending on the size of the DMI change, is still required for the transition to a new steady

state in faecal chromium concentration. This has important implications for the faecal sampling regime adopted when animals are under rotational grazing management and the level of feed intake changes between days. Raymond and Minson (1955) investigated the effect of this on the recovery of chromium within days as sheep grazed down a sward under rotational grazing. There were large differences in the amount of chromium recovered from the faeces at the beginning and end of grazing, although the average recovery was close to 100%. This indicates, as do the results from the present trial, that faecal sampling over at least 3 days is required to account for the fluctuations in DMI that are more common under rotational grazing management.

If the plunger travel and chromium recovery results are considered together, it can be concluded that optimum performance of CRC for predicting DMI will occur when animals are maintained at a uniform level of intake, such as under "fixed" sward height grazing management (Bircham 1981). The effects of continuous grazing of sheep at different pasture heights on CRC plunger travel are addressed in the next Chapter. However, even when DMI are widely different, as in this trial, flexible faecal sampling routines between days can be adopted because diurnal variation in faecal chromium concentration is low due to the continuous mode of  $Cr_2O_3$ release from CRC (Ellis et al. 1981; Chapter Three).

# **CHAPTER EIGHT**

# FACTORS AFFECTING THE RATE OF CHROMIUM RELEASE FROM INTRARUMINAL CRC IN SHEEP CONTINUOUSLY GRAZED AT PASTURE

# INTRODUCTION

Where chromium CRC are used in grazing studies it will be necessary to determine the average daily chromium release rate for treatment groups. This information may be obtained by the serial slaughter of animals grazed with the treatment animals, from changes in chromium concentration in the facees as matrix extrusion from the CRC ends (Ellis et al. 1988), indirectly by the measurement of plunger travel in capsules recovered at regular intervals from the rumen of fistulated animals, or by extrapolating from the results of previous trials where data have been obtained by one of the above methods<sup>1</sup>. Measurements from rumen-fistulated animals are more readily obtained and can be made repeatedly on the same capsule. However, two comparisons of intact and fistulated sheep at Massey University have indicated that chromium release rates were 11.6 and 13.0% slower in the rumen-fistulated animals (see Chapter Six). While the same manufacturing batch of capsules was used in these comparisons, and herbage allowances to the groups were similar, the intact and rumen-fistulated animals were grazed separately. It is possible, then, that differences in chromium release rate were due to differences in herbage intake rather than to rumen fistulation <u>per se</u>.

Trials using chromium CRC with sheep grazing at pasture at Massey University have shown that other factors may influence the rate of chromium release because the recovery of chromium in the faeces has varied with the level of herbage intake (Chapter Four and Chapter Seven). Thus the recovery of chromium has consistently been low or high when sheep have been grazed at low or high herbage allowances, respectively. There are two possible explanations for this result. Either lower herbage intakes reduce rumen turnover and hence the passage of chromium from the rumen to the anus (Moran et al. 1987) or the rate of chromium release from CRC is reduced by as yet unknown factors when intakes are lower. It has not been possible to determine the relative contributions of these factors to the lower recovery of chromium in the previous experiments because of the confounding effect of changes in feeding level at relatively short intervals (5-7 days).

<sup>&</sup>lt;sup>1</sup>An unsuccessful attempt to measure plunger travel in sheep CRC by realtime ultrasound scanning is reported in Appendix II.

The results of experiments where chromium CRC were recovered by serial slaughter from ewes continuously grazed at uniform herbage allowances, and comparisons of rates of CRC plunger travel in rumen-fistulated and intact sheep grazed together, are presented in this Chapter.

# MATERIALS AND METHODS Experiment 9

The experiments were conducted at the Haurongo Block of the Sheep and Beef Cattle Research Unit at Massey University in August-September, 1988. Eighteen mixed age (MA) Border Leicester x Romney ewes, weaned from an early lambing study, were allocated to three equal sized groups balanced for liveweight ( $61.4\pm0.9$  kg,  $61.1\pm2.7$  kg,  $58.7\pm1.9$  kg). Each ewe was then dosed, using the Captee capsule gun, with two 65% chromium QS capsules (3.0 cm pressed tablet core, 9.00 mm orifice; Captee (NZ) Ltd, Auckland), and the groups immediately allocated to continuously grazed pasture swards representing high (H), medium (M) or low (L) herbage allowances. Two CRC were administered to each ewe to enable between-capsules within-sheep variation in capsule performance to be assessed. The respective pasture masses and average compressed heights of the swards were: H.  $\geq 1250$  kg DM/ha,  $\geq 5.5$  cm; M, 850-1100 kg DM/ha, 3.5-4.6 cm; L, 435-650 kg DM/ha, 1.6-2.6 cm. These herbage allowances equated to <u>ad libitum</u>, maintenance (M) and 0.7 M intakes respectively (Rattray et al. 1987). Average pasture height was recorded three times weekly (80 readings/paddock) with an Ellinbank Pasture Meter (Earle and McGowan 1979). The relationship between pasture masses (y) and height (x) was assumed to be:

y = 79 + 223x; n = 52, r = 0.947

This regression equation was derived from calibration pasture cuts taken from similar swards in an adjacent grazing trial midway through the present experiment (see Chapter Eleven). Sward conditions were maintained on the L and M treatments by grazing additional sheep with the treatment animals. This was not critical for the H allowance where ewes were on <u>ad libitum</u> feed intakes.

One ewe from each group was slaughtered between 1400 h and 1600 h on each of days 4, 8, 12, 16, 21 and 24 after the administration of CRC. CRC were recovered from the rumen within 10 minutes of slaughter and the position of the plunger was measured by callipers (Mitutoyo, Tokyo) to within 1 mm. Readings were taken from opposite sides of each barrel to account for any uneven plunger displacement. A sample of rumen contents (c.50 ml) was retained from each ewe before the rumen paunch and reticulum were washed out with water and the wall linings inspected for signs of capsule wing damage. The rumen sample was freeze dried to determine DM content. Duplicate 1.0 g samples of the dried material were ashed overnight at 550°C to measure OM content. These data provided an indication of the "abrasiveness" of the

#### rumen contents.

Three MA rumen-fistulated wethers  $(77.9\pm 5.9 \text{ kg})$  were each fitted with a single 65% chromium 3.0 cm QS capsule (of the same specifications as those used in the ewes), on the same day as administration of capsules to the ewes. The capsules were attached by nylon string to the cannula plug (Harrison et al. 1981). The wethers were then grazed with the M group for the following 24 days. CRC were recovered from the wethers at 1100 h on each ewe slaughter date and plunger displacement was measured in the same manner as described for those recovered by slaughter. The capsules were measured directly out of the rumen environment and were returned within two minutes to minimise the effects of changes in temperature.

#### Statistical analysis

The mean plunger travel data for each ewe from the date of insertion  $(day_0)$  was initially fitted to a univariate regression model (y=a+bx) to estimate the linearity of chromium release rate within a herbage allowance. A second regression model including terms for date of slaughter  $(d_i)$ , feeding level  $(l_j)$ , and an interaction term  $(dl_{ij})$  was used to test for homogeneity of regression lines between the different herbage allowances (Snedecor and Cochran 1967). For the second model each CRC was assumed to represent an independent observation of plunger travel, the error variance being that between CRC within sheep plus the between-sheep variation. The same test of homogeneity of regression lines was used to compare plunger travel data from the M ewes and the fistulated wethers. Within-sheep differences in plunger travel were expressed as a coefficient of variation (CV, %) and were also analysed for homogeneity of variance using Bartlett's test. Regression analyses were conducted using the 'REG'statistical package (Gilmour 1985). Other analyses were performed with the 'SPSSX' programme (SPSSX 1983).

# RESULTS

#### Recovery of CRC and Condition of Rumen Contents

All except two capsules, one per ewe on two slaughter dates from the L group, were recovered at slaughter. One missing CRC was found in the drenching race, apparently having been regurgitated by the ewe soon after administration. The remaining capsule was not discovered. No obvious evidence of capsule wing damage to the recticulo-rumen was found in any of the ewes, although the reticular lining in one ewe was marked at one site. However, this damage was not consistent with the normal pattern of CRC injury, where opposing walls of the reticulum are both marked by muscular contractions of the reticulum against the extended wings of the capsule. The different feeding levels resulted in rumen contents which differed significantly (P < 0.001) between groups in the percentage of OM (Table 8.1). Thus, the intake of soil was apparently inversely related to the height of the pasture grazed. The percentage DM of rumen contents, although not significantly different, showed the same trend.

Fceding level	DM (%)	OM (%)
Low	10.38 ± 0.58	77.78 ± 1.23
Medium	9.37 ± 0.28	81.82 ± 0.70
High	9.19 ± 0.23	84.17 ± 0.36
Significance <sup>a</sup>	NS	***

**Table 8.1** Proportions of dry matter (DM) and organic matter (OM) (mean ± sem) in rumen contents from ewes grazed at different feeding levels, experiment 9.

<sup>a</sup>Significance of feeding level effect (by ANOVA).

# Rate of CRC Plunger Travel

Average daily plunger travel (d 4 to d 24) was 0.95, 0.98 and 0.99 mm/d for the L, M and H herbage allowances respectively (Table 8.2). This equates to chromium (Cr) release rates of 136, 139 and 142 mg/d, or a 4% difference between the feeding level extremes.

**Table 8.2** Regression analysis of plunger travel (y) on time (x) at different herbage allowances and estimated average daily release rates of chromium from CRC in intact ewes and rumen-fistulated wethers, experiment 9.

Feeding level		Chromium releasc			
	n	а	b	r	mg Cr/d
Low	6	-0.887 ± 0.626	$0.951 \pm 0.040$	0.995	136
Medium	6	-0.598 ± 0.740	$0.979 \pm 0.047$	0.995	139
	6 <sup>c</sup>	-1.137 ± 0.222	$0.883 \pm 0.014$	0.999	126
High	6	$0.381 \pm 1.083$	0.994 ± 0.069	0.991	142

<sup>c</sup>Mean reading for CRC in three rumen-fistulated wethers.

Plunger travel was shown to differ significantly between feeding levels at the 5% level, but inspection of the mean square values indicated that virtually all (c.99.7%) of the variation in plunger travel was explained by the day of capsule recovery. The within-feeding level regressions of plunger travel on measurement date were not significantly heterogeneous. Despite the regression lines being based on data from 6 different animals, the linearity of chromium release was very high ( $r \ge 0.991$ ).

#### Plunger Travel in Rumen-Fistulated vs Intact Sheep

The average daily rate of plunger travel in the rumen-fistulated wethers from d 4 of 0.88 mm/d (126 mg Cr/d) was significantly (P<0.001) lower than the 0.98 mm/d (139 mg Cr/d) recorded in the M ewes i.e an 11% difference (Table 8.2). Differences between sheep type in the regression coefficient of plunger travel on time were also close to being significant (P<0.1). The correlation (r) between plunger travel and date of measurement in the fistulates of 0.999 indicates that the rate of chromium release was linear between days 4 and 24 of grazing. The marginally lower correlation (r=0.995) in the M ewes includes the effects of different capsules and sheep.

### Variation Between CRC Within Ewes

The mean CV between plunger displacements of CRC within sheep was 2.5, 6.2 and 5.8% respectively in the L, M and H feeding level groups (Table 8.3). In one instance teeth damage to the CRC barrel during administration may have contributed to a high CV between CRC. Capsule plunger travel exhibited a similar level of variation within each feeding level and variation in plunger travel did not significantly increase through time (i.e. variances were not significantly heterogeneous by Bartlett's test).

	-	Feeding level			
Day of slaughter	L	М	Н		
4	0.0	9.0	2.0		
8	_a	3.0	7.9		
12	_a	16.0 <sup>b</sup>	7.0		
16	6.0	5.0 <sup>b</sup>	2.0		
21	1.0	1.0	15.0		
24	3.0	4.0	2.0		
Mean (± sem)	2.5 <sup>c</sup>	6.2	5.8		

Table 8.3 Within sheep variation (CV, %) in plunger displacement between two chromium CRC recovered by slaughter, experiment 9.

<sup>a</sup>Only one CRC recovered at slaughter.

<sup>b</sup>CRC barrel damaged by teeth during administration.

<sup>c</sup>Bartlett's test of homogeneity of variance between feeding levels and within slaughter days across feeding levels indicated variances were not significantly heterogenous.

DISCUSSION

This experiment has demonstrated that feeding levels ranging from approximately 0.7 M to <u>ad</u> <u>libitum</u> for non-lactating ewes continually grazed at pasture significantly affected of the rate of chromium release from intraruminal CRC. Thus, release rates increased with the amount of herbage available to the ewes. This was probably associated with differences in rumen DM and OM contents between groups on different sward types. In practice the 6 mg Cr/d (4%) difference between release rates at the lowest and highest levels of intake recorded in this trial could mean that faecal output would be incorrectly estimated by c.2% because of a feeding level effect if an overall average rate of chromium release was used in calculations. This error is smaller than the between-sheep CV of 4-8% in chromium release rates when sheep are grazed together (Ellis and Rodden 1987). The results confirm those of the slaughter trials reported in Chapter Six which showed that if feed intakes are reduced to very low levels (<0.6 M) for 5-7 days, capsule performance will be compromised because of changes in the rumen environment. These low levels of intake may be encountered in high stocking rate rotational grazing experiments with 7-10 day intervals between pasture shifts (Sheath 1982).

The effects of herbage allowance on low recoveries of CRC chromium from the facces of sheep, as reported in Chapters Four and Seven, could therefore be due to a combination of the change in rate of chromium release and differences in the passage of chromium from the rumen to the anus (Lambourne 1957b).

Chromium release rates were 11% lower in the CRC inserted into rumen-fistulated wethers than those in the intact ewes when both groups of animals were grazed together. Gender differences are unlikely to have contributed to this difference because both ewes and wethers were in a similar physiological state during the trial. The lower rate of plunger travel in the fistulated animals could be explained by the different gaseous conditions in the rumen of fistulated sheep vs intact animals (Laby 1986). This result confirms the earlier findings and indicates that plunger travel data obtained from fistulated sheep should be used with caution in intake studies. However, if differences in plunger travel between fistulated and intact animals are consistently lower by 10-13% (as suggested in Chapter Six and by Laby (1986)), then it would be possible to adjust travel by a correction factor of around 1.12. Further work is also required to establish whether it is appropriate to extrapolate the plunger travel data from rumen-fistulated sheep grazed at a CRC testing station to an experimental site at a different location.

The loss of two CRC from the ewes slaughtered and teeth damage to two capsule barrels can be attributed to the dosing procedure used, although both the technique and equipment were as recommended by the manufacturer. The recommended procedure of releasing CRC at the back

of the tongue has two potential disadvantages. First, the sheep is able to bite and damage a capsule if it becomes dislodged from the gun during application. In some cases it will be possible to recover the damaged CRC before swallowing and replace it with a new capsule, but in other cases (as in this trial) it may not be apparent that damage has occurred. It should be noted that not all teeth damage to the barrel will affect CRC performance. The second disadvantage of "back of the tongue" application is that an "apparently" swallowed capsule can be regurgitated from the oesophagus some time after dosing. Australian experience suggests that this happens with increased frequency where more than one capsule is being administered (Rodden 1989). Waiting for a short period after dosing (c. 1 min.) to ensure that the capsule has been retained in the rumen is a worthwhile practice. Ocsophageal application using a flexible and soft rubber hose with an end configuration that protects most of the CRC until it is foreibly displaced at the thoracic inlet overcomes both of these problems. However dosing by this method is slower and is physically more demanding on both the operator and the sheep (see Chapter Five).

In conclusion, the plunger travel data obtained in this study provide further evidence of the efficacy of CRC for delivering chromium uniformly and safely into the rumen. This suggests that if all chromium is recovered in the facees an accurate estimate of faecal output in grazing animals should be obtained. In the next Chapter a comparison between faecal output measured by total collection and estimated by faecal chromium concentration is described.

# **CHAPTER NINE**

# INTRARUMINAL CHROMIUM CRC FOR MEASUREMENT OF FAECAL OUTPUT BY SHEEP AT PASTURE

# INTRODUCTION

Indoor feeding experiments involving sheep fitted with intraruminal chromium controlled release capsules (CRC) indicate that faecal outputs can be reliably predicted from the concentration of chromium in the faeces (Laby et al. 1984; Chapters Five and Six). No comparable studies of the application of CRC for predicting faecal outputs in sheep at pasture have yet been published. The objective of the experiments reported in this Chapter was to determine the suitability of CRC for estimating faecal output and feed intake in sheep grazed on pastures of different heights.

In addition the sward ring faccal sampling method (Raymond and Minson 1955), which has advantages of lower labour requirements and less animal disturbance, was compared with sampling facces per rectum as a means of estimating faccal output.

MATERIALS AND METHODS Experiment 10 Animals

Ten eight-month old Romney and Romney cross ram lambs (average liveweight  $39.4 \pm 0.8$  kg; mean $\pm$ sem) were divided into two groups of 5 and introduced to the experimental area on April 28, 1988 (d 0). On the same day each sheep was fitted with a faecal collection bag harness, dosed with a single 65% chromium CRC (3.0 cm pressed tablet core, 9.00 mm orifice, Mark II design) and drenched with an ivomeetin-based anthelmintic for internal parasite control.

The ram lambs in group 1 were set stocked onto a medium (M) pasture allowance treatment, and those from group 2 onto a high (H) pasture allowance, four days after capsule administration (see Table 9.1 for details of sward characteristics). Faecal collections, with plastic bag linings changed at 0900 and 1600 h cach day, then commenced for a 3 day animal familiarisation period. The initial 5 day faeces collection period (period 1) began on the sixth day after capsule insertion. Period 2, also of 5 days duration, commenced on d 13, 2.5 days after the groups switched grazing treatments.

A third collection period of 8 days (period 3), with all animals grazed together on the H pasture allowance, commenced 18 days after capsule administration. During this period one group was not bagged while the other group was fitted with faecal collection bags on alternate days. Faeces from the unbagged animals were collected at 0900 h each day from within 12 rings Faeces from the unbagged animals were collected at 0900 h each day from within 12 rings marked by pegs (2m radius, 3 per quarter and equivalent to 3.4% of the sward area). Rings were randomly allocated within each quarter of the plot. Thus faecal output was measured by bags on 4 days and estimated indirectly from the chromium concentration in the faecal ring samples on 4 days for each group during period 3.

Morning (0900 h) and evening (1600 h) faecal samples, equivalent to rectum grab samples (which are not readily obtained on a routine basis from immature sheep), were collected from the top of the excreta at each bagging change during the middle 3 days of periods 1 and 2, and daily from each bagged group during period 3. These samples were individually identified as  $\underline{am}$  and  $\underline{pm}$  samples.

Ram lambs were weighed at the commencement of each grazing treatment and prior to slaughter 26 days after capsule insertion. CRC were recovered at slaughter from each animal and plunger displacement from the time of insertion was measured by dial callipers (Mitutoyo, Japan). Reticulum and rumen wall linings were inspected for evidence of capsule wing irritation.

#### Pasturcs

Two 0.45 ha plots of ryegrass-browntop-white clover pasture were prepared to represent medium and high pasture allowances over a 4 week period prior to the commencement of the trial. This included the removal of all stem material by forage harvester. Pasture mass was measured by nine 0.24  $m^2$  quadrats (3 per quarter) cut to ground level with a shearing handpiece on d 7 and d 18 of the trial. The harvested material was washed and oven dried at  $80^{\circ}$ C for 24 h to determine DM yield per hectare. These pasture cuts were also used to calibrate Ellinbank Pasture Meter (EPM) height readings in order to estimate DM yield. EPM readings (50 readings/plot) were recorded twice weekly during the experimental period. Average green leaf contact height was recorded on the same days using an HFRO sward stick (45-60 readings/plot). EPM and sward stick measurements were always taken across the same diagonal of each plot.

Sward composition was determined by partitioning approximately 50 g of fresh herbage, cut to ground level and collected at random across the plots (days 7 and 18), into grass, clover, weed and dead components. Material which was more than 50% senesced was classified as being dead (Thomas 1980).

#### Herbage digestibility

Herbage extrusa samples were collected from 4 oesophageally fistulated wethers (OF:2 wethers/treatment/collection period) during each period. One of each pair of wethers was switched between treatments within periods to obtain an estimate of between-animal variation in herbage digestibility estimates. Extrusa was collected, into clear plastic bags fitted around the neck of each OF, following the procedure outlined by Hodgson and Rodriguez (1971). Collection of a 50 to 200 g sample took 10 to 15 minutes, but for the medium treatment this required the OF to be grazed on a low pasture allowance for 12 h prior to sampling. Extrusa (including saliva) was sealed in plastic bags and placed into crushed iced immediately after removal from the OF to minimise biodegradation of the sample. Samples were stored frozen before being freeze dried then ground through a 1.00 mm sieve for <u>in vitro</u> digestibility determination using a method modified from Roughan and Holland (1977). Samples were assayed against 6 pasture standards of known <u>in vivo</u> digestibility collected from wether sheep fed indoors.

A subsample of extrusa (c. 2 g/sheep) was dissected by point analysis (see Appendix IV) into the same categories used for botanical composition for each collection. A count of 100 points was made for each duplicate sample.

#### Faccal chromium analysis

Faeces from the two bagged collections each day were bulked and weighed to determine the fresh weight of total daily output. Duplicate sub-samples of 120-180 g were oven dried at 80°C over a 72 hour period to a constant weight. Approximately 50 g of the dried sample was retained for chromium analysis using the procedure described in Chapter Two. Sward ring samples from period 3 were bulked within days but otherwise were processed by the same routine.

Faeces were prepared for analyses by different methods, as detailed below, to identify the most effective means of estimating faecal output.

 Across days within periods for individual animals (BULK-G): Sub-samples of 30 g faecal DM from each animal's daily output were combined across the 5 days of periods 1 and 2. The bulked sample was ground through a 1.00 mm sieve (Cranston, England) and 1.00 g duplicates from this composite were analysed for their chromium content. Intact facces were bulked on the same basis (1 g/sheep/day) prior to assay and the 5.0 g composite was digested to determine chromium content (BULK-I):

- 2. Daily estimates for individual animals within periods (DAILY-I): One gram sub-samples of intact faeces from the bagged daily collections of each animal were analysed for each day within each treatment period. Individual daily estimates were averaged within periods to provide mean output per period (g/animal/d; DAILY-C).
- 3. Simulated "rectum" grab samples (AM-PM): Morning and evening simulated "rectum" grab samples, collected as described previously, were analysed separately for individual animals within days and periods. These data also provided an estimate of diurnal variation.

Analyses for chromium were repeated for samples where the CV between duplicates exceeded 5%, or where the calculated recovery of chromium lay outside the range 85 to 115%. The recovery of chromium and daily release rate of chromium were determined by the formulae detailed in Chapter Three.

To estimate chromium recoveries for the sward ring samples, the mean group bagged faecal output by the same animals on the day prior to sward collection was used as a proxy for the weight of facces produced on the day of ring sampling.

Predicted group mean daily herbage organic matter intakes during periods were derived using the corresponding mean in vitro digestibilities of the OF extrusa samples as follows:

OMI	=	<u>(FO-S)</u>
		1-OMD

where	OMI	=	predicted herbage OMI (g/d)
	FO	=	$faecal\ output\ predicted\ from\ the\ concentration\ of$
			chromium in the faeces (g/d)
	S	=	Soil OM content of faeces (see Appendix IV).
	OMD	=	in vitro OM digestibility of herbage

# Experiment 11

# Animals

Two groups of eighteen month (3 per group) and mixed age (4 per group) first cross Border Leicester-Romney ewes were selected from the animals used by Walsh (1989) in an earlier study (see Appendix III). The average liveweights of groups 1 and 2 were  $62.4\pm 2.4$  kg and  $62.9\pm 2.1$  kg respectively on April 29, 1988 (d 0), when each ewe was dosed with a single chromium CRC (3.0 cm pressed tablet core, 65% chromium matrix, 9.00 mm orifice, Mark II design). Group 1 animals were then assigned to the H herbage allowance and Group 2 to the M herbage allowance (d 0). Herbage allowances are described below. One mixed age ewe was removed from the treatment on d 3 of the trial to maintain sward height but no further adjustments were made to stocking rates over the ensuing 16 days. Two ewes (1 eighteen month and 1 mixed age) were removed from the H plot from d 17 until the end of the trial (d 22) for the same reason.

Facces were collected either from the rectum at 0830 h each day or from the sward surface around six rings of 2.0 m radius marked by pcgs in each of the plots. These collections were divided into two periods of 5 days and one period of 4 days, commencing on days 6, 12, and 18 of the experiment (periods 1, 2 and 3 respectively). Faeces from the sward rings were oven dried at 80°C over at least 72 h until they reached a constant weight. The total daily faecal DM output per plot was then recorded. The entire dried sample from each day's collection was ground through a 1.00 mm sieve (Cranston, England) and thoroughly mixed. A subsample of c. 50 g of the ground material was retained for chromium analysis.

<u>In vitro</u> digestibility of herbage was estimated from extrusa collected from oesophageally fistulated wethers. Collections were made from 2 wethers during each period using the technique described for Experiment 10.

#### Pasturcs

Two 0.45 ha plots of predominantly rycgrass (L. perenne), browntop (A. tenius) and white clover (T. repens) swards were prepared to represent high (H) and medium (M) pasture allowances for ewes over a 4 week period prior to the commencement of the trial. The H and M swards were grazed to maintain uniform average EPM heights of 5.0 and 3.0 cm respectively. EPM readings (50 per plot) were recorded every 3-4 days. Pre-treatment herbage mass in each plot was determined on d 0 from the mean of nine 0.24 m<sup>2</sup> quadrats cut to ground level with an electric shearing handpiece. At the same time samples were collected from sites adjacent to the quadrat cuts to determine the botanical composition (% dry weight) of the swards. The height of first green leaf contact was measured by taking 50 readings on each plot with an HFRO sward stick (Bartham 1986) on the same day that EPM height readings were recorded.

#### Statistical Analysis

Predicted faecal outputs were compared with bagged outputs by pairwise Student's t-tests using the SPSSX routine (SPSSX 1983). Diurnal variation was expressed as a percentage deviation (CV%) from the mean <u>am</u> and <u>pm</u> readings and analysed by a split-plot model which included terms for group, animal, pasture allowance, day, sampling time and their interactions. Level, day, and time effects were tested against the level x animal x group, day x level x group and time x day x level x group mean square values respectively.

#### RESULTS

#### Experiment 10

#### Sward conditions and herbage digestibility

Pasture EPM heights were maintained at an average of c. 2.8 and 4.5 cm respectively during the trial (Table 9.1), but pasture mass declined on the H sward during period 2 as the effect of selective grazing by the ram lambs decreased the uniformity of the sward. The proportion of dead material was more than twice as great on the M sward compared with the H sward during both treatment periods. This was reflected in a higher intake of dead material by the OF wethers, and lower <u>in vitro</u> digestibility values for this pasture than on the H sward. Although the clover content of the H treatment was lower, OF wethers appeared to consume a higher proportion of clover than on the shorter pastures of the M treatment. This response can probably be attributed to the larger and more erect clover plants in the H sward.

**Table 9.1** Sward characteristics of high and medium grazing allowance treatments, experiment10.

Grazing Allowance					
Mo	cdium	High			
1	2	1	2		
2.94	2.71	4.61	4.54		
3.11	2.93	4.86	4.79		
1395	1293	2407	1753		
39.5	39.2	75.8	70.6		
2.6	3.0	2.2	2.2		
0.0	0.0	1.1	0.0		
57.9	57.8	21.0	27.2		
18.68	18.16	15.99	14.71		
71.10	73.39	76.28	75.66		
61.97	64.25	68.16	68.21		
74.91	76.83	79.46	78.92		
63.0	73.8	68.5	88.3		
1.5	1.5	4.5	2.0		
0.0	0.0	1.5	0.0		
35.5	25.0	25.5	10.0		
	1 2.94 3.11 1395 39.5 2.6 0.0 57.9 18.68 71.10 61.97 74.91 63.0 1.5 0.0	Mcdium           1         2           2.94         2.71           3.11         2.93           1395         1293           39.5         39.2           2.6         3.0           0.0         0.0           57.9         57.8           18.68         18.16           71.10         73.39           61.97         64.25           74.91         76.83           63.0         73.8           1.5         1.5           0.0         0.0	McdiumH121 $2.94$ $2.71$ $4.61$ $3.11$ $2.93$ $4.86$ $1395$ $1293$ $2407$ $39.5$ $39.2$ $75.8$ $2.6$ $3.0$ $2.2$ $0.0$ $0.0$ $1.1$ $57.9$ $57.8$ $21.0$ $18.68$ $18.16$ $15.99$ $71.10$ $73.39$ $76.28$ $61.97$ $64.25$ $68.16$ $74.91$ $76.83$ $79.46$ $63.0$ $73.8$ $68.5$ $1.5$ $1.5$ $4.5$ $0.0$ $0.0$ $1.5$		

<sup>a</sup>The regression of pasture mass (y) on EPM height reading (x) was:

y = 330 + 368 x, r = 0.94, n = 38.

<sup>b</sup>Average of quadrat cuts on days 7 and 18 respectively.

Plunger travel (d 0 to d 26) and expected daily quantities of chromium (Cr) released are summarised in Table 9.2. Mean plunger travel was 16.75% (P<0.001) faster in group 1 animals which were initially assigned to the H pasture allowance. The pattern of chromium recovery from twice-daily rectum grab samples taken during period 3 from the four sheep with empty capsules indicated that the tablets expired within the final 24 h of the trial. Overall chromium release rates were estimated on this basis. The chromium matrix in the CRC of sheep 7 had travelled 15 mm, but had subsequently croded internally. The release rate of chromium in sheep 7 must therefore have differed substantially from that of the other sheep during the trial.

Group Chromium Reticulum Grazing Livc-Plunger travcla rclcasc injury Scquence Shccp Wcight (mg Cr/d)(mm)(**k**g) Minor MHH 1 37.5 30.5 167 1 2 30.5 40.0 167 3 42.0 30.5 167 4 37.0 30.5 167 5 26.0 143 36.5 26.0 143 42.5 6 Ycs 7 37.5 2 НМН 8 37.5 26.0 143 Yes 26.0 Ycs 9 143 40.0 Minor 26.0 10 43.0 143 Mean (scm) 39.4 (0.8)

**Table 9.2** Plunger travel in CRC from insertion until slaughter and daily chromium release rates, experiment 10. Evidence of capsule injury to the reticulum and pre-treatment (d 0) liveweights are also shown.

<sup>a</sup>All capsules had an initial plunger reading of 30.5 mm.

Within-group CV for plunger travel was 6.7% and 0.0% (excluding sheep 7) in groups 1 and 2, respectively.

Minor surface damage to papillac on opposing walls of the reticulum indicated that contractions had occurred against the CRC wings. This action depresses voluntary herbage intake (see Chapter Five). It is not clear why more evidence of reticulum damage was found in the group 2 animals or why plunger travel was more rapid in CRC in group 1 sheep.

#### Recovery of faccal chromium

The percentage recoveries of chromium from the faeces during periods 1 and 2 are presented in Table 9.3. Recoveries of chromium from samples tested at a CSIRO laboratory in Australia (Ellis 1988) were not significantly different from those analysed at Massey University. The mean recoveries for groups within periods suggest that an interaction between faecal chromium concentration and level of herbage intake occurred.

Table 9.3 Recovery of chromium from BULK-G faecal samples, experiment 10. Figures inbrackets are the recoveries of chromium for samples analysed by CSIRO, Australia.

		Group 1			Group 2			
Shccp		iod 1 edium)	Period 2 (High)	Ѕһсср	Pcrio (High		Pcriod 2 (Mcdium)	
1	91.6	(99.8)	105.5	6	103.8	(87.7)	97.0	
2	93.8	(96.4)	102.5	7	95.8	(92.5)	84.4	
3	93.2	(103.9)	112.1	8	126.8	(136.8)	82.7	
4	96.5	(98.0)	112.2	9	129.4	(140.7)	100.0	
5	93.4	(89.6)	118.4	10	109.7	(117.7)	100.3	
Mean	93.7	(97.5)	110.2		111.1	(115.1)	91.5	
Overall 1	nean	101.2				103.0		

#### Within-day variation in chromium concentration

The variation between morning and afternoon faccal chromium concentrations for individual animals exceeded 10% on 16 of the 54 days in which samples were collected (Table 9.4). Differences between <u>am</u> and <u>pm</u> sampling times within and across days at each feeding level were not significant for both groups. The effect of pasture allowance was significant at P < 0.1. No consistent trend for individual animals was evident but variability was greater on day 14 in group 1 animals. This probably reflects the fact that faceal chromium had not stabilised at a new steady state (see Chapter Seven) following the transfer of these animals from the medium to high feeding allowance. Consistently lower chromium concentrations for the afternoon of d 14 support this view.

**Table 9.4** Mean within-day coefficient of variation (CV,%) between  $\underline{am}$  and  $\underline{pm}$  faecal chromium concentrations, experiment 10 (sheep 7 is excluded).

		Day of rectal sampling					
	8	9	10	14	15	16	
Group 1	7.92	6.71	7.75	17.89	6.68	5.75	
Group 2	7.83	11.99	11.24	7.56	7.89	3.94	

Prediction of faecal output

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# 1. BULK-G, BULK-I and DAILY-C Estimates

The faecal outputs for individual animals predicted from chromium concentrations for different types of facces samples are summarised in Table 9.5. Predicted group means within periods were within  $\pm$  12.2% of the values obtained by total collection. DAILY-C samples provided the most consistent estimates of faecal outputs for individual animals.

Table 9.5 Actual and predicted faecal outputs (g DM/d) over 5 days from daily samples of intact faeces (DAILY-C), and faccal samples bulked across days (BULK-G and DAILY-I), experiment 10. Figures within brackets are the predicted values expressed as a percentage of actual (bagged) mean faecal outputs.

	Gro	up 1	Group 2		
	Pcriod 1	Period 2	Period 1	Period 2	
Actual faccal output	342±19	356±11	353±20	255±21	
Predicted faecal output	ıt <sup>a</sup>				
DAILY-C	$343 \pm 16$	361±21	338±25	$271 \pm 16$	
	(100.5)	(101.4)	(95.8)	(106.3)	
BULK-G	$354 \pm 20$	326±16**b	327±31	277±15	
	(103.5)	. (91.6)	(92.6)	(108.6)	
BULK-I	366±17	389±28* <sup>b</sup>	337±29	281±17	
	(107.0)	(109.3)	(95.5)	(112.2)	

<sup>a</sup>See Materials and Methods for definition of sample preparation.

<sup>b</sup>Significance values refer to paired t-tests of differences between predicted and actual outputs. All other differences were non-significant.

#### 2. Prediction of Faecal Output from Rectum Grab Samples

The predicted and actual daily faecal outputs for alternative rectum grab sampling routines over the final 3 days of period 1 and period 2 are shown in Table 9.6. Predicted faecal outputs for the less intensive rectum sampling routines (3 samples) were similar to those derived if grab samples were obtained twice-daily, except for group 2 animals in period 2. Overall these results indicate that the effects of diurnal variation on the estimation of group mean faecal output were small.

Sampling	Samples	Group 1	(n = 5)	Group 2	(n=4)
routine	(n)	Period 1	Period 2	Period 1	Period 2
Actual	3	352±13	355±9	366±15	241±13
Predicted					
AM-PM	6 <b>a</b>	$360 \pm 13$	334±15	367±21	274±17**
		(106.3)	(94.1)	(100.3)	(113.7)
AM-PM-AM	3	348±12	341±16	366±26	267±17
		(98.9)	(96.1)	(100.0)	(110.8)
PM-AM-PM	3	373±14+ <sup>b</sup>	326±12**	368±34	282±17*
		(106.0)	(91.8)	(100.6)	(117.0)
AM only	3	356±12	346±17	$364 \pm 32$	$268 \pm 18$
	2	(101.1)	(97.4)	(99.4)	(111.2)
PM only	3	363±15	322±18**	$370 \pm 30$	280±16*
	5	(103.1)	(90.7)	(101.1)	(115.7)

**Table 9.6** Actual and predicted group mean faecal outputs (g DM/d) for alternative 3-day rectum grab sampling routines, experiment 10. Figures in brackets are predicted values expressed as a percentage of actual (bagged) values.

<sup>a</sup>Number of samples collected per animal.

<sup>b</sup>Significance values refer to paired t-tests of differences between between predicted and actual (bagged) faecal output.

The poorer prediction of mean output in group 2 can be attributed mainly to the low recovery of chromium from the facees of sheep 8. Data for chromium recovery (Table 9.3) show the same effect. It therefore seems likely that some of the small hard pelleted facees produced by sheep 8 were not collected, resulting in underestimation of true faceal output for this animal.

# 3. Prediction of Faecal Output by Sward Ring Sampling

Group mean daily bagged faecal outputs of group 1 and 2 animals during period 3 are summarised in Table 9.7. Differences between groups over the 4 collection days were small and non-significant, indicating that the presence of the collection bag did not affect intakes during this period. Daily faecal outputs across the 8 days of period 3 were uniform (CV = 4.4%) and, although average pasture height declined from 4.3 to 3.6 cm during this time, the increased ingestion of soil (as shown by the 5 to 7% rise in faecal ash content) compensated for this in terms of faecal DM production.

Daily faecal outputs predicted from the chromium concentration of sward ring samples were not significantly different from the actual (bagged) outputs (Table 9.7), indicating that the sward sampling technique can be used successfully with CRC. This is despite the low release rate in sheep 7, suggesting that either the faeces of sheep 7 made a relatively small contribution to the total ring sample each day or that higher than average CRC release rates occurred in some of the remaining sheep in group 2. Because the slaughter data indicated that CRC performance could be unreliable during the final two days of the trial, 10 g DM of ground faecal material from the sward rings was bulked across the first 3 days of collection for each group. Predicted faecal outputs for these chromium analyses were within  $\pm 4.3\%$  of the mean measured outputs for both groups.

Table 9.7 Faecal ash contents, and actual (bagged) and predicted group mean faecal outputs (g DM/d) for sward ring sampling (sward) on individual sampling days, experiment 10. Faecal outputs predicted from <u>am</u> and <u>pm</u> rectum grab samples (grab) on the days of total collection are also shown. Figures in brackets are predicted values expressed as a percentage of actual (bagged) output.

Group Day	Ash	Fa	Faccal output measurement			
	(%)	Actual	Sward	Grab		
1	20	19	357	378	422	
	22	21	347	344	315	
	24	22	347	344	338	
	26	26	402	398	343	
Mean		22	363 <sup>a</sup>	366	355	
				(100.8)	(97.7)	
2	19	19	357	371	386	
	21	19	358	333	363	
	23	21	342	308	432	
	25	24	384	380	371	
Mean		21	360	347	388	
				(96.4)	(107.8)	

<sup>a</sup>All differences between faccal sampling methods and between groups were non-significant.

Rectum grab samples (0.5 g/collection) were bulked for morning and evening collections across sheep in each group for each of the 4 days in period 3 (Table 9.7). Faecal samples for sheep 7, which had chromium concentrations that were approximately 50% of those for the remaining sheep in group 2, were included. This provided a measure of faecal chromium comparable with that obtained by sward ring samples the following day. Estimates of mean faecal output provided by rectum grab samples were not significantly different from those obtained by sward ring sampling or bagging. Average diurnal variation during period 3 was much lower in group 2 animals (3.6% vs 20.2% in group 1) because capsules were still in a "linear" release phase at the time of faeces collections.

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# Herbage intake estimated from total faecal collections and predicted faecal output

Herbage intakes of ram lambs (Table 9.8) were derived from actual faecal outputs and from faecal outputs estimated using the concentration of chromium in rectum grab samples (Table 9.5) together with the mean in vitro digestibility of extrusa collected from the oesophageal fistulated wethers during each grazing period (Table 9.1). The correlation (r) between the 18 estimates derived from total collection and rectum grab sample  $Cr_2O_3$  concentration estimates was 0.87. Group mean estimates of OMI were therefore similar and differed by a maximum of 8.6% in group 1 animals while on the H allowance. Intakes on the M sward were equivalent to 0.9 to 1.1 M while those on the H sward were estimated to be 30-50% above maintenance requirements for a 40 kg ram lamb (Rattray 1986).

Table 9.8 Estimates of faecal output and feed intake based on total collection or the concentration of  $Cr_2O_3$  in rectum grab samples from ram lambs grazed at two pasture allowances, experiment 10 (sheep 7 is excluded).

Herbage allowance	Μ			Н		
Method	Total		Total		Sign. <sup>a</sup>	
	Collection	Cr <sub>2</sub> O <sub>3</sub>	Collection	Cr <sub>2</sub> O <sub>3</sub>	М	Н
Group 1 (n = 5)			_			-
Faecal output (g DM/d) Feed intake:	361±16	360±14	355±12	334±15	NS	+
- (g OM/d)	$1009 \pm 60$	1005±43	1262±43	1161±45	NS	ŵ
-(kg DM/d)	$1.20 \pm 0.03$	$1.20 \pm 0.04$	$1.45 \pm 0.03$	$1.33 \pm 0.03$	NS	*
- (MJ ME/d)	12.12 <sup>b</sup>	12.12	16.12	14.79		
Group 2 (n = 4)						
Faecal output (g DM/d)	241±213	274±17	366±27	367±43	+	NS
Feed intake						
- (g OM/d)	739±76	818±57	1317±95	$1292 \pm 146$	NS	NS
-(kg DM/d)	$0.87 \pm 0.09$	$0.97 \pm 0.07$	$1.53 \pm 0.11$	$1.50 \pm 0.17$	NS	NS
- (MJ ME/d)	8.79	9.80	16.02	15.74		
		,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,				

<sup>a</sup>Significance values refer to paired t-tests of differences between actual faecal output and estimates derived from faecal Cr<sub>2</sub>O<sub>3</sub> concentration.

<sup>b</sup>Energy value of daily feed intake = (DOMD x 16.3 x DMI).

#### Experiment 11

# Sward conditions and herbage digestibility

Sward conditions and measures of herbage quality during experiment 11 are summarised in Table 9.9. Except for a slight decline in pasture mass and height during period 3 on the H treatment, pasture conditions were maintained in a uniform state during the trial. The <u>in vitro</u> digestibility of extrusa from the shorter pastures grazed by group 2 ewes was significantly (P < 0.05) lower than for the herbage samples collected from the area grazed by group 1.

		Group	1	Group 2		
Pcriod (P)	P1	P2	P3	P1	P2	P3
Sward characteristics						
Average EPM height (x;cm)	4.6 <sup>a</sup>	4.6	4.2	2.8	2.9	2.8
Average HFRO height (cm)	4.7	4.1	4.0	3.0	2.8	2.6
Pasture mass (kg DM/ha)	2023	2023	1876	1360	1397	1360
Pasture composition (% DM)						
Grasses	58.7	62.2	-	37.7	45.4	-
Clover	2.5	3.0	-	2.8	7.3	-
Wceds	1.0	0.5	-	0.0	0.6	-
Dead	38.1	34.8	-	59.5	44.7	-
In vitro digestibility (%)						
DMD	75.94	76.18	-	70.87	70.45	-
OMD	79.37	79.56	-	75.41	75.00	-
DOMD	68.22	68.66	-	61.96	63.84	-

Table 9.9 Pasture mass, composition and in vitro digestibilities of herbage, experiment 11.

<sup>a</sup>Average pasture residual =  $380 (\pm 107) + 368 (\pm 22)x, r = 0.94, n = 38.$ 

# Comparison between sward ring and rectum grab sampling

Differences in the mean chromium concentration in the faeces obtained either by sward ring sampling or per rectum were small (Table 9.10). The apparent OMI derived from the two sampling regimes were therefore similar (maximum CV = 5.4%). Contamination of sward ring samples by soil was low as indicated by the small variation in ash content of faeces collected by the two methods. This reflected the generally dry weather conditions experienced during the trial.

	(1) (1) (1) (1) (1) (1) (1) (1) (1) (1)	Period 1						Period	2	
	Gre	Group 1			up 2		Gro	up 1	Group 2	
	SR	RG		SR	RG		SR	RG	SR	RG
Faecal chromium										
(ppm)	6.89	6.60	(3.0)	5.87	5.77	(1.2)	6.15	6.33 (2.0)	5.97	5.87 (1.2)
Faecal ash										
(% DH)	20.3	22.5	(7.3)	20.3	22.9	(8.5)	23.9	21.2 (8.5)	22.5	20.8 (5.6)
Faeces										
(g DM/d)	378	410	(5.7)	445	469	(3.7)	426	423 (0.0)	437	445 (1.3)
Feed intake										
(g DM/d)	1462	1579	(5.4)	1372	1418	(2.3)	1638	1646 (0.3)	1324	1329 (0.0)

**Table 9.10** Comparison between sward ring (SR) and rectum grab (RG) sample estimates of faccal output and OMI, experiment 11. Figures in brackets are coefficients of variation between estimates.

A significantly (P < 0.01) greater dry weight of faeces was collected from the high allowance rings during periods 1, 2 and 3 (Table 9.11). The stocking rate on this plot was approximately 15% higher than on the M sward. The mean daily weight collected represented a minimum of 1.2% of the estimated mean total daily faecal output on each sward. This compares with a sampling fraction of approximately 0.25% under a once-daily rectum grab sampling routine.

 Table 9.11
 Total weight, DM content and proportion of daily output of faceal samples

 collected from sward rings on the high (H) and (L) pasture swards each day, experiment 11.

	Period 1		Period 2	2	Period 3		
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	
	Righ	Righ Nedium		High Medium		Nedium	
Paeces (g DM/d)	105 <u>+</u> 14	69 <u>+</u> 19	55 <u>+</u> 9	32 <u>+</u> 7	92 <u>+</u> 19	54 <u>+</u> 14	
(%DM)	21.7	25.4	17.1	20.1	20.4	18.9	
Sampling							
fraction (%)	14.3ª	2.6	1.9	1.2	4.3	2.0	

<sup>a</sup>Sampling fraction ~ mean daily sward ring sample weight (g DM/d) expressed as a percentage of mean daily total faecal output (g DM/).

Ring sites equivalent to 3.4% of the sward area therefore allowed faecal samples comparable to those collected per rectum (Table 9.6) to be obtained. These results indicate that the intensity of sward rings could be adjusted according to stocking rate with fewer sites being required at higher stocking rates.

## Predicted faceal outputs and feed intakes of ewes

The predicted faecal outputs and apparent OMI and DMI of ewes during experiment 11 are presented in Table 9.12. Daily mean intakes of OM and DM differed significantly (P<0.001) between groups only during period 2. Even at the low allowance, apparent intakes were approximately 45% above the estimated maintenance requirements of a 60 kg ewe during both intake measurement periods. The quality of pasture ingested was high in both swards (Table 9.9) probably because animals were stocked at a rate which enabled them to consume primarily new pasture growth.

	Period 1		Period	2
	Group 1	Group 2	Group 1	Group 2
Herbage allowance	High	Medium	High	Medium
Faecal chromium (ppm)	$6.60 \pm 0.31$	$5.77 \pm 0.33 + a$	$6.33 \pm 0.30$	$5.87 \pm 0.10$
Faecal Ash (% DM)	$22.46 \pm 0.55$	$22.94 \pm 0.44$	$21.17 \pm 0.23$	$20.80 \pm 0.23$
Faeces output (g D.M/d)	$410 \pm 26$	469 ± 27	423±18	445 ± 7
Digestibility (% OM)	79.37	75.41	79.56	75.00
Feed intake				
-(g O.M/d)	$1580 \pm 100$	1418±82	1646 ± 73	1329 ± 33 ***
$-(g OM/kg^{0.75})$	71.66 ± 3.22	64.43 ± 2.57 +	66.1±3.5	55.8±1.4 *
-(kg D.M/d)	$1.83 \pm 0.12$	$1.63 \pm 0.09$	1.97±0.09	1.52±0.04***
- MJ ME/d <sup>b</sup>	20.35	16.46	22.05	15.81
- xM <sup>c</sup>	1.8	1.5	2.0	1.4

Table 9.12 Predicted faccal outputs and apparent herbage intakes of ewes, experiment 11.

<sup>a</sup>Significance of differences between groups within periods by Student's t-tests.

<sup>b</sup>Energy value of daily feed intake = (DOMD x 16.3 x DMI).

<sup>c</sup>Feed intake as a proportion of maintenance requirements of a 60 kg ewe (11 MJ ME/d; Rattray 1986).

#### DISCUSSION

Group mean faecal outputs of ram lambs estimated from the faecal concentration of chromium, were between 91 and 117% of the values obtained by total collections. This result was achieved despite variable CRC performance which resulted in significantly faster matrix expiration in one group of animals, even though feed allocation to the two groups during the trial was similar. The CRC used in this study were manufactured some 15 months prior to their use and may not have received correct storage treatment during this period. In addition, the stiff-wing configuration of the Mark II capsule caused damage to the reticulum wall lining. This would have depressed feed intakes, as shown in Chapter Five, but any such effect should have been similar for both groups. The failure of the CRC in sheep 7 may have been precipitated by damage during dosing, although no obvious damage to the plastic barrel was apparent when it was recovered at slaughter. The capsule deficiencies noted in experiment 10 are less likely to occur in the commercially available QS version which has a more flexible wing configuration (Chapter Eight), but care should be taken to ensure that capsules are stored in a dark dry environment prior to their use (Laby 1986).

The apparent interaction between recovery of chromium and herbage allowance, evident in this and earlier trials (Chapter Four), may have occurred partly because plunger travel was influenced by 2 to 4% by the level of intake on the M treatment (Chapter Eight). Chromium recovery is also likely to have been affected because faecal sampling started 6 days after CRC administration and within 3 days of feeding level changes. Both of these events mean that faecal sampling probably occurred prior to a steady state concentration of chromium being achieved in the faeces of all the sheep (Chapter Seven). Presentation of chromium in the faeces would therefore have been influenced by feed intake through changes in the rate of rumen turnover and hence the rate of passage of faeces (Chamberlain et al. 1988).

The alternative methods of preparing faecal samples for chromium determination generated similar estimates of faecal output irrespective of the procedure followed, confirming the findings reported earlier for experiment 5. Bulking faeces across days reduced the number of samples assayed but variation between duplicates was greater with intact than with ground faeces. This is because identical daily sample weights are more difficult to produce with intact faeces. Intact faeces also require extra attention during ashing to ensure that there is complete removal of organic matter (see Chapter Two).

The generally low diurnal variation in faecal chromium concentration when CRC are used (see also Chapters Three and Seven) means that a flexible rectum sampling routine can be adopted to suit the availability of labour and to minimise disturbance of animals when CRC are used. Thus, if feeding levels are reasonably uniform between days and the capsule is established in its linear release phase, it should also not matter if faecal samples are obtained on alternate days or at less regular collection intervals. A 3-day sampling period, as was shown for indoor conditions (Chapter Five), provided satisfactory estimates of faecal chromium levels and hence faecal output. However, a 5-day sampling period would yield a more reliable estimate of faecal output since the variation in faecal output between days is often high.

Sampling of faeces from the sward provided an effective way of obtaining estimates of group mean faecal output in both experiments. This technique saves substantial labour and minimises animal disturbance, an important consideration when ewes with young lambs are being sampled or when the trials involve small animals for which rectum grab sampling is inappropriate. Furthermore, if sward ring collections are made over 15 to 20 days and the samples are analysed independently, the variation in predicted faecal output between days should theoretically equate to the variation between animals within days (Blair 1988).

The reliability of sward ring sampling in experiment 10 was based on a proxy for actual faecal output, namely the estimate derived by total collection from the previous day. Faecal output for the groups on the day of ring sampling may therefore have differed, but the low variation between mean outputs for the 4 days of total collection during period 3 (CV = 7.2% and 4.8\% respectively for groups 1 and 2) suggests that this was unlikely. Using the mean value of total faecal outputs for the days preceding and following ring collections provided no advantage for determining chromium recovery.

Sward sampling may reduce the number of samples for chromium analysis since facees of individual animals cannot be assayed. However, experimental designs with more replicates of plots may be required to compensate for the loss of faecal output data from individual animals. If sward ring sampling is adopted, a minimum programme of rectum grab sampling (of 3 days) is recommended to establish that CRC are delivering chromium correctly in individual sheep.

It is difficult to establish the validity of the herbage intake estimates obtained for the ram lambs in this trial because of the paucity of intake data reported for this class of animal under New Zealand conditions (Rattray 1986). In addition, the short treatment periods and variation in gut-fill between treatments precluded the reliable measurement of liveweight gain. However, Scales et al. (1981) estimated that lambs of 26 kg liveweight consumed 0.7 kg OM/d when grazing pastures of 1700 kg DM/ha (800 kg green DM/ha) with a low clover content. The 0.74-1.00 kg OM1/d estimates calculated for the medium allowance treatment for 40 kg hoggets therefore appear reasonable. During et al. (1980) suggest that lamb intakes will just exceed maintenance requirements during the autumn on swards with 800 kg DM/ha of live herbage and that this will increase to allow liveweight gains of around 100 g/d on pastures with 1400-1800 green DM/ha. The respective estimates of intakes of 1.0 to 1.1 M and 1.5 M for the medium and high allowances in the present study correspond to these findings.

The OMI of the high allowance ewes (c 1.5 kg OM/ewe/d) in experiment 11 equates to a DMI of 3.0% of liveweight. Under these conditions liveweight gains of 50 to 100 g/d could be expected (Rattray 1986), but liveweights recorded off pasture at the commencement and end of the trial (not corrected for gutfill) indicated that the 5 ewes which were on the high allowance throughout the trial lost 61 g/d. Ewes on the medium allowance lost 143 g/d over the same period. There are several possible explanations for the discrepancy between apparent energy intakes and measured liveweight gains. First, liveweights reflect differences in gutfill of up to 11% in bodyweight and these could easily mask the 0.5 to 1.0 kg increase in liveweight expected during the trial (Hughes 1976). Second, the "on-off" grazing sequence used for the oesophageal fistulated wethers to collect extrusa samples may have resulted in <u>in vitro</u> digestibilities being higher than the sward average (resulting in OMI being overestimated). Third, currently

published estimates of intake by ewes and their relationships with liveweight gain may not be correct. In addition, while no estimates of energy expended to harvest feed were obtained directly, visual observations indicated that ewes on the M sward spent considerably more time grazing than those on the H treatment. This would have increased their maintenance requirements relative to those of ewes on the H sward.

Ewe intakes obtained by the difference technique, at various pasture allowances suggest, that intakes of around 0.9 to 1.0 kg DM/cwe/d at a pre-grazing green herbage mass of 500 to 600 kg DM/ha (corresponding to the medium treatment) should have been achieved (Rattray and Clarke 1984). The estimated intakes of ewes on the M sward are therefore high relative to these findings. However, British studies with ewes continuously grazed at pasture suggest that their herbage intakes are higher than the New Zealand estimates for low sward heights (e.g. Hodgson and Maxwell 1984), possibily because ewes adapt to grazing at reduced herbage masses when continuously grazed (Wadsworth 1979).

In summary, the results of these experiments provide an indication of the accuracy with which voluntary herbage intakes can be estimated for groups of animals grazing a specified herbage allowance. If the maximum error in predicting faecal output using chromium CRC is  $\pm 10\%$  and that for herbage digestibility is  $\pm 3\%$  (Hodgson and Rodriguez 1971) predicted intakes will lie within  $\pm 20\%$  of actual intakes. Chromium CRC therefore provide group mean estimates of faecal output at least as good as those obtained through the traditional methods of chromium administration, but with substantially less labour and interference to animals. Reduced labour requirements and simplified management for marker administration will also permit the use of larger treatment groups of animals for the same input of resources. This factor, along with further refinements in CRC field trial management as described in Chapter Eleven, will enable the accuracy which which feed intake can be estimated to be further improved. In the next Chapter the application of CRC to the measurement of faecal output in ewes of different pregnancy and rearing status is described.

# **CHAPTER TEN**

# HERBAGE INTAKES AND PRODUCTIVE PERFORMANCE OF EWES OF DIFFERENT PREGNANCY STATUS AND REARING RANK:PILOT STUDY

# INTRODUCTION

New Zealand sheep farmers frequently face feed shortages during periods which coincide with late pregnancy and lactation in their ewes and so are required to make decisions as to how limited feed reserves should be allocated between competing livestock classes (Rattray 1978; Parker 1984). Information describing the production responses of animals to different herbage allowances and the likely financial consequences of differential feed allocation is important for the achievement of efficient grazing management during this period. However, relatively little is known about the relationship between herbage intakes and productivity of ewes of different pregnancy status or rearing rank under New Zealand pastoral grazing conditions (Rattray 1986). The primary purpose of the trial reported here was to characterise the pattern of feed intake of ewes bearing and rearing single and twin lambs from mid-pregnancy until nine weeks post-partum. The trial provided an opportunity to further evaluate Captec chromium controlled release capsules (CRC) in a longer term trial and under conditions more extensive than those normally encountered in herbage intake experiments. In both respects, the trial acted as a pilot study for a larger scale study of the interaction between feed allocation and rearing rank of ewes planned for the 1988 season (Chapter Eleven).

MATERIALS AND METHODS

Experiment 12

## **Pastures and animals**

The trial, conducted at Massey University's Sheep and Beef Cattle Research Unit, commenced on June 3, 1986 (day 74 of pregnancy, P 74) with the selection of 20 single- and 20 twin-bearing mixed aged (MA) Romney ewes by a realtime ultrasound scanner (Carter 1986). The individually identified ewes were then approximately balanced across birthrank groups for birthdate on the basis of mating harness crayon markings and ultrasound foetus image size. Selected ewes were 54 to 74 days pregnant. At 1430 h on P 91 a single chromium CRC (3.0 cm pressed tablet core, 65% Cr2O3 matrix, 9.00 mm orifice, Captec (NZ) Ltd) was orally administered to 10 of the single- and twin-bearing ewes, respectively. The remaining 20 ewes were fitted with a CRC of the same type and at the same time of day on P 109. This approach to CRC management was adopted because longer-life 6.0 cm core CRC (see Chapters Four and Five), which would have enabled the last 6 weeks of pregnancy to be monitored with a single capsule, were not available at the time. Treatment ewes were grazed with a larger mob (c.400 ewes) using an on-off grazing system until ewes were set stocked for lambing (P 136). This system provided access to fresh pasture for 2-3 h per day from 0900 h each morning. For the remainder of the day the ewes were restricted to a "sacrifice" paddock with a residual dry matter (RDM) of 400 to 500 kg DM/ha. Faecal grab samples were obtained from ewes at 7 to 10 d intervals from P 96 until P 145. Liveweights were recorded on the same day as faecal sampling. Rectum grab samples were collected at a similar time each day (1430 h), except on P 101 (0930 h). This minimised the effect of variation in gutfill on liveweight (Hughes 1976).

At the expected expiration of the 3.0 cm core CRC (P 117 and P 136 respectively) a second custom-made CRC with a 6.0 cm core (65% Cr<sub>2</sub>0<sub>3</sub> pressed tablet matrix, 9.00 mm orifice) was inserted into each ewe to facilitate the monitoring of faceal output from late pregnancy through to 9 weeks post-partum.

Ewes were set stocked for lambing (15 ewes/ha) onto pastures of 800-1000 kg DM/ha (4.0 - 5.0 cm average pasture height). Lambing commenced on P 145 (August 14 = lactation day 1 (L 1)). Lamb birthweights were recorded within 24 h of parturition. Lambs were individually tagged and were weighed again at approximately 6 weeks of age (L 49) and at weaning (L 102). Lactating ewes were weighed at docking (L 49) and nine days after weaning (L 111). Ewe fleeces were weighed at shearing one week after weaning.

The height of pasture grazed by ewes from lambing until weaning was recorded on the same day as faceal grab sampling using an Ellinbank Pasture Meter (50 readings/paddock). The relationship between pasture mass (y; kg DM/ha) and meter reading (x;cm) was determined by taking 0.2 m<sup>2</sup> quadrats cut to ground level from the pastures grazed by the cwes post-lambing on L 26. This provided the linear calibration equation:

y = 17.4 + 200.0 x, r = 0.96, n = 17.

## Chromium analysis

The rate of plunger travel in CRC can be estimated by the pattern of disappearance of added chromium from the faeces (Ellis et al. 1988). For this to be achieved with an accuracy of  $\pm 1$ day, rectum grab samples should be collected daily over the period when expiration of the capsules is anticipated. In the present experiment faecal samples were generally collected at intervals of 7 to 14 d. The release rate of CRC chromium matrix can therefore be only crudely estimated from the chromium concentration in faeces on the sampling day nearest to the time of expected expiration. Serial slaughter trials using ewes fitted with CRC from the same manufacturing batch as the current experiment (experiment 6, Chapter Six) were therefore used to supplement the faecal sampling data. The first slaughter trial was run with non-pregnant ewes, at times corresponding to P 91 to P 117 in the pregnant group, to establish the rate of chromium release in the 3.0 cm core CRC. The slaughter ewes were grazed with the pregnant single- and twin-bearing ewes. A second serial slaughter trial using barren ewes fitted with the 6.0 cm core CRC commenced in September (experiment 7, Chapter Six). These ewes were grazed separately from the lambed flock and were subjected to wider extremes of feeding conditions, both at pasture and indoors. The serial slaughter trials resulted in estimated chromium release rates (Y<sub>i</sub>) of  $143\pm 6$  and  $122\pm 4$  mg Cr/d for the 3.0 and 6.0 cm core CRC respectively.

Faecal chromium concentration in the faeces  $(Y_0)$  was determined by atomic absorption spectrophotometry (see Chapter Two) on samples of 0.3 to 1.0 g DM/ewe from each faecal collection date. The small amount of faeces which could be collected precluded preparation of duplicate samples for assay in approximately 60% of the ewes. The organic matter (OM) content of the faeces was estimated in duplicate using faecal samples bulked on an equal dry weight basis across sampling times within ewes.

Faecal output (FO, g DM (or OM)/d) of the ewes fitted with CRC was therefore calculated as:

 $FO = \underline{Y}_{i} \qquad (\underline{mg Cr/d})$  $Y_{o} \qquad (\underline{mg Cr/g DM(OM)})$ 

Pre-lambing and post-lambing herbage organic matter digestibility were estimated to be 68% and 78% respectively, from <u>in vitro</u> analyses of herbage collected from the experimental area during winter and spring (see experiment 2, Chapter Three and Chapter Five). Dry matter intake (DMI) was estimated from the equation:

DMI (g/d) = (OMI + (OMI x plant ash content) + soil DM intake) where OMI = organic matter intake = <u>(faecal DM x (1-faecal ash content))</u> (1-OMD) and soil intake = <u>faecal soil</u>; assuming 2% soil DMD (Appendix IV). (1-0.02)

Statistical analysis

Student's t-test was used to assess the significance of differences in ewe liveweight and faecal DM output between single- and twin-bearing ewes prior to lambing, DM output being expressed in terms of ewe metabolic liveweight  $(kg^{0.75})$  at the first faecal sampling (P 91). Although ewe liveweights corresponding to later faecal sampling dates were available, they were substantially

influenced by the weight of the products of conception. Ewe faecal outputs post-lambing were expressed in terms of ewe metabolic liveweight at docking. Ewe liveweights during lactation were subjected to MANOVA after correcting for the effects of mid-pregnancy liveweight and lambing date. The effects of sex and rank on lamb birthweights were tested after fitting midpregnancy ewe liveweight and date of birth as covariates in the MANOVA model. For the analysis of lamb liveweight gains, mid-pregnancy ewe liveweight was fitted first in the model as a covariate. Assessment of birth and rearing rank of both ewes and lambs was based on the actual lambing record. Ewes losing lambs, and any surviving lambs reared by these ewes, were excluded from post-lambing analyses. All analyses were carried out using the SPSSX (1983) statistical package.

# RESULTS

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## Pregnancy Diagnosis and Lambing Performance

Of the 20 ewes diagnosed in mid-pregnancy as bearing a single lamb, 3 (15%) gave birth to twins (Table 10.1). Two (10%) of the designated twin-bearing group gave birth to a single lamb. Six of the 39 ewes which survived lambing lost all of their lambs and two lambs born as twins were reared as singles.

	Ultrasound d	Ultrasound diagnosis				
Actual lambing record	Single	Twin				
Reared single	12	2				
Reared twins	2	15				
Single lamb died	5	2				
Twin lambs, one died	0	0				
Twin lambs, both died	1	0				
Ewe death	0	1				
	20	20				

Table 10.1 Pregnancy diagnosis and actual lambing performance of ewes, experiment 12.

## Lamb Birthweights and Growth Rates

Birthweights were obtained for 55 of the 58 lambs born. Lambs born as singles were significantly (P < 0.001) heavier than those born as twins (5.47 kg vs 4.28 kg) and ram lambs weighed more (P < 0.001) than their female contemporaries (Table 10.2). Compared to lambs

reared as twins, those reared as singles grew faster (P < 0.001) from birth to docking (279 vs 236 g/d) but not from docking until weaning (239 vs 233 g/d, P > 0.10). Ewe lambs grew less rapidly than ram lambs from birth to docking (P < 0.05) and from docking until weaning (P < 0.001). Rank by sex interactions were not significant.

Table 10.2 Effects of rank and sex on lamb birth and weaning weights and average daily liveweight gains (mean  $\pm$  sem), experiment 12. The numbers of lambs with liveweight data in each category are shown in brackets.

Rank (R):	Single		Twin		Sign.	
Sex (S):	licmale	Male	Female	Maic	R	s
Birthweight (kg)	5.11 ± 0.27	5.99±0.24	4.24±0.15	4.34±0.13		
	(10)	(7)	(20)	(18)		
Weaning weight (kg)	27.0S±1.28	32.82 ± 2.69	$23.73 \pm 0.72$	$27.03 \pm 0.76$	NS	
	(9)	(5)	(16)	(15)		
Livewcight gain:(g/d)						
Birth-docking	262 ± 15	309 ± 15	219±9	245±9		•
	(9)	(5)	(18)	(16)		
Docking-weaning <sup>a</sup>	211 ± 13	290 ± 27	209±9	247±9	NS	•••
Birth-weaning <sup>a</sup>	231 ± 12	292 ± 18	213±6	243±8		•••

<sup>a</sup>Numbers per group as for weaning weight.

## Ewe Livewcights and Wool Production

Single- and twin-bearing ewes were of a similar mean liveweight (P>0.1) until the final week of gestation, when the increased foetal weight of the twin-bearing ewes became most apparent (Table 10.3). By docking, ewes rearing twins were 5.4 kg lighter (P<0.05) than those rearing singles. This difference persisted until weaning (P<0.10), although both groups of ewes gained liveweight from docking until weaning.

	Day	Operation <sup>a</sup>	Pregnancy/rearing Status		Sign.
			Single	Twin	
Liveweights (kg)	P 96		60.5±1.5	62.4±1.2	NS
	P 107		62.4±1.5	63.8±1.1	NS
	P 136	Set stocking	$60.8 \pm 1.1$	62.5±1.2	NS
	P 145	Start lambing	$66.2 \pm 1.2$	69.0±1.0	+
	L 49	Docking	62.5±1.9	57.1±1.4	×
	L 111	Weaning <sup>b</sup>	65.1±2.1	60.2±1.2	+
Wool weight (kg)	L 109	Shearing	$5.06 \pm 0.17$	4.53±0.11	×

Table 10.3 Effect of pregnancy status and rearing rank on ewe liveweights and wool production (mean  $\pm$  sem), experiment 12.

<sup>a</sup>Management operation on the day weights were recorded.

<sup>b</sup>Including weight of wool clipped from ewes at shearing on L 109.

Ewes which reared a single lamb produced, on average, 0.53 kg (greasy) more wool (P < 0.05) than those which had reared twins. There were insufficient data to estimate the effects of pregnancy or lamb loss on ewe wool production.

## CRC Performance

Three 3.0 cm core CRC from the first group of 20 capsules had expired by P119, 28 days after their insertion (i.e. faecal chromium concentrations were within 24 to 48 hours of returning to the background level). The remaining CRC were still in the linear release phase on d 28 or about to commence final expiration (as evidenced by a 100% increase in chromium concentration in the faeces of two of the ewes). Thus the average CRC life was around 30 d, equivalent to an average plunger travel of 1.03 mm/d. This compares with the average rate of  $0.97\pm0.3 \text{ mm/d}$  measured in the ewes grazed in the same flock and serially slaughtered over the same time period. The average chromium release rate of 143 mg Cr/d during the slaughter trial was therefore used to estimate average faecal outputs. The first faecal sample for ewes with the first CRC was taken as recommended by the manufacturer, 5 days after CRC insertion. Subsequent results (Chapters Two and Seven) indicate that steady state levels of chromium are unlikely to be achieved in all animals until d 8. The faecal chromium concentrations for d 5 (P 96) were therefore increased by 15% to represent a more typical equilibrium situation (see Chapter Two). As a result, estimated faecal outputs for P 96 should be interpreted with caution. Faecal samples were not taken until eight days after insertion of the second 3.0 cm core CRC. Mean faecal chromium concentrations were similar at the subsequent two samplings - d 18 (P 129) and d 25 (P 136) of CRC life. All CRC for which faecal samples were obtained were apparently still in a linear release phase on d 25. The 143 mg Cr/d release rate was therefore assumed to apply to the second group of 3.0 cm core CRC. One CRC from this group of 20 capsules failed.

Of the 19 initial 6.0 cm core CRC inserted on P 129, 7 had expired 65 days later, 9 expired between d 65 and d 78 and 1 was still running on d 78. Two CRC from this group of capsules had failed, as assessed by the pattern of chromium recovery. In one case, the ewe may have been able to regurgitate the capsule (despite her 51 kg initial liveweight) because her previous 3.0 cm CRC had also failed. An average linear release phase of 70 days was assumed, giving a release rate of 0.87 mm/d (or 133 mg Cr/d). The corresponding rate from the serial slaughter trial was 0.71 mm/d (122 mg Cr/d) over 65 d. However, the slaughtered ewes were subjected to more extreme feeding conditions (0.6 M to <u>ad libitum</u>) and also had to adapt to indoor feeding midway through the capsule's life. While the slaughtered ewes grazed at pasture, release rates were up to 129 mg Cr/d

The majority of the second group of 6.0 cm core CRC (9 of the 14 CRC for which faecal samples were obtained) which were inserted at the start of lambing (L 7) were still in the linear release phase after 62 days. Two CRC each had expired by L 49 and L 62 respectively. One CRC from this group failed.

In summary, of the forty 3.0 cm core CRC which were inserted, 2 (5%) failed or were regurgitated. Three of the 39 longer-life 6.0 cm core capsules (8%) failed and average plunger was more variable than in the 3.0 cm core CRC.

## Faecal Output and Herbage Intake prior to Lambing

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The estimated faecal DM outputs of single- and twin-bearing ewes during the last trimester of pregnancy are presented in Table 10.4. Faecal DM output (per unit metabolic liveweight) of single-bearing ewes was generally higher than that of twin-bearing ewes but the difference was significant (P < 0.05) only on the final day of gestation. The low number of ewes which finally yielded reliable faecal chromium data reflects both the incomplete sampling of ewes and inappropriate sampling times in relation to the pattern of CRC chromium release. Sampling of 40 ewes took about two hours, and generally only 50 to 60% of the ewes yielded adequate grab samples, probably because of the combination of disturbance during yarding, the low level of feed intake and the period of time ewes were removed from pasture (4-5 hours).

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Day of Eve Pregnancy <sup>a</sup> stat	Eve status <sup>b</sup>	Number of	Eve liveweight <sup>d</sup>	Paccal output <sup>e</sup>	Soil <sup>f</sup> content	Herbage intake		Sign. <sup>g</sup>
			(kg)	(g DM/d)		kg OM/đ	kg DM/đ	
96	Single	7	62.5±1.5	704±58	39	1.08	1.46	NS
	Twin	4	63.6±2.5	649±114		0.98	1.32	
103	Single	8	60.4±1.7	530±61	11	1.24	1.41	NS
	Twin	8	62.4±2.3	557±93		1.48	1.68	
111	Single	7	62.9±2.9	481±109	26	0.95	1.17	NS
	Twin	8	63.3±1.5	291:42		0.53	0.65	
119	Single	4	55.6±2.9	716±162	34	1.18	1.53	NS
	Twin	6	60.0±1.6	521±125		0.85	1.11	
129	Single	6	57.7±2.3	707±100	39	1.04	1.40	NS
	Twin	9	61.3±1.4	545185		0.77	1.04	
136	Single	11	62.4±1.6	628±63	59	0.51	0.87	NS
	Twin	11	64.6±2.0	605±57		0.58	0.98	
145	Single	6	67.7±1.9	660±48	29	1.25	1.56	٠
	T⊌in	8	69.3±1.2	513±35		0.96	1.20	

Table 10.4Effect of pregnancy status on faecal output and estimated dry matter intake ofewes during the last trimester of pregnancy, experiment 12.

<sup>a</sup>Day 1 equals start of mating, March 20. Chromium concentrations were increased by 15% for P 96, because equilibrium conditions had not been attained.

<sup>b</sup>Based on number of lambs born.

<sup>c</sup>Ewes sampled with valid chromium readings.

<sup>d</sup>Average liveweight of ewes (including wool), sampled at each faecal collection (mean±sem).

<sup>e</sup>Estimated average faecal output of sampled ewes (mean ± sem).

<sup>f</sup>Soil content (%) in the faces = 91.978 - (1.096(1-faecal ash)).

 $g_{Student's t-test}$  of differences between single- and twin-pregnant ewes in faceal output expressed as g DM/kg<sup>0.75</sup>.

The estimated DMI indicate that on most days the ewes received 0.8 to 1.5 kg DM, including soil. Variation in herbage intake estimates during pregnancy reflect differences in grazing conditions prior to each sampling, as indicated by the amount of soil in the faeces. Soil intakes exceeded 26% of faecal DM, except on one occasion when faecal samples were taken at 0900 h after ewes had been introduced to a new pasture break. Soil contents reached 59% when the ewes were sampled after 18 hours on muddy pastures with a residual herbage mass of 600-700 kg DM/ha (P 136). OMI were therefore much lower than DMI and suggest that the ewes, particularly those bearing twins, received inadequate nutrition. This can be confirmed by comparing the average liveweight of ewes on P 91 and P 145 (Table 10.3) after correcting for the products of conception (c.7 and 12 kg for ewes with singles and twins respectively, Table 10.2) and wool growth (54 d x 10 g/d = 0.54 kg, Table 10.3). Single-bearing ewes therefore lost c.1.8 kg (66.2 - 7.0 - 0.5) - 60.5 kg)) and twin-bearing ewes c. 5.9 kg (69.0 - 12.0 - 0.5)-62.4 kg)) over the pre-lambing study period. Gutfill effects between weighings were probably minor because weights were recorded at the same time and apparently after similar levels of DMI.

# Faecal Output and Herbage Intake After Lambing

Estimated faecal outputs, OMI and DMI for ewes rearing single and twin lambs are shown in Table 10.5. Faecal outputs (g  $DM/kg^{0.75}$ ) were significantly (P < 0.05) higher in the twin-rearing ewes between weeks 6 and 8 after lambing. Soil contents of faeces were generally lower (21-30%) than those observed prelambing, because of the higher herbage allowance under set stocking (residual herbage mass >750 kg DM/ha) but still reflect the wet ground conditions typical of Tokomaru silt loam soils over the spring period.

Herbage intakes increased to a maximum of 2.43 kg DM/d (4.1% of liveweight) in the ewes with twins and 2.17 kg DM/d (3.6%) in the ewes raising singles 39 days after the start of lambing (mean lactation day = 28). DMI exhibited a curvilinear relationship with time over the period of sampling. OM1 of ewes rearing twins were generally greater than for ewes rearing a single lamb, the reverse of the situation which existed during the last trimester of pregnancy.

Day of Ewe Lactation <sup>a</sup> rank <sup>b</sup>	Ewe rank <sup>b</sup>	Number of eves	Ewe liveweight <sup>C</sup>	Faecal			Herbage intake	
			(kg)	(g DM/d)	۲	kg OM/d	kg DM/d <sup>e</sup>	
15	Single	4	63.5±2.9	427±75	30	0.73	0.95 <sup>d</sup>	NS
	Twin	2	62.0±2.5	453±30		0.80	1.01	
21	Single	9	63.0±3.0	587±90	26	1.72	2.00	NS
	Twin	9	56.3±2.0	573±115		1.68	1.95	
8	Single	8	60.0±2.7	654±50	21	1.85	2.17	NS
	Twin	8	59.8±1.3	732±41		2.07	2.43	
9	Single	9	63.6±2.7	601±47	24	1.60	1.91	٠
	Twin	12	57.4±1.5	705±24		1.87	1.24	
9	Single	4	64.1±3.3	482±67	28	1.45	1.70	•
	Twin	7	57.7±2.3	628±63		1.92	2.21	
2	Single	5	61.7±3.0	556±49	28	1.47	1.76	NS
	Twin	4	60.3±1.6	492±52		1.30	1.56	

Table 10.5Effect of rearing rank on faecal output and estimated DMI of ewes during the firstnine weeks of lactation, experiment 12.

<sup>a</sup>Day 1 of lactation = August 14; mean lambing date L 11.

<sup>b</sup>Based on actual lambs reared to docking.

<sup>c</sup>Average docking liveweight (L 49) of ewes sampled (mean ± sem).

dSoil contnet (%) in the faces - 91.978 - (1.096(1-faecal ash)).

<sup>e</sup>Dry matter intake based on a herbage OMD of 78%.

<sup>f</sup>Student's t-test of significance of differences between rearing ranks in faecal output (g  $DM/kg^{0.75}$ ).

#### DISCUSSION

This experiment achieved the original objective of providing information about how CRC should be managed in field trials but a number of shortcomings in the original experimental design, especially in relation to faecal sampling programmes and the collection of herbage digestibility data, were evident.

Although the original treatment groups each comprised 20 ewes, the final number of faecal samples that provided usable chromium data was low, for three main reasons:

- 1. Not all ewes provided a faecal sample at each collection. Some ewes had voided faeces during yarding and, especially over the pregnancy period, daily faecal outputs were generally low. Sampling ewes directly off pasture or holding ewes for a second attempt at sampling 1-2 hours later would increase the success rate. However, there will always be some problems with larger groups of animals because of the time required to complete sampling. In this respect, running the treatment ewes with a larger flock provided a realistic grazing situation, but created more animal disturbance (and associated loss of faecal material) because treatment animals had to be shifted to a central holding yard for drafting off at each sampling interval.
- 2. Some faecal collections did not coincide with the attainment of equilibrium chromium levels in the faeces (e.g. group 1, P 96) or were taken outside the period of linear release of chromium (e.g. group 1, P 119). The sampling regimes adopted were based on the manufacturer's recommendations available at the time of the experiment. These problems can now be readily overcome because the performance characteristics of CRC have been more precisely defined. For example, the first faecal samples should have been delayed until 10 days after insertion when intakes are equivalent to 0.6 0.8 M (see Chapter Seven). Similarly for the 3.0 cm core CRC, collections should not be taken beyond d 25 of capsule life.
- 3. Only 27 of the 40 original ewes (68%) reared lambs true to their predicted pregnancy status based on ultrasound.

More cost-efficient and accurate use of CRC will be obtained where fewer animals (of known status) are used and faecal samples are taken more frequently (to reduce large between-day variation). Treatment animals could continue to be grazed with a larger flock but labour requirements would be reduced and faecal collections simplified if the ewes under study were managed separately during faecal sampling periods of 4 to 5 days duration. Pastures similar to those grazed by the larger flock, or

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within the paddock occupied by the larger flock, would need to be prepared and the same method of grazing management maintained. Separate grazing would allow sward ring sampling to be combined with rectum sampling and would also facilitate the collection of more representative samples for in <u>vitro</u> digestibility analysis. Monitoring herbage digestibility under a large flock management system is difficult, especially under rotational grazing, because of the range of herbage conditions encountered.

If a farmer had adopted differential grazing management of single- and twin-rearing ewes during pregnancy using the ultrasound data collected, then based on actual lambs born (Table 10.1), 85% of the ewes in each group would have received the appropriate nutrition. Post-lambing efficiency of selection by ultrasound decreases because of lamb losses but, in practice, wet/dry ewes which have lost all their lambs and twin-bearing ewes which have lost one lamb can be readily screened off within 7-14 days of lambing if feed supplies are low.

The pattern of ewe liveweights from late pregnancy until weaning indicate that the ewes rearing twins were placed under more nutritional stress than their single-rearing counterparts. It is not known. however, what proportion of this weight loss can be attributed to the inability of the twin-rearing ewes to physically consume sufficient feed to meet the energy requirements of foctal growth, lactation and wool production (Hadjipieris and Holmes 1966). The DMI values (Tables 10.4 and 10.5) suggest that underfeeding occurred during late pregnancy, as well as in early lactation. However, twin-rearing ewes were apparently able to consume more than the assumed maximum of 3.0% of bodyweight (Ulyatt et al. 1980) by the sixth week of lactation. OMI in the ewes with a single lamb reached a maximum of 31 g OM/kg LW/d during lactation. This compares favourably with the 32 g OM kg LW/d measured by Bircham (1981) for ewes rearing a single lamb, the 30 g OM/kg LW/d intake of Greyface ewes suckling single lambs in a trial by Maxwell et al. (1979), and the 27 and 32 g OM/kg LW/d consumed by single- and twin-rearing Border Leicester x Romney ewes respectively, in the study by Coop and Drew (1963).

Differences in lamb birthweights and growth rates between singles and twins, and between sexes, were of the same magnitude as those recorded by other researchers (Duff 1981; Rattray et al. 1981; McEwan et al. 1983). Lamb growth rates indicate that the major period of nutritional shortfall in twin lambs occurs in the first 6 weeks of lactation. This corresponds to the period of high feed requirements of the twin-rearing ewe (Rattray 1986). Greatest benefits from differential grazing will therefore be likely to occur during early lactation (Garrick 1984), but this trial and the work by Scales et al. (1986) suggest that the period of late pregnancy should not be neglected. A detailed study of the effect of continuous grazing of ewes rearing either single or twin lambs at different pasture heights is reported in the next Chapter. That experiment was designed to provide ewe feed intake and **associated** production data that would enable benefits of differential grazing during the first 6 weeks of lactation to be assessed.

# CHAPTER ELEVEN

# THE APPLICATION OF CHROMIUM CRC FOR ESTIMATING THE HERBAGE INTAKE OF EWES OF DIFFERENT REARING RANKS DURING LACTATION

# INTRODUCTION

The production of the ewe and her progeny is sensitive to the level of nutrition over the period from parturition to weaning, and particularly the first six weeks of lactation (Cooper al. 1972; Rattray et al. 1982b; Munro and Geenty 1983). Nutritional regimes imposed during pregnancy generally have a lesser effect (Coop and Clark 1969; Monteath 1971; Rattray et al. 1982a; Smeaton and Rattray 1984). However there is evidence that such regimes may affect lamb survival and growth rates through factors other than lamb birthweight (McClymont and Lambourne 1960; Khalaf et al. 1979; Scales et al. 1986) as well as affecting ewe fertility and production (Everitt 1967; Fletcher 1974; Lewer et al. 1983; McEwan et al. 1983; Cahill et al. 1984). In all cases the effects of under-feeding are greatest in multiple-rearing ewes and their lambs.

For these reasons considerable effort has been directed towards the development of practical methods of identifying the foetal status of the ewe during pregnancy, in order that differential management systems favouring the multiple-bearing ewe might be imposed over the winter and spring months which are usually the most limiting in terms of pasture growth (Thwaites 1981; Beach 1984). Technological advances during the last decade in the field of "realtime" ultrasonic imaging have provided farmers with equipment allowing the pregnancy status of a ewe at 40-50 days of gestation to be reliably predicted at a modest cost (Fowler and Thompson 1985; Blair 1986; Carter 1986). This technology has particular relevance to management systems for out-of-season lambing where the patterns of intake associated with changes in physiological status typically cannot be matched with the seasonal pattern of pasture production (Rattray 1978; Andrewes and Taylor 1986). In addition to an improved efficiency of feed allocation, farmers can expect to benefit through choosing better paddocks for twin lambings, the earlier sale of barren ewes, improved selection of flock replacements, and different weaning and drafting regimes for single and twin lambs (Garrick 1984).

Little is known about the amount of herbage which should be offered to post-parturient ewes of different rearing ranks under continuous grazing management, mainly because of the difficulties associated with measuring herbage intake under field conditions. In practice farmers have based the allocation of feed mainly on feed requirement tables derived from indoor trials (Rattray 1986) and a limited number of outdoor pasture allowance trials (Rattray and Jagusch 1977; Geenty and Rattray 1987; Rattray et al. 1987).

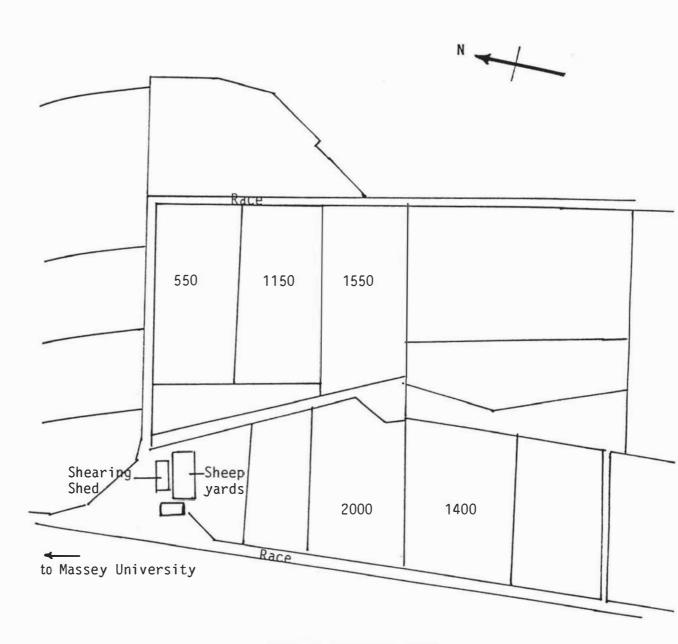
In this study chromium CRC were used to estimate the herbage intake of ewes rearing either single or twin lambs over a range of pasture conditions during a nine week period from parturition. The feed intake data generated, together with other data, could then be used to develop improved grazing management systems for ewes of different rearing ranks.

#### MATERIALS AND METHODS

#### **Experiment 13**

#### Experimental design

The trial commenced 10 days after the mid-point of lambing (lactation 10 (L 10)) on 29 August 1988 and involved the continuous stocking of pastures held at a steady pasture mass (measured indirectly as herbage height) by ewes rearing either single or twin lambs. Five different paddocks, each of c. 1.0 ha, were prepared at Massey University's Sheep and Beef Cattle Research Unit Haurongo Block during a 16 week period before the commencement of the trial. Average sward heights aimed for were 3.0, 5.0, 7.0, 9.0 and 11.0 cm (Figure 11.1). These are subsequently referred to as the 550, 1150, 1400, 1550 and 2000 swards, values which corresponded to the average herbage mass (kg DM/ha) on the swards during the trial. To ensure that sufficient pasture mass was achieved in the highest sward, 23 kg nitrogen/ha (in the form of urea) was applied to the entire experimental area in early May. Each sward was subsequently spelled from grazing until late July when rotational grazing with ewes and yearling bulls was adopted to adjust sward heights to the desired levels.



# HAURONGO RESEARCH FARM



# Selection and management of trial animals

Trial ewes were chosen from a larger flock of 235 first cross Border Leicester x Romney ewes, in which oestrus had been synchronised with progesterone-impregnated controlled internal drug release devices (CIDR's, Type G, AHI Plastic Moulding Company, Hamilton, New Zealand). The selected ewes were all mated during the first three days of tupping (Walsh 1989). The flock was pregnancy-diagnosed by realtime ultrasound scanning (Carter 1986) at 50-53 days of pregnancy (P 50-53), and ewes bearing single and twin lambs were identified. During pregnancy the flock was rotationally grazed at maintenance feeding levels around paddocks adjacent to the trial area. A group of 96 ewes (45 single-bearing and 51 twin-bearing) was selected as potential final treatment animals on P 140. Lambing commenced in this group of ewes the following day and was completed 9 days later. The mid-point of lambing for the treatment ewes was 20 August (L 0).

Swards were stocked with ewes and lambs between L 4 and L 8. Each treatment was allocated 12 ewes comprising equal numbers of two tooth and mixed age (MA) ewes balanced within age groups for rearing rank (single or twin, n = 3). The resultant stocking rate of 12 cwes/ha, approximately 15% lower than the normal farming practice for these pastures, was adopted to ensure that the initial sward height conditions could be maintained without removing treatment animals, especially during the first half of September. Local pasture growth rates in early September historically averaged about 30 kg DM/ha/day, but were expected to increase to 50 kg DM/ha/day by the end of September and to peak at 65-70 kg DM/ha/day by mid-October (Matthew et al. 1988). Pasture growth should therefore have approximately matched the feed demand of ewes and lambs during September and exceeded requirements during October. Buffer groups of livestock, consisting of 12 yearling bulls and 10 dry ewes, were formed at the commencement of the trial to control anticipated surplus pasture growth.

Detailed measurements of pastures and animals, as described below, commenced on P 144 (August 15).

### Animal measurements

## 1. Liveweights

Ewes were weighed at least monthly over the period from flushing until lambing as part of the studies of Walsh (1989) and Gao et al. (1990). These liveweight records were incorporated in the present study to correct for pre-treatment effects. The final liveweight during pregnancy was obtained the day before lambing commenced (P 144). The next weight was obtained on L 10 when each of the final treatment groups had been established and allocated to their respective treatment swards. Subsequently, ewe liveweights were recorded on L 33, 45, 56, 70

and 76 (i.e. 24, 33, 47, 61 and 66 days after set stocking, respectively). With the exceptions of the 24 h fasted mid-pregnancy (P 50) and final (L 76) weighings, all liveweights were obtained within 1 hour of removal of the ewes from grazing at pasture.

Lambs were tagged and weighed within 24 hours of birth and were subsequently weighed on the same day and at the same times as their dams, commencing from L 33. A 24-hour fasted liveweight was also obtained for lambs on L 76.

# 2. Condition Scoring and Ultrasonic Backf at Depth

Ewes were condition scored using the 5-point scale of Jeffries (1961) on L 14, 59 and 70 (24, 50 and 61 days after set stocking). Condition was assessed by hand over the region of the backbone and twelfth rib in graduations of 0.5 units. Ultrasonic backfat depth C measurements (Delphi A-mode ultrasound, Auckland) were recorded from the left and right hand sides on L 70 (Purchas and Beech 1981). The mean value for the two readings was used in subsequent analyses.

# 3. Wool production

Midside sampling sites  $(c.20 \text{ cm}^2)$  were clipped on each of the 96 potential final treatment ewes with Oster 001 clippers (size 40 blades) on P 144 according to Bigham (1974). Lambs (n = 90) were prepared in the same manner on L 10, after they had been allocated with their dams to treatment groups.

Wool regrowth samples from these sites were clipped over a period of two days (L 76,77), 80 and 66 days after preparation in the ewes and lambs, respectively. The final area clipped (c. 10 cm<sup>2</sup>) was determined from calliper (Mitutoyo, Tokyo) measurements of the four sides and the diagonal. Greasy and clean weights and colour (Elgabbas 1986) were measured on each midside sample. Fibre diameter was measured by air flow (Ross 1958) for the lamb samples. A greasy contralateral midside wool sample from each ewe (collected on L 76 and L 77) was tested for fibre strength using a "Hounsfield Tensometer" (Horton 1978). Full details of the methods used for wool measurement are provided in Appendix IV.

#### 4. Faecal Output

Treatment ewes were dosed with a single CRC (3.0 cm core of pressed tablet matrix with 65%  $Cr_2O_3$  and 9.00 mm orifice; Captec (NZ) Ltd, Auckland) on two occasions to facilitate the indirect estimation of faecal output. The first capsule (CRC 1) was administered on L 10 (29 August) and the second (CRC 2) on L 45 using the Captec capsule applicator (Phillips, Australia). From earlier trials (see Chapters Five and Seven) it was estimated that steady state rumen conditions for  $Cr_2O_3$  would be achieved by day 8, and final expiration of the  $Cr_2O_3$  matrix would commence after 27 days. Faecal sampling was therefore carried out between days 8 and 25 following administration of each capsule. Although it would have been possible to have used custom-made 6.0 cm core CRC with a greater than 70 day linear release phase (see Chapter Six), the expected release rate of 100-110 mg Cr/day from these CRC would have resulted in potential faecal DM chromium concentrations of only 1.0-2.0 mg/g in the ewes on the high pasture allowances. These levels were considered to be unacceptably low relative to the environment background levels of 0.15-0.30 mg Cr/g DM (see p. 45). Furthermore, the results of cxperiment 12 results (Chapter Ten) suggested that the malfunction rate was higher in the 6.0 cm core CRC than in the commercial capsules with a 3.0 cm core.

Faecal samples were collected both per rectum and from sward rings (Raymond and Minson 1955). A typically wet soil conditions during September (see Table 11.1) prevented the intensive handling of ewes and lambs required for rectum sampling. Rectum grab samples were therefore obtained from ewes (48/60) only on L 19 and L 20 to check that CRC were delivering  $Cr_2O_3$ . A more intensive sward ring sampling regime was therefore substituted. Samples were collected daily from rings of 2.0 m radius at 16 pegged sites in each treatment sward from day 8 after CRC 1 administration until the expected final expiration of chromium on L 45. To obtain estimates of feed intake for the different rearing ranks, single- and twin-rearing ewes and their lambs were grazed separately, using temporary electric fences, within each treatment over a 5 day period commencing from L 27 (approximately 3.5 weeks post-partum). Temporary fences were erected to ensure that single- and twin-rearing ewes had access to approximately the same amount of pasture during this period.

During the life of CRC 2, faeces were collected by ring sampling from L 53 to L 56 (days 8-11 after CRC administration) and from L 63 to L 70 (days 18-25). Two corresponding 4 day rectum sampling periods commenced on L 56 and L 63 (47 and 54 days after ewe set stocking, respectively). Ewes from each paddock were penned at 0900 h and all ewes were sampled per rectum over a period of 10-15 minutes. Samples were most difficult to obtain from the ewes grazing the low sward and, on some days, ewes were retained in the pen for a further 30-40 minutes before attempts were made to obtain a second sample. The minimum number of grab samples obtained on any one day for CRC 2 was 53 (out of 60 ewes).

Ring sampling continued from day 25 to day 33 after insertion of both CRC's to monitor the pattern of chromium expiration from the faeces and hence to estimate the endpoint of CRC chromium delivery (Ellis et al. 1988). An average daily chromium release rate for treatment groups was determined from this endpoint and was supplemented by data obtained from serial slaughter of ewes grazing pastures adjacent to the experimental area (Chapter Eight). The combined data indicated that an average rate of release of 139 mg Cr/d should be applied to the calculation of faecal output. Full details of the derivation of this release rate are presented in Appendix IV.

Rectum grab samples were oven dried at 70°C for 72 hours and analysed for chromium as described in Chapter Two. Sward ring samples were oven dried to a constant weight and ground through a 1.00 mm sieve (Cranston, England). After thorough mixing, a 10.0 g sub-sample was retained for chromium analysis. Rectum grab samples, and sward ring samples from day 26 of CRC life, were analysed as single samples of 1.0 g faecal DM for chromium. Remaining samples were assayed in duplicate. Correction for background chromium was made using the chromium concentrations of faeces collected from non-treatment sheep grazing on the experimental area. The intra- and inter-assay coefficients of variation (CV) were 6.2 and 4.7% respectively.

#### 5. Pasture Digestibility

Four oesophageal-fistulated (OF) mixed age wethers, were used to collect extrusa samples for in vitro determination of herbage digestibility. OF wethers grazed pastures adjacent to the trial paddocks, except on sampling days. Below-average pasture growth rates prevented the rotation of these animals around the treatment areas during the experiment as described by Bircham (1981). However, the sward grazed by the OF wethers was of similar botanical composition to the treatment paddocks.

Extrusa samples were collected during a grazing period of 10-20 minutes into 800 x 250 mm bags(Wait 1972). Collections from three OF wethers per sward were obtained from paddocks 1, 2 and 3 on L 24, L 52 and L 68. Paddocks 4 and 5 were sampled on the subsequent day at the same intervals. These sampling times corresponded approximately to the sampling of single-and twin-rearing ewes for faeces. To account for between-animal variation, at least two of the OF wethers were common between collections for individual paddocks. Collections were readily made from the OF wethers on all except the low sward. To ensure adequate extrusa samples were obtained from the 550 sward, it was necessary to withhold the fistulated animals from grazing for 4-8 hours. Even with this imposition, collections were not made from some wethers on the lowest pasture allowance on L 52 and L 68. Extrusa samples were divided into two parts - 67% for in vitro digestibility analysis and the balance for botanical separations to determine diet compositions - and sealed in plastic bags. The saliva fraction was retained with the sample (Hodgson and Rodriguez 1971; Armstrong et al. 1989). Samples were placed on

crushed ice immediately after collection and then frozen at -12°C until required for further analysis.

For the <u>in vitro</u> digestibility assay, samples were freeze dried (Cuddon F57, New Zealand) to a constant weight and ground through a 2.00 mm sieve. Duplicate 1.0 g samples were then assayed by the cellulase incubation method described by Roughan and Holland (1977). Six standards of known <u>in vivo</u> digestibility, collected from wether sheep fed indoors, were run with each batch. Percentages of DMD, OMD, DOMD and OM in each sample were calculated.

## 6. Botanical Composition of the Ewe's Diet

Extrusa samples were bulked on an equal fresh weight basis (1 g/sheep) for each paddock and thoroughly mixed before being separated into grass, clover, weed and dead material (including senesced herbage which was defined as that containing more than 50% choloritic material) using the technique described by Clarke and Hodgson (1986). Extrusa was floated in a white tray marked into 100 x 1 cm<sup>2</sup> quadrats and point counts of the herbage component appearing over each square intersect were made. These were expressed as a percentage of the total number of counts. Blank intersects were excluded from the analysis. Full details of the procedure are presented in Appendix IV.

#### Pasture measurements

#### 1. Pasture Mass

Twelve 0.25 m<sup>2</sup> ground level cuts were made using an electric shearing handpiece in paddock at intervals of 14-17 days from L 10. Sampling was stratified with two cuts being taken from each quarter of the paddock. Corresponding Ellinbank Pasture Meter (EPM) readings (n = 2) were taken for each quadrat to derive the regression equation (y = a + bx) of pasture height (y) on pasture mass (x) (Earle and McGowan 1979). The high positive correlation (r = 0.94) for the first calibration provided justification for using the EPM to obtain additional estimates of pasture mass (1-2 sites per quarter) using the double-sampling technique described by O'Sullivan et al. (1987). This improved the precision with which pasture mass was estimated. Each quadrat sample was washed to remove soil contamination and dried at 100°C for 48 hours before being weighed to determine the dry weight. Duplicate sub-samples (2.0 g/cut) of the dried material were bulked within paddocks, ground through a 2.00 mm sieve and ashed overnight at 500°C to determine herbage OM content. Herbage DM mass was determined by multiplying the dried sample weight by 40, and herbage OM mass determined as the product of DM mass and average OM content of the herbage.

## 2. Pasture Accumulation

Four exclosure cages (1.0 m x 0.4 m) were placed, one per quarter, in each paddock and pasture growth was recorded by the difference technique (Radcliffe 1971). Within each cage, ground level cuts of  $0.25 \text{ m}^2$  were taken at 14-17 day intervals. These measurements therefore corresponded to (and were part of) those obtained for pasture mass determination. Residual herbage mass was measured on a matching sward area immediately adjacent to the exclosure cage at each shift. Pasture accumulation was calculated as the difference between the final cage yield and the initial estimate of residual herbage. The relatively short interval between pasture cuts was adopted to minimise the effects of exclusion of herbage from grazing (Brougham 1959).

#### 3. Pasture Composition

The proportions (%) by dry weight of grasses, legumes, weeds and senesced and dead material in the respective swards were estimated from a sub-sample of herbage collected adjacent to the pasture accumulation cuts in each treatment paddock. No distinction was made between leaf and stem material as visible stem elongation associated with inflorescence (Thomas 1980) did not appear until the final week of the trial.

# 4. Pasture Height

Pasture height was measured in terms of compressed height using an EPM (Earle and McGowan 1979) and of green leaf contact height using the HFRO sward stick (Barthram 1986). EPM measurements (80 per paddock) were taken on the same diagonal across each treatment three times weekly. The slower and more labour intensive HFRO sward stick readings (30-40 per paddock diagonally) were taken weekly. Calibration of the EPM height readings with pasture mass (described previously) allowed changes in pasture mass to be monitored more intensively than by pasture cuts alone. It was not possible to process sufficient numbers of herbage mass cuts at the speed required for making once-to twice -weekly decisions on sward management. Adjustments to stocking rate on each treatment sward were therefore made on the basis of trends in average EPM height readings.

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#### GENERAL OVERVIEW OF TRIAL IMPLEMENTATION

# Weather Conditions

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Weather conditions during September and October were considerably worse than average (Table 11.1). September was the dullest on record for the Manawatu and had the fourth-lowest ever total hours of monthly sunshine (Heerdegen 1988). Wind run and rainfall were 38 and 94% above average respectively, but temperatures of the waterlogged soil at 10 cm were 20% above average. October's rainfall was 22% above average, sunshine hours 13% below average and wind run 83% above the long term average. Soil temperatures, at 10 cm, were  $0.5^{\circ}C$  (6%) above average.

Table 11.1 Summary of September and October 1988 weather conditions at Massey University, experiment 13 (Source: Heerdegen 1988).

Parameter recorded	Scpte	October		
Temperature:				
Mean daily ( <sup>0</sup> C)	12.6	(11.0) <sup>a</sup>	17.1	(16.8)
Average overnight ( <sup>0</sup> C)	9.5	(6.9)	10.3	(8.4)
Average 10 cm soil ( <sup>0</sup> C)	11.8	(9.8)	13.1	(12.4)
Frosts:				
No. of ground frosts	3	(6)	1	(3)
No. of air frosts	1	(1)	0	(0)
Rainfall:				
Total (mm)	144	(74)	98	(87)
Raindays (> 1mm)	16	(11)	14	(12)
Days with no rain	9		13	
Sunshine:				
Total (h)	68	(129)	138	(158)
Wind:				. ,
Total wind run (km)	10391	(7507)	14714	(8029)
Average wind speed (km/h)	14.4	(10.4)	19.8	(10.8)

<sup>a</sup>Figures in brackets are the long term averages for the period 1951-1980.

The poor weather had a significant impact on both pasture production and animal health during the trial. Low sunshine hours and saturated soils (e.g. 25% of the 550 sward was covered with surface water for a 24 hour period on two occasions) meant that pastures rarely grew faster than the level required to sustain 12 ewes/ha. Thus, the major pasture management problem was maintaining pasture heights, rather than controlling them as had been anticipated.

## Animal Health

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Wet underfoot conditions contributed to outbreaks of foot scald in both ewes and lambs during September and the first half of October. All lambs were therefore treated with an 8% (v/v)solution of formalin at each weighing. Affected ewes were treated individually with an iodinebased foot spray. Additional treatment of some lambs was required between weighings, particularly on the 1450 sward. Two ewes (one each in the 550 and 1150 swards) contracted mastitis. Treatment of the ewe on the 550 sward was successful, although growth of her twin lambs was checked from L 29 to L 38. Growth rates of these lambs then recovered to a level similar to that of their counterparts on the same sward. The single lamb of the second ewe with mastitis died before the antibiotic treatment was successful. This ewe, and a ewe which had lost one of a set of twin lambs through inclement weather on L 27, were replaced with ewes of the same rearing rank and similar birth dates on L 45. This enabled sward ring sampling to be continued and faecal samples were obtained from these ewes during the second CRC collection The effect of previous post-parturient nutrition on the performance of periods (L 53-70). these ewes during measurement periods was not considered to be significant since their residual grazing levels had been similar to those of ewes maintained on the 1150 pasture. Liveweight and wool production data for these two ewes and their lambs were excluded from all analyses.

A record of all animal health problems was maintained and was subsequently compared with individual performance records. Where poor performance was clearly attributable to poor animal health, rather than the imposed sward conditions, the affected records were removed from analysis. These are noted in the results section.

Wet ground conditions also made animal handling more difficult than would normally be the case. The main sheep yards were heavily pugged in areas not concreted and temporary yarding of sheep in their paddocks quickly resulted in mud 5-10 cm deep. This mainly affected the proposed rectum grab sampling regime for CRC 1. After attempts on L 19 and L 20, it was evident that the muddy conditions placed too much stress on the ewes and their 2-3 week-old lambs. Daily sward ring sampling, as described previously, was therefore adopted. It should be noted that this option existed only because the CRC circumvented the need for daily administration of the faecal marker:

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#### STATISTICAL ANALYSIS

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Feed intake data obtained by repeated measurements from the same animals across periods were analysed by split-plot analyses (Gill and Hafs 1971; Rowell and Walters 1976). The effects of rearing rank, ewe age and the rearing rank x treatment interaction were tested against an "animal across treatments" error term where individual animal data were available. Differences between intake measurement periods were tested using "animals within a period" as the error term. Since the non-replicated design meant that no pure treatment error existed for the five pasture conditions, a regression approach was used in the ANOVA where the first and second order effects of pasture height were fitted, leaving the remaining 2 df as an error term for between treatment (pasture sward) comparisons. Although this enabled an estimate of between treatment statistical significance to be derived, the (1,2) df meant the test had low power. Ewe liveweight at the commencement of continuous grazing (L 10) was initially included in the model as a covariate but was later excluded because it was highly confounded with rearing rank.

Ewe and lamb wool production and liveweight gain data were analysed using linear models. The ewe models included main effects for age, rearing rank and treatment, together with their associated interaction terms. The treatment effect, which was fitted last in model, was estimated by the regression approach described previously. Initial ewe liveweight (L 10) was fitted as a covariate. The lamb models were of the same form but included birthweight as the covariate term. Differences between group means across treatments were assessed by Duncan's Multiple Range tests.

Pasture mass was regressed on EPM height readings by fitting linear and first order polynomial models both for individual harvest data and data pooled across harvests. Calibrations were based only on quadrats cut from the grazed portion of the sward because inclusion of the non-grazed pasture mass cuts from the pasture growth cages generated regression equations with different slopes (Grant et al. 1983; Stockdale 1984). Outliers were tested for using Mahalanolois' and Cook's distance values (Norusis 1985). Double sample estimates of pasture mass (n=6-9 per paddock depending on sward variability) were estimated from the regression equations for the individual harvests.

In vitro digestibility data were analysed by ANOVA with pasture height partitioned into first and second order effects as described previously. All analyses were carried out using the SPSSX (SPSSX 1983) and SAS (SAS 1985) statistical packages.

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#### RESULTS

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#### Pastures

## Sward height

Average weekly sward heights during the trial for the EPM and HFRO sward stick measurements are summarised in Figures 11.2 and 11.3, respectively. EPM weekly readings (the mean of the three recordings made each week for individual swards) indicate that, with the exception of the longest (2000) pasture, sward heights were maintained at approximately their initial levels through September (i.e. until L 42), but then decreased gradually until the cessation of the trial. Relative height differences were, however, generally maintained throughout the trial (i.e. heights of the different treatments declined in parallel). HFRO readings indicate the same trends, but are c. 1.0 cm higher overall because they represent the first point of green leaf contact rather than a compressed height of herbage under a 0.1 m<sup>2</sup> plate.

Actual sward heights were therefore lower than the initial targets, especially on the three longest swards. This partly reflected changes in sward structure brought about by the grazing behaviour of the ewes, as well as the unfavourable weather conditions which meant that pastures grew less vigorously than normal. Uneven grazing, particularly of the 1550 (9 cm) and 2000 (11 cm) swards, resulted in "clumpiness" and increased variability in height readings within these swards as the trial progressed. Two management strategies were adopted to improve sward uniformity. First, yearling bulls which preferred the longer pastures left by the ewes were introduced for periods of 2-4 days. Second, trial ewes and lambs were restricted to the longest pastures within these paddocks for 3 days (L 45 to L 48) by temporary electric fencing on one occasion. These strategies were only partially successful in reducing the unevenness of grazing.

An increase in stocking rate was required to control growth surplus to ewe and lamb requirements only between L 34 and L 49. Four yearling bulls grazed the 1150 (5 cm) and 1400 (7 cm) swards for 5 day periods from L 43 and L 35 respectively, and 20% of the 550 (3 cm) sward was fenced off from the treatment ewes and maintained at the same height as the treatment area with dry ewes from L 34 to L 49. The gradual reduction in pasture height during October was accepted in preference to removing treatment ewes and lambs, primarily because the expectation was that pasture growth rates would follow the normal pattern and increase with the advancement of warmer spring weather. A further consideration was that six weeks of measurements had already been made on these animals.

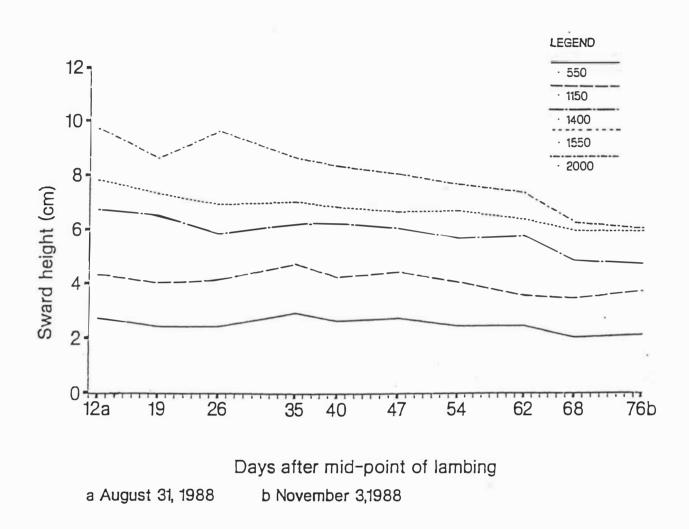


Figure 11.2 Mean weekly Ellinbank Pasture Meter heights (cm) on each of the five swards, experiment 13.

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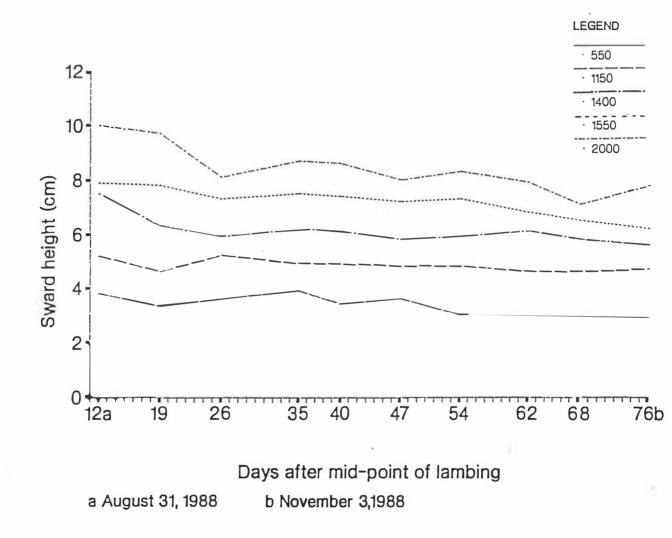


Figure 11.3 Mean weekly HFRO sward stick green leaf contact heights (cm) on each of the five swards, experiment 13.

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#### Pasture mass

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Sward pasture mass (kg DM/ha and kg OM/ha) exhibited the same trends as the sward height measurements although differences between the 1400 and 1550 swards were smaller (Table 11.2). The major feature of pasture mass measurement was the approximately 100% difference between the 550 and 1150 swards. Management of these adjacent swards (Figure 11.1) was dentical until three weeks before the trial, when the electric fence subdivision was erected.

	Harvest Date								
Sward	L 12	L 26	L 41 <sup>a</sup>	L 52 <sup>b</sup>	L 69	Mean			
Dry matt	er yield (kg DN	A/ha)							
550	516 ± 47	592 ± 54	792 ± 46	$400 \pm 30$	572 ± 34	574			
1150	$1132 \pm 64$	1242 ±121	1368 ± 76	902 ± 77	$1160 \pm 51$	1161			
1400	1372 ±112	1397 ±114	1603 ±132	1259 ±107	1486 ±155	1423			
1500	1753 ± 93	1506 ±158	1724 ±154	1310 ±120	1486 ±155	1556			
2000	2260 ±138	1928 ±176	2131 ±167	1764 ±150	1895 ±207	1996			
Organic	matter yield (k	g OM/ha)							
550	438 <sup>c</sup>	441	621	345	493	469			
1150	958	992	1036	780	1009	955			
1400	1080	1168	1223	1112	1260	1169			
1550	1473	1259	1315	1143	1287	1295			
2000	1898	1673	1786	1529	1647	1707			

Table 11.2Herbage mass (mean± sem) on each of the swards at five harvestsduring the trial period, experiment 13.

<sup>a</sup>Pasture cuts made immediately after heavy rain.

<sup>b</sup>Pasture cuts made during very windy conditions.

<sup>c</sup>DM yield x ash content of herbage DM.

Net herbage accumulation rates

Net herbage accumulation rates (NHA, kg OM/ha/d), estimated by the difference technique, are presented in Table 11.3. NHA was defined by Bircham (1981) as being the difference between new growth (G) and the disappearance of herbage through senescence and decay (D) (i.e. NHA = G-D). NHA values were more consistent on the 550 and 1150 swards. This probably reflects the greater uniformity of sward conditions on these treatments which enabled differences in pasture production to be more easily determined, compared with the longer swards where identical sward conditions for the before and after harvests were more difficult to identify. NHA apparently declined between L 42-56, but these estimates probably include a larger error component because of the strong wind conditions encountered during the L 56 harvest.

		Period of Growt	Period of Growth		
Sward	L 14-26	L 27-41	L 42-56	L 57-69	
550	26	33	29	30	
1150	59	50	20	59	
1400	16	65	23	59	
1550	11	20	12	44	
2000	15	52	30 <sup>a</sup>	47	

**Table 11.3** Net herbage accumulation rates (kg OM/ha/d) on the five swards during the trial period, experiment 13.

<sup>a</sup>Estimated from pre- and post-harvest height differences.

The NHA values indicate why difficulties were experienced in maintaining the initial sward heights. On the grazed area of the sward NHA = G-(C+D), where C is the daily consumption of animals on the treatment area. If a sward is maintained in a steady state, NHA = 0 = G-(C+D). At a stocking rate of 12 ewes/ha, and C = 1.5 kg OM/ewe/d, NHA (before D) had to at least exceed 18 kg OM/ha/d if pasture mass (height) was to be maintained. This value increased as the lambs progressively contributed to daily C. G therefore mainly exceeded (C+D), as explained previously, during the period L 27-41.

### Relationship between herbage mass and pasture height

The relationship between pasture mass (y) and EPM sward height (x) determined by quadrat cuts was best explained by a linear equation of the form y = a + bx (Table 11.4). Fitting a first order polynomial regression ( $y = a + bx + cx^2$ ) only marginally improved the power of prediction and did not reduce overall residual errors. While the slopes of the regressions for individual harvests were not significantly different, the intercepts were (P< 0.001). Only one value of the 192 cuts was excluded as an outlier.

Regression parameter									
Harvest	n	a	_		b		RSD	r	
L 12	34	76 ±	81	NS	220 ± 13	***	194	0.946	
L 26	40	226 +	107	***	193 ±16	* * *	305	0.887	
L 41	39	425 ±	94	***	179 ±14	***	285	0.909	
L 52	39	60 ±	110	NS	209 ± 18	***	306	0.881	
L 69	39	382 ±	88	***	$211 \pm 16$	***	312	0.897	
Pooled <sup>c</sup>	191	281 ±	44	***	192 ± 7	***	295	0.897	

**Table 11.4** Parameters of linear regression calibration equations (± sem) of herbage mass and EPM sward height for individual harvest dates, experiment 13.

<sup>c</sup> First order polynomial:  $y = 84 + 260x - 5x^2$ , r = 0.904.

#### **Botanical composition**

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The percentage DM of the major components in cut herbage remained relatively constant during the trial (Table 11.5). Full details for individual harvests are included in Appendix V. Grasses were the predominant plant species in each of the swards, comprising more than 75% of the DM. Ryegrass (Lolium species) cultivars were the most common grass species followed by browntop(Agrostis tenuis), poa annua (in the 550 sward) and Yorkshire fog (Holcus lanatus, in the 2000 sward). Clover (mainly Trifolium repens) content was highest (c. 11.0% DM) on the 550 sward and diminished once pasture height exceeded c. 6.0 cm. Dead material content showed the reverse trend and was, as expected, highest in the longest pastures.

Herbage Component								
Method	Sward	Grass	Clover	Weeds	Dead			
Cut herbage	550	75.3 ± 2.5	11.3 ± 1.4	5.2 ± 1.6	8.2 ± 1.4			
	1150	81.0 ± 3.2	8.1 ± 2.2	$2.5 \pm 1.1$	8.4 ± 1.8			
	1400	79.4 ± 2.0	$3.4 \pm 0.5$	$1.2 \pm 0.4$	$16.0 \pm 2.2$			
	1550	78.3 ± 2.1	$3.0 \pm 0.6$	$1.4 \pm 0.2$	17.3 ± 2.7			
	2000	78.1 ± 1.6	$3.1 \pm 0.7$	$1.1 \pm 0.5$	17.7 ± 2.1			
Extrusa	550	79.3 ± 1.5	6.7 ± 1.9	2.5 ± 1.9	12.7 ± 1.0			
	1150	79.0 ± 1.7	$11.5 \pm 4.1$	$0.5 \pm 0.0$	9.0 ± 3.3			
	1400	76.7 ± 2.3	3.8 ± 2.2	$0.5 \pm 0.0$	$19.0 \pm 2.0$			
	1550	80.3 ± 2.0	$2.6 \pm 0.4$	0.0	17.1 ± 1.7			
	2000	71.3 ± 3.3	$3.0 \pm 1.1$	$0.8 \pm 0.0$	23.4 ± 3.1			

Table 11.5 Botanical composition of swards, estimated from cut herbage (% total DM, mean±sem for five harvests) and by point analysis of oesophageal fistulated sheep extrusa (% occurrence, mean±sem of three collections from three wethers), experiment 13.

The herbage components of extrusa generally paralleled those obtained by dissection of cut herbage (Table 11.5), although the point analysis refers to the frequency of occurrence of diet components rather than to the proportions of DM consumed. Grasses were the major component of herbage intake. More dead material appeared on the extrusa collected from the 1400, 1550 and 2000 swards, while more clover was evident in the samples obtained from the 550 and 1150 treatments.

# Invitro digestibility of herbage

Variation between estimates of <u>in vitro</u> herbage digestibility for the three collections from oesophageal fistulated sheep (L 24, 52 and 68) was low (Appendix V). Therefore only mean results are presented in Table 11.6. These provide an indication of differences in feed quality between the swards. Actual values for each collection were used for the derivation of herbage intake. The CV between the mean values of OMD for the five swards was 0.68%. Differences in OMD were not significantly different between treatments, periods, or sheep. The inorganic (ash) fraction was significantly (P < 0.05) greater on the 550 and 1400 swards. With the exception of the 1400 treatment, which included areas with poor drainage, ash decreased as the residual height of grazing increased. The higher ash component of the shortest sward depressed the DMD and DOMD values (Nicoll 1982), but differences between the swards were otherwise small.

			Herbage parameter								
Sward	na	Ash	DMD	OMD	DOMD						
550	7	24.21 ± 1.47	75.68 ± 1.11	80.63 ± 0.67	65.13 ± 0.70						
1150	9	16.97 ± 0.77	78.81 ± 0.27	81.68 ± 0.47	$70.48 \pm 0.40$						
1400	9	18.07 ± 0.70	77.86 ± 0.35	81.47 ± 0.24	$69.60 \pm 0.42$						
1500	9	16.36 ± 1.25	78.47 ± 0.42	81.21 ± 0.37	$70.32 \pm 1.02$						
2000	9	$15.31 \pm 0.66$	$77.22 \pm 0.44$	80.39 ± 0.36	$69.92 \pm 0.37$						

**Table 11.6** Ash content (mean±sem) and <u>in vitro</u> digestibility values of extrusa collected by oesophageal fistulated wethers from each sward, experiment 13.

<sup>a</sup>Number of extrusa samples collected per sward.

## Animals

#### Eweherbageintakes

Predicted daily OMI and DMI of ewes rearing one or two lambs during three stages of lactation are presented in Figure 11.4 (see Appendix V for data). Values for the period L 27-32 were derived from the chromium concentration in the faeces collected from sward rings while ewes of different rank were grazed separately within treatment paddocks. These show that OMI were similar between ewes across treatments, and between rearing ranks within treatments, and equated to 28.9-32.7 g OM/kg ewe liveweight (LW)/d (Table 11.7). Although rearing rank x treatment interactions were not significant, estimates of OMI per kg of ewe LW (at the time of faecal sampling) were consistently higher (5.1-14.3%; P<0.05) for the ewes rearing twins. Differences in DMI between treatments were small because of soil ingestion, particularly on the 550 and 1400 swards (Table 11.8).

During the second and third intake measurement periods (L 59-62 and L 66-70, respectively) differences in OMI (and DMI) between treatments were small and non-significant on all except the 550 sward. Thus ewes on the 1150 and higher swards consumed around 2.00 kg OM/ewe/d while those on the 550 sward had intakes of c. 1.58 kg OM/ewe/d. With one exception, ewes rearing twins consumed more OM/kg ewe LW/d (P < 0.05) than those rearing singles, although the magnitude of the difference (1.3-11.2% in period 2 and 0.9-17.6% in period 3) was inconsistent across treatments. When intakes were expressed in terms of DM consumption, differences between treatment groups were reduced, as for the sward ring sample estimates, because of the variation in soil ingestion by ewes on the different sward heights (Table 11.8).

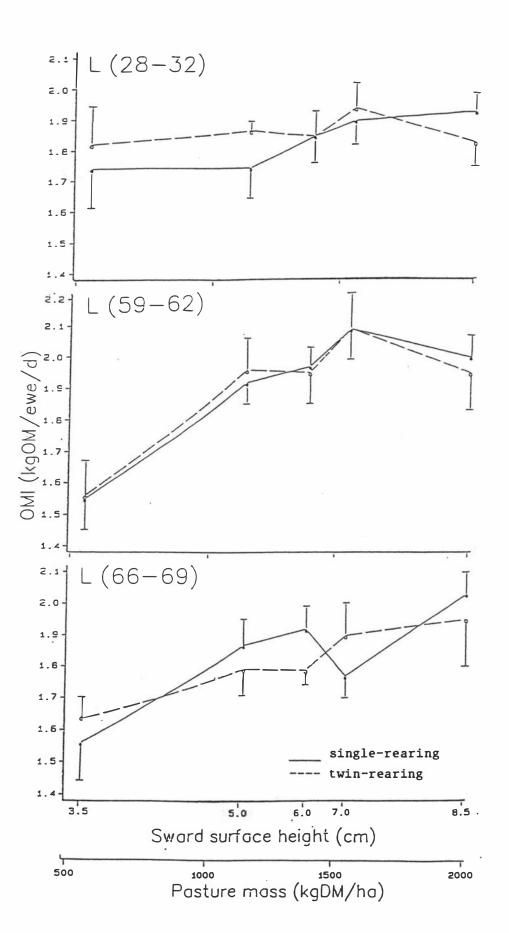


Figure 11.4 Effect of sward surface height on organic matter intake by lactating ewes, experiment 13.

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	Per	iod 1 (L 28-32)	Period	2 (L 59-62)	Period	3 (L 66-69)
Sward	Single	Twin	Single	Twin	Single	Twin
550	31.20	33.59	28.38 ± 1.74	28.37 ± 1.73	29.13 ± 1.55	29.39 ± 1.24
1150	28.12	32.20	31.49 ± 1.65	34.30 ± 2.26	29.90 ± 1.55	35.16±2.42
1400	28.73	32.57	$31.33 \pm 1.63$	34.29 ± 1.27	30.05 ± 2.03	31.31 ± 1.01
1550	29.64	31.48	$34.30 \pm 0.74$	$34.76 \pm 0.74$	$28.54 \pm 1.41$	31.26 ± 2.35
2000	28.97	30.95	$33.44 \pm 1.31$	$37.18 \pm 1.42$	29.21 ± 1.76	33.79 ± 2.60
Overail me	an	30.71	32.78	8	30.	77
-s <sup>a</sup> b		2.88	5.3	7	5.	93
-Sw		1.68	3.70	6	4.	42
Effects						
Rearing ran	k	•	•		,	
Eweage		NA <sup>b</sup>	•			
Treatment		NS	NS	5	2	NS .
Treatment x	rank	NS	NS	5	1	NS .
Period (P2v	s P3)	2	Ň	•••		

Table 11.7 Effect of sward surface height on herbage intakes of ewes rearing single (S) or twin (T) lambs at three stages of lactation (g OM/kg cwe LW/d; mean ± sem), experiment 13.

<sup>a</sup>The "animal between sward" (treatment) standard deviation  $(s_b)$  and the "within sward" standard deviation  $(s_w)$ . These correspond to the terms used in the denominator of the F-tests. The same definitions apply to subsequent tables.

 $^{b}NA = Not applicable.$ 

**Table 11.8** Effect of sward surface height on ingestion of soil (g DM/ewe/d) by ewes of different rearing ranks, experiment 13.

	Period 1 (	L 28-32) <sup>a</sup>	Period 2 (L	, 59-62) <sup>b</sup>	Period 3 (L 66-69) <sup>b</sup>		
Sward	Single	Twin	Single	Twin	Single	Twin	
550	253	182	143 ± 19	144 ±26	111 ±11	111 ± 16	
1150	71	77	44 ± 3	41 ± 5	41 ± 5	45 ± 3	
1400	108	122	66 ± 2	82 ± 20	92 ± 10	87 ± 8	
1550	81	78	37 ± 6	34 ± 3	45 ± 3	40 ± 4	
2000	35	67	$21 \pm 3$	22 ± 4	34 ± 5	33 ± 4	

<sup>a</sup> Group mean soil intake estimated from sward ring samples.

<sup>b</sup> Mean ± sem soil intake for individual ewes during each period.

The group mean OMI of ewes, predicted from faecal samples collected from sward ring sites during lactation, are presented in Table 11.9. These estimates indicate that differences in daily OMI were small across the sward treatments (P < 0.1) but were generally lowest on the 550 sward. Intakes were highest during the initial intake period (L 18-22) when the ewes had been on their respective swards for seven days. Pasture heights were at or near their maximum for the trial at this stage (Figure 11.2). OMI decreased most in the ewes on the 550 sward, a reflection of both reduced ewe liveweight (Table 11.10), and the lower herbage availability on this pasture.

Stage of lactation (L):									
Sward	18-22	23-27	28-32	33-36	53-57	58-63	66-70		
500	2.14	1.82	1.78	1.78	1.65	1.54 (1.55)	1.53 (1.60)		
1150	2.05	2.08	1.80	1.83	2.17	2.02 (1.94)	1.85 (1.60)		
1400	2.16	1.95	1.84	1.72	2.02	2.01 (1.97)	2.19 (1.86)		
1550	2.18	2.14	1.91	1.70	1.94	2.31 (2.09)	1.71 (1.84)		
2000	2.14	1.96	1.87	1.73	2.24	2.34 (2.20)	1.99 (1.99)		
Mean	2.13	1.98	1.84	1.75	2.00	2.04	1.80		
-sb			151						
-s <sub>w</sub> Effects			127						
Period			*						
Treatment			+						
Treatment			NS						

**Table 11.9** Organic matter intakes of ewes (kg OM/ewe/d) predicted from the concentration of chromium in faeces collected from ring sites on swards with different herbage mass (experiment 13). Figures in brackets are the comparable group means estimated by rectum grab sampling over the same period.

The correlation (r) between group mean OMI estimated by sward ring and rectum grab sampling methods for the final two intake periods was 0.88. The sward ring sampling technique can therefore be assumed to have provided estimates of group mean OMI of similar reliability during the earlier stages of lactation.

The multiple regressions of OMI on ewe liveweight (LW), rearing rank, ewe age (two year old ewe dummy variable = 1, mixed aged = 2) and sward height (h) for the periods L 59-62 and L 66-69 were:

OMI  $_{59-62} = 190$  (± 284) + 95 (± 18) h + 67 (± 59) rank + 19 (± 4) LW - 13 (± 64) age. n = 56, r<sup>2</sup> = 0.57. NS \*\*\* NS \*\*\* NS OMI  $_{66-69} = 794$  (± 350) + 37 (± 72) h + 88 (± 72) rank + 13 (± 6) LW - 49 (± 75) age. n = 56, r<sup>2</sup> = 0.20. + + NS \* NS

Ewe liveweight and sward conditions had the most significant effects on feed intake.

# Ewe liveweight

Ewe liveweights (unfasted and corrected for wool weight) increased by 2-4 kg/ewe on all except the 550 sward, where the ewes lost a similar amount of liveweight, over the 66 d of continuous grazing on the respective treatments (Table 11.10). Twenty-four hour fasted liveweights, obtained on L 76, indicated that gutfill contributed to  $10.45\pm0.26\%$  of the unfasted ewe liveweight, with a maximum difference of 1.6% between ewes on the 550 and 1400 swards (P >0.1). The impact of gutfill between treatments and at different weighings could therefore have been expected to be small, since ewes were weighed at the same time at each measurement date and off comparable levels of residual herbage mass. However, the pattern of liveweight change between L 10 and L 33 suggests that this may not have been the case for the first weighing after set stocking (Table 11.11). Differences in ewe liveweight which could be attributed to the amount of wool per ewe were small, as indicated by the average fleeceweights at shearing (L 97, Table 11.16).

			Liveweigl	nt (kg):
	Rearing			
Sward	rank	n	L 10 <sup>a</sup>	L 76
550	Single	6	55.35 b	50.68 b
	Twin	5	53.16	51.62
1150	Single	6	55.80 b	57.78 c
	Twin	4	49.08	52.49
1400	Single	6	58.93 b	62.81 d
	Twin	6	51.63	54.70
1550	Single	5	56.91 b	61.55 d
	Twin	6	54.92	58.45
2000	Single	6	59.26 b	64.99 e
	Twin	6	52.38	57.82
Overall m	eans		55.04	57.51
-sb			2.67	0.76
-sw			5.02	5.96
Effects				
Rank			* *	*
Ewe age			* * *	* *
Treatment	t - linear		NS	**
	t - non-linear		NS	+
Treatment			NS	NS

**Table 11.10** Effect of sward treatment and rearing rank on ewe liveweight at the commencement (L 10) and end (L 76) of continuous grazing, experiment 13.

<sup>a</sup> Corrected for weight of wool clipped on L 97.

b,c,d,e Different letters indicate treatment group means differed significantly at the 5% level by Duncan's Multiple Range Test using a between treatment (2 df) error term.

Treatment differences in ewe liveweight at the commencement of continuous grazing (L 10) were small (P > 0.1) but mixed age (P < 0.001) and single-rearing ewes (P < 0.01) were heavier than two year old and twin-rearing ewes respectively. By L 76 treatment differences had become significant (P < 0.01) and ewe liveweights showed a marked increase with increasing sward height. With the exception of those on the 550 sward, single-rearing ewes were heavier than twin-rearing ewes (59.5 vs 55.3 kg, P < 0.05) at L 76. Two year old ewes were lighter than mixed age ewes (59.7 vs 55.1 kg, P < 0.01) at this time.

Ewe liveweight change during the first 45 days of lactation was highly variable, especially on the four longest swards (Table 11.11). This suggests that ewe gutfill may have been adapting to the new levels of pasture availability, particularly on the 1550 and 2000 swards. During the final third of this period (L 34 to L 45) all ewes except those rearing twins on the 550 sward lost liveweight. Subsequently only one period of weight loss, in twin-rearing ewes on the 1550 sward from L 46 to L 59, was recorded on the 1150 and greater swards. This weight loss may have been associated with foot scald problems. However, weight losses on the 550 sward from L 46 to L 76 were consistent and clearly not sustainable in the long term. Liveweight change was highly variable for both single- and twin-rearing ewes (P > 0.1). Two year old ewes generally grew faster than mixed aged ewes but this effect was not consistent.

					Pe	riod	of lactatio	n (L):				
	L 10	)-33	-		L 34-45		L 46	-59	<		0-76	
Sward	Single	Twi	n	Singl	le Tv	vin	Single	Twin		Single	Т	win
550	-90	-36	а	-53	27	а	-107	-69	a	-28	-5	а
1150	99	75	a	-50	-4		31	50		4	31	ab
1400	83	54	а	-44	93	а	71	114	а	88	78	b
1550	191	119	а	-140	-8	ab	11	-20	а	104	68	b
2000	175	171	а	-108	-151	b	95	63	а	98	143	С
sb	50				55		19	91		45	5	
sw	92			1	54		9	96		89	)	
Effects												
Rank	NS			1	NS		N	S		NS	5	
Ewe age	+			1	NS		4	k 🕸		NS	5	
Treatment	*				+		N	S		4		

Table 11.11 Effect of sward treatment on liveweight change (g/d) in ewes of different rearing rank, experiment 13.

<sup>a,b,c</sup>Different letters indicate treatment group means differed significantly at the 5% level by Duncan's Multiple Range Test using a between treatment (2 df) error term.

Multiple regression equations were fitted to establish the effect of sward surface height (linear and quadratic terms), ewe age (two year old ewe dummy variable = 1, mixed aged ewe = 2) and rearing rank on ewe liveweight change (Table 11.12). The period L 10 to L 33 was excluded because of the possible confounding effects of gutfill.

ίπ.		Pe	eriod of 1	actation			Ewe	liveweig	ht:
		L 34-4	5		L 46-7	6		. 76	
Parameter	ßa	seß	Sign.	B	seß	Sign.	ß	seß	Sign.
Constant	18	247	NS	-234	108	¥	-11.74	6.39	+
Ewe age	-44	37	NS	-51	16	**	-2.53	1.03	*
Rearing rank	-27	37	NS	4	16	NS	-0.30	0.86	NS
h	7	82	NS	94	36	* *	6.12	1.72	***
<u>h</u> <sup>2</sup>	2	7	NS	-6	3	+	-0.37	0.14	*
Initial LW							0.94	0.08	***
Model r <sup>2</sup>			0.11			0.48			0.85

Table 11.12 Coefficients for the multiple regression ( $\beta \pm se\beta$ ) of ewe liveweight change (g/d) during lactation (n = 56) and ewe liveweight (LW, kg) at the end of continuous grazing on sward surface height (h and h<sup>2</sup>), rearing rank and ewe age, experiment 13.

 ${}^{a}\beta$  = Partial regression coefficient; se $\beta$  = standard error of regression coefficient.

For the period L 34 to L 35 the standardised partial regression coefficients indicated that sward height had less effect than ewe age or rearing rank on changes in ewe liveweight. However, for the period L 46 to L 76 sward height had a large and primarily linear effect (P < 0.01) on ewe liveweight change. The impact of ewe age (P < 0.01), was larger than the effect of rearing rank (P > 0.1) with two year old ewes gaining more weight than the older ewes. Final ewe liveweights (L 76) were most affected by sward height (P < 0.001) and ewe age (P < 0.05) during the period of continuous grazing. Rearing rank had only a small (P > 0.1) negative effect.

#### Ewe condition

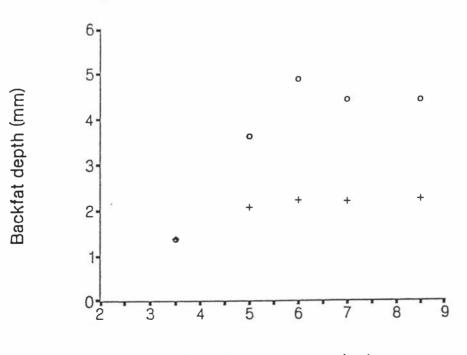
The general trend was for subjectively assessed ewe condition score to decline from L 33 to L 70 with the largest decreases occurring in the twin-rearing ewes (Table 11.13). Thus ewe condition was consistently lower in the ewes rearing two lambs at L 33 (P<0.001), L 59 (P<0.05) and L 70 (P<0.01). Ewes on the 550 sward were thinner than the ewes on the remaining treatments but this difference was significant (P<0.05) only at L 33. Condition scores at L 33 suggest that the ewes on the 550 sward had lost more body condition than the remaining trial ewes during the period L 10 to L 33. This is compatible with the results for ewe liveweight change (Table 11.11).

Near the end of the trial (L 70) ewes rearing twin lambs had lower (P < 0.01) ultrasonic backfat depth measurements than those rearing singles (Table 11.13). Backfat depth was at least 1.5 mm greater in the single-bearing ewes on the four longest pastures, but on the 550 sward single- and twin-rearing ewes had similar amounts of fat cover (Figure 11.5).

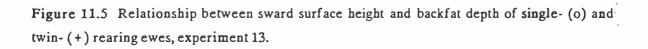
	D	Co	ndition Score	:	Backfat depth (mm):
Sward	Rearing rank	L 33	L 59	L 70	L 70
550	Single	2.75 a	2.58 a	2.33 a	1.37 a
	Twin	2.70	2.60	2.30	1.38
1150	Single	3.42 b	3.00 a	3.17 a	3.63 b
	Twin	3.63	2.38	2.38	2.08
1400	Single	3.50 b	3.33 a	3.33 a	4.87 b
	Twin	3.75	3.00	2.67	2.22
1550	Single	3.30 b	3.10 a	2.90 a	4.42 b
	Twin	3.08	2.67	2.58	2.20
2000	Single	3.25 b	2.83 a	3.08 a	4.41 b
	Twin	2.96	2.83	2.67	2.25
Mean		3.04	2.84	2.76	2.91
-sb		0.17	0.60	0.43	0.54
-s <sub>w</sub> Effects		0.44	0.44	0.56	1.99
Rearing	rank	***	*	**	* *
Ewe age		NS	+	NS	NS
Treatmo		*	NS	NS	*
Treatm	ent rank	NS	NS	NS	NS

Table 11.13 Effect of sward treatment and ewe rearing rank on condition score and backfat depth, experiment 13.

a, bDifferent letters indicate treatment group means differed significantly at the 5% level by Duncan's Multiple Range Test using a between treatment (2 df) error term.



Sward surface height (cm)



The correlations between ewe liveweight (unfasted) and condition scores on L 33, 59 and 70 were 0.49, 0.52 and 0.57, respectively (all significant at P<0.001). Backfat depth was more highly correlated with L 70 liveweight (r = 0.75, P<0.001), and with condition score recorded on the same day (r = 0.78, P<0.001).

## Lamb liveweight and growth rates

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Data for 82 of the 90 lambs originally allocated to the treatment pastures were suitable for analysis (Table 11.14). The average birthweight of lambs, lay within the range 4.34 kg (1150 sward) to 4.52 kg (2000 sward). Single lambs were 0.93 kg heavier at birth than twins (5.02 vs 4.09 kg, P < 0.001), and ram lambs were heavier than ewe lambs (4.50 vs 4.36 kg, P < 0.1).

Up to L 45 growth rates of lambs were not significantly affected by sward conditions, but single lambs grew more rapidly than those reared as twins (317 vs 245 g/d, P<0.001). Sex effects were not significant during this period. From L 46 to L 70, lambs on the 1150 and longer swards achieved higher average rates of liveweight gain (240 g/d) than those on the 550 sward (197 g/d). Single (P<0.001) and ram lambs (P<0.01) had more rapid rates of growth from L 46 than twins and females respectively but there were significant sex x treatment and rank x treatment interactions. Thus the difference in growth rates between singles and twins was not the same for ram and ewe lambs for all treatments. At L 76, lamb weights were 4.5 kg greater in singles than in twins (P<0.001) and 1.4 kg (P<0.01) greater in rams than in ewe lambs respectively. However, treatment differences in the final lamb liveweight (unfasted), which ranged between 21.50 kg on the 550 sward and 22.96 kg on the 1150 sward, were not significant.

Variation in gutfill, as a percentage of the L 76 lamb liveweight, was greater than recorded in the ewes and ranged from 5.4% for lambs from the 550 sward to 8.6% from lambs from the 1550 sward (P < 0.1). Percentage gutfill was greater on average in the lighter twin lambs than in those reared as singles (7.26 vs 6.25\%, P < 0.01). Analysis of the fasted L 76 liveweights indicated that treatment differences remained non-significant if the effect of gutfill was excluded.

	D .			Weight (l	kg) at:	Daily gain	(g/d) fro	m:
Sward	Rearing <b>rank</b>	Sex	п	Birth	L 76	L 0-45	L 46-7	76
550	Single	Ram	3	5.00 a	25.27 a	340 a	208	a
	0	Ewe	3	4.60	20.57	261	174	-
	Twin	Ram	5	4.04	20.22	246	205	
		Ewe	4	4.13	20.98	269	196	
1150	Single	Ram	2	5.50 a	30.80 a	381 a	298	at
	0	Ewe	4	4.60	24.80	305	265	
	Twin	Ram	5	4.46	21.72	232	252	
		Ewe	3	3.87	17.37	202	166	
1400	Single	Ram	3	4.97 a	26.00 a	312 a	282	b
	0	Ewe	3	5.40	24.07	281	247	
	Twin	Ram	7	4.50	22.80	265	259	
		Ewe	4	3.48	18.10	212	203	
1500	Single	Ram	4	5.05 a	25.40 a	311 a	248	ab
	5.5	Ewe	2	5.50	29.70	370	296	
	Twin	Ram	5	4.34	20.78	235	221	
		Ewe	7	3.86	20.57	244	215	
2000	Single	Ram	1	5.00 a	30.20 a	385 a	303	ab
	0	Ewe	5	5.04	24.30	312	230	
	Twin	Ram	8	3.94	21.94	256	238	
		Ewe	4	4.13	20.90	261	218	
Overall	mean (total							
	single		(30)	5.02	25.37	317	248	
	twin		(52)	4.09	20.86	245	223	
-sb				0.04	1.80	8	42	
-sw				0.71	2.44	46	34	
Effects								
Sex				NS	-	NS	-	
Rank				* * *	-	* * *	-	
Treatm	ent -linear			NA	NS	+	NS	
	- quadı	atic		NA	NS	+	NS	
Rank x	treatment			NS	+	NS	+	
Sex x tr	eatment			NS	*	NS	-	

**Table 11.14** Lamb birthweights and effects of sward conditions, rearing rank and sex on final weight and growth rates, experiment 13.

<sup>a,b</sup>Different letters indicate treatment (sward) group means, across rearing ranks and sexes, differed significantly at the 5% level by Duncan's Multiple Range Test using a between treatment (2 df) error term. NA = not applicable.

The relative impact of sex, rearing rank and sward height on lamb growth rates and L 76 liveweights was estimated by multiple regression (Table 11.15). This confirms that sward, conditions had no positive impact on lamb production from L 0 to L 45, but became more important from L 46 to L 76. Lambs which had higher birthweights achieved greater L 76 liveweights, as did ram and single-reared lambs, compared with ewe and twin-reared lambs respectively. Sward conditions had only a small positive impact on L 76 weights.

		L 0-4	45		L 46-	76		L 76	
Parameter	ß	seß	Sign.	ß	seß	Sign.	ß	seß	Sign.
Constant	465	71	* * *	138	56	**	22.44	4.54	* * *
Sex	-17	11	NS	-28	8	* *	-1.73	0.59	**
Rearing rank	-75	11	* * *	-31	9	* * *	-3.74	0.72	* * *
Sward height	-21	23	NS	59	18	* *	0.92	1.27	NS
Birthweight	-	-	-	-	-	-	1.16	0.41	**
Model r <sup>2</sup>			0.38			0.31			0.52

Table 11.15 Multiple regression coefficients for lamb growth rates from L 0-45 and L 46-76 ( $\beta \pm se\beta$ ) of lactation on rearing rank, sex and sward surface height, experiment 13. Coefficients for the same regression, but including birthweight, on L 76 liveweight are also shown (Dummy variable for sex; ram = 1, ewe=2).

# Ewe wool production

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Midside wool samples were obtained from 58 of the ewes allocated to treatment pastures. Ewes which had previously been excluded from the liveweight analyses were not removed from the wool data set because wool production of these animals was above the respective treatment averages (i.e. short-term effects of disease had a larger impact on liveweight than on wool growth).

Total wool weight (excluding wool removed for midside samples) collected on L 97, after the ewes had grazed together as a single group from L 77, averaged  $3.43 \pm 0.10$  kg for 55 of the trial ewes (Table 11.16). No effect of the grazing treatments on total wool production was apparent. Two year old ewes, last shorn in March, produced 2.85 kg of wool (2.88 kg in the single-rearing and 2.83 kg in the twin-rearing ewes, respectively). Mixed age ewes, shorn 12 months previously, produced 3.99 kg (3.92 vs 4.08 kg for single- and twin-rearing ewes, respectively).

			Mid-side sam	ple	Contral	ateral samp	le	T
Sward	Rearin; rank	g n	Wool growth (mg/cm <sup>2</sup> /d)	Colour (Y-Z)	Diameter (µm)	Strength (N/ktex)	Colour (Y-Z)	Tota Woo (kg)
550	Single	6	0.98 a	0.97 a	38.98 a	17.29 a	2.78 a	3.52
	Twin	6	1.16	1.45	38.55	16.33	2.72	3.18
1150	Single	6	1.81 a	1.23 ab	40.67 a	17.83 a	3.20 a	3.62
	Twin	4	0.99	1.63	38.88	11.51	2.95	3.22
1400	Single	6	0.98 a	1.43	38.88 a	21.43 a	2.65 a	3.29
	Twin	6	1.16	1.55	38.13	12.37	3.43	3.47
1550	Single	6	1.23 a	1.53 ab	40.15 a	18.45 a	5.03 a	3.37
	Twin	6	1.10	1.50	38.95	19.59	3.73	3.52
2000	Single	6	1.01 a	2.08 b	40.70 a	18.34 a	3.40 a	3.44
	Twin	6	1.09	1.51	39.50	17.17	3.36	3.43
-sb			0.19	0.34	2.85	<b>4</b> .91	0.71	0.35
-sw			0.18	0.57	2.71	6.38	0.78	0.42
Effects								
Rearing ra	ank		-	NS	NS	+	NS	NS
Ewe age			***	NS	NS	+	***	***
Treatment	t		-	NS	NS	NS	NS	NS
Treatment	t x rank		+	NS	NS	NS	NS	NS

**Table 11.16** Effects of treatment and rearing rank on clean wool growth, fibre diameter, fibre strength and colour, and total wool production since the previous shearing, experiment 13.

<sup>a</sup>Different letters indicate treatment group means differed significantly at the 5% level by Duncan's Multiple Range Test using a between treatment (2 df) error term.

Wool on the midside patch grew at an average rate of 1.09 mg (clean)/cm<sup>2</sup>/d. The effects of increased pasture availability and rearing rank on wool growth were inconsistent. The rearing rank x treatment interaction (P < 0.1) indicates that the effects of treatment on wool growth varied between rearing ranks. Clean wool growth was faster in the two year old than in the mixed aged ewes (1.30 vs 0.88 mg/cm<sup>2</sup>/d, P<0.001). Although the overall level of discolouration of wool grown from lambing until L 76 was low, the Y-Z values were higher for the ewes from the three longest pastures.

A similar pattern for colour was evident for the contralateral midside samples of wool grown since the previous shearing. A higher degree of discolouration was also evident in the two year old than in the mixed age ewes (Y-Z = 3.54 vs 2.84, P<0.001). Fibre diameter, as assessed by airflow, did not differ between rearing ranks, ewe age groups or pasture treatments. Staple strength of the contralateral samples was generally greater in the single-rearing than the twin-rearing ewes (18.7 vs 15.5 N/ktex, P<0.1), and in the two year old than the mixed aged ewes (18.6 vs 15.7 N/ktex, P<0.1).

# Lamb wool production

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Midside clean wool growth in the lambs  $(1.72 \text{ mg/cm}^2/\text{d})$ , was inconsistent between rearing ranks across treatments (Table 11.17). There was no relationship between lamb wool production and pasture availability. Airflow measurements indicated that wool from the twin lambs was significantly finer than from the single lambs (31.71 vs 33.49  $\mu$ m, P<0.001). Wool was also finer (P<0.05) for the lambs reared on the two shortest pastures. Wool was almost pure white (mean Y-Z=0.38) for all of the pasture treatments.

Sward	Rcaring rank	п	Wool Growth (mg/cm <sup>2</sup> /d)	Diameter (µm)	Colour (Y-Z)
550	Single	6	1.74 a	33.73 a	0.50 a
	Twin	9	1.73	32.70	0.47
1150	Single	6	1.82 a	34.65 b	0.32 a
	Twin	8	1.43	31.05	0.76
1400	Single	6	1.91 a	33.75 c	0.35 a
	Twin	11	1.58	31.21	0.31
1550	Single	6	1.83 a	32.63 c	0.00 a
	Twin	12	1.72	31.62	0.20
2000	Single	6	1.74 a	32.50 c	0.13 a
	Twin	12	1.82	31.98	0.63
Overall n	nean	(84)	1.72	32.36	0.38
sь			0.26	0.27	0.76
SW			0.32	1.74	0.57
Effects					
Sex			按安安	NS	NS
Rank			-	* * *	NS
Treatmen	nt		-	*	NS
Treatmen	nt x rank		*	NS	NS

**Table 11.17** Effects of sward treatment and rearing rank on lamb midside wool growth, fibre diameter and colour, experiment 13.

a,b,cDifferent letters indicate treatment group means differed significantly at the 5% level by Duncan's Multiple Range Test using a between treatment (2 df) error term.

#### DISCUSSION

The primary purpose of this experiment was to quantify the relationships between pasture availability, herbage intake and the productivity of ewes of different rearing ranks under continuous grazing management during lactation. This was achieved by set stocking six singleand six twin-rearing ewes onto each of five pastures of different sward heights for a period of 66 days from the tenth day after the mid-point of lambing.

#### Experimental Design

The five pastures were established at random and without replication. The decision to adopt a non-replicated design was based primarily on the physical limitations of land area, animals and availability of labour. It was known a priori from the literature that herbage availability affected ewe intakes. Sufficient animals therefore had to be included in each treatment group to obtain reliable measures of herbage intake. Cruickshank et al. (1987) showed that under field conditions the minimum number of animals required to have a 95% chance of detecting a 10% difference in DOMI at the 10% level of significance was 6, due to errors associated with estimating faccal output using CRC (CV c. 4%) and herbage digestibility (CV c. 7.7%). Thus a minimum of 12 ewes (6 of each rearing rank) were required for each treatment arca or a total of 72 animals for a replicated design for three herbage heights. Furthermore, insufficient New Zealand research results were available to indicate which three pasture heights should be chosen, since the response curves for single- and twin-rearing ewes were not expected to cover the same ranges of herbage mass (Rattray et al. 1982b; Rattray 1986). Bircham (1981) adopted a non-replicated design for a range of four herbage masses, but maintained separate records for quartiles within each paddock, to generate treatment replication. However, Bircham's primary interest was sward dynamics under continuous grazing, rather than the productivity of the ewes rearing single lambs grazing each of the treatments (although herbage intakes were estimated by drenching with Cr<sub>2</sub>O<sub>3</sub>-impregnated paper). In those experiments ewes were managed under a "put and take" system, and were removed if their liveweights fell below 50 kg (Bircham 1981). In contrast, the main focus of the present experiment was ewe productivity. Ewes and their lambs were therefore required to remain on their respective swards for the duration of the experiment. A form of replication was provided by animals within swards. For this reason, the stocking rate selected for the trial area was conservative, and non-treatment animals were used to maintain swards at the required heights. It was not anticipated, at the commencement of the trial, that ewes would need to be removed from their plots because of malnutrition since the lowest sward height was above that frequently encountered on New Zealand sheep farms over the lambing period (Parker and Townsley 1986). An alternative experimental design would have been to replicate at least one of the sward heights (Garrick 1989). This would have provided an error term for testing between sward parameters (not a primary objective) but,

given constraints on land and other resources, would have precluded use of one of the treatment heights and hence diminished the opportunity to define the herbage intake - productivity relationship. The design of this experiment therefore posed the classical dilemma between the need for sufficient scale and range of treatments to provide a holistic appreciation of system performance (which by virtue of scarcity of resources leads to non-replication) and the statistical requirement for replication to test whether treatment differences are real.

Although careful consideration was given to the selection of trial paddocks to minimise variation in sward parameters and climate, microsite differences in soil fertility, drainage and pasture composition meant that the five swards were not identical. The absence of treatment replication did not enable an estimate of these effects to be made, except by using a regression approach to partition the treatment degrees of freedom. This provided a statistical test of low power. Thus some treatment differences which were statistically non-significant would be of practical significance. In these instances the nature of the relationship can be interpreted from a practical farming viewpoint

# Variation Between Swards and Maintenance of Pasture Conditions

The swards were approximately maintained at their initial heights for the pastures with surface heights of 3.5, 5.0 and 6.0 cm but the selective grazing behaviour of the ewes (Clarke and Harris 1985), together with relatively low seasonal pasture growth rates, meant that sward height was more variable on the 7.0 and 8.5 cm pastures. The latter two pastures became more clumpy as the trial progressed, and the ewes and their lambs had the opportunity to graze pastures within the sward considerably below the initial targets. Bircham and Hodgson (1983) and Grant et al. (1983) noted similar increases in variability of height measurements on the longer pastures of their fixed height grazing experiments. This suggests that sward surface height for ryegrass-white clover pastures should be restricted to less than 6.0 cm for sheep studies in early lactation. The findings of Bircham (1981) support this view, as do the herbage intake results for this trial which show that intakes were maximised at a sward height below this level during the lactation period.

Pasture cuts to ground level were more difficult to obtain where pasture height was < 3.0 cm because, as also noted by Hodgson et al. (1981), herbage became more prostrate and increased in density, relative to the longer pastures, as the trial progressed. Post-harvest herbage residual was therefore greatest on the low sward quadrat cuts. Pasture mass may therefore have been underestimated on the low swards (as indicated by the pasture height-mass calibrations, Table 11.5). Grant et al. (1983) measured similar large increases in sward mass at three experimental sites with clover-free ryegrass-dominant continuously grazed swards (i.e. 358 kg OM/ha between 2.1 and 2.9 cm average sward heights at site 1, 393 kg OM/ha between 1.1 and 2.1 cm at site 2, and 441 kg OM/ha between 2.0 and 3.1 cm at site 3).

The NHA results (Table 11.3) show that sward differences existed, notably in the 1550 sward. A sward height of 2.5-3.5 cm did not disadvantage NHA, which is in agreement with the findings of Tainton (1973) and Clarke (1978). Smetham (1975) suggested that NHA would not be compromised unless pasture heights fell below 2.5 cm.

All the swards had, and maintained, a similar proportion of the predominant grass species (<u>L</u>, <u>perenne</u>) but clover contents were higher on the 550 and 1150 swards. <u>In vitro</u> OMD exhibited little difference between treatments. This indicates that while ewes had less opportunity to graze new pasture growth on some of the swards, the quality of the diet selected by the ewes was probably similar across the range of sward heights and did not change significantly through time. The small variation in pasture digestibility is likely to have been associated with the swards remaining in a vegetative state until the final week of the trial and the selective grazing behaviour of sheep (Hughes 1983). Bircham (1981) recorded larger differences between sward heights in OMD on predominantly <u>Lolium perenne</u> swards, especially at the lowest sward height where animals consumed significant amounts of pseudostem material.

It is concluded that variation between the swards, in attributes other than pasture mass, was not sufficiently large to compromise interpretation of animal response data across the range of treatments.

# CRC Performance

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A total of 120 capsules were administered to the ewes during the trial. For the 60 CRC involved in the second intake period no indication of capsule failure was evident from the chromium concentration in faecal grab samples from individual animals. Chromium levels in a single grab sample on L 19 and L 20 from 48 of the ewes showed that capsule failure probably did not occur during the first intake period.

Analysis of the chromium end point data (Appendix IV) indicated that the rate of CRC chromium release for the different pasture heights was similar to that measured by serial slaughter of ewes in experiment 9 (Chapter Eight). In that experiment, differences in chromium release rates occurred when ewes were grazed at pasture heights of 2.0 - 2.5 cm, compared to 3.5 cm and longer pastures, but the differences were not significant. The assumed common release rate of 139 mg Cr/d used to derive faecal DM output for all treatments in this study may therefore have marginally ( $\pm$  5%) under- and over-estimated actual outputs, particularly on the 550 and 2000 swards.

#### Ewe Herbage Intakes

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The main feature of the predicted herbage intakes was that intake in both single- and twinrearing ewes was maximised at a sward surface height of around 5.0 cm. This corresponds to a herbage mass of 900-1000 kg OM/ha or a compressed sward height of 4.0 - 4.5 cm. Milne et al. (1981) demonstrated that the asymptote for herbage intake by ewes rearing twin lambs and continuously grazed on swards of 500 (sward surface height = 2.0 cm), 750 (3.0 cm) or 1500 (6 cm) kg OM/ha during the first six weeks of lactation was also around 1000 kg OM/ha (5.0 cm). In another British trial, where ewes with twin lambs were continuously grazed over the first seven weeks of lactation, Penning and Hopper (1985) measured average OMI by ewes of 1.60, 2.98, 2.75 and 2.54 kg/d at sward surface heights of 3, 6, 9 and 12 cm respectively. These results also suggest that ewe intakes were maximised at a sward height of around 6.0 cm.

Similarly the predicted DMI, which include a highly variable amount of soil ingestion (especially on the 550 sward), correspond to the 2.10 kg DMI/d at a residual of 500 kg DM/ha estimated by McEwan et al. (1983) by the difference technique. However, they are lower than the 3.35 kg DMI/d estimated for a 900 kg DM/ha residual for single- and twin-rearing Romney ewes continuously grazed during lactation.

The sward ring samples suggest that OMI peaked at or during the third week of lactation but then remained at a similar level until the ninth week of lactation. This contrasts with the results of Clarke (1978) who recorded a gradual decline in intakes, measured by the difference method with ewes shifted daily, as lactation progressed. However, Gibb and Treacher (1980) found only a slight decrease from peak intakes at weeks 5-7 of lactation until week 15. A similar response was found in their 1978 experiment when intakes peaked 2-3 weeks after parturition. Geenty and Sykes (1986) recorded maximum intakes between weeks 2 and 4 of lactation in Dorset ewes at pasture.

On a per kg liveweight (LW) basis the predicted ewe intakes of 28-37 g OM/kg LW/d compare favourably with other intake studies of lactating ewes. Coop and Drew (1963) measured intakes of 27 and 32 g OM/kg LW/d in Border Leicester x Romney ewes (57 kg LW) rearing single and twin lambs respectively, when pasture was not limiting. Intakes of 22.0, 29.0 and 38.0 g OM/kg LW/d by ewes rotationally grazed with their twin lambs on allowances of 25, 33, 47, 73 and 120 kg OM/ewe/d were recorded by Gibb and Treacher (1978). In a latter trial by Gibb and Treacher (1980) peak intakes in twin-rearing ewes corresponded to 33-38 g OM/kg LW/d. Single-rearing ewes consumed 32 g OM/kg LW/d at a sward surface height of 4.5 cm (1700 kg OM/ha) in Bircham's (1981) trial. Total daily OMI of 1.74 to 1.93 kg/d in week 4 of lactation are similar to the values reported by Geenty and Sykes (1986) for ewes on daily allowances of more than 5.7 kg DM/ewe/d.

Twin-rearing ewes consumed 0.9 - 17.6% more herbage per day on a liveweight basis than single-rearing ewes. This difference is smaller than the 20-30% suggested in feeding tables for New Zealand ewes (Geenty and Rattray 1987) and is also less than the 18-33% higher intakes of twin-rearing ewes recorded by Coop and Drew (1963). However, Maxwell et al. (1979) recorded a difference of only 6% with ewes grazing ryegrass-white clover pastures, while Owen and Ingleton (1963) reported no difference in estimated intake for ewes at pasture suckling single or twin lambs. Similarly, Peart (1967) found that intakes of twin-rearing ewes were not consistently greater than those of ewes rearing singles and by week 12 of lactation all rearing rank differences had disappeared. McEwen et al. (1983) found no difference between the intakes of single- and twin-rearing ewes measured by the pasture disappearance method. Energy intake of pasture and concentrates by twin-rearing cwes at weeks 5 and 6 of lactation was only 4% more than that of the ewes rearing singles, despite the significantly lower milk production by the ewes suckling singles (Newton and Orr 1981).

The trial results therefore suggest that the capacity of ewes of different rearing ranks to consume pasture was reasonably similar on each of the sward height treatments, especially on the 550 sward where intakes were almost identical from L 59. Thus, intakes of both single- and twin-rearing ewes were showing signs of restriction at a sward surface height of 3.5 cm, and the physical capacity to harvest feed (biting rate x bite size x grazing time; Allden and Whittaker 1970), limited intakes to a comparable level. Nevertheless, total DMI, as a proportion of fleece-free bodyweight, equated to 3.4 to 4.1% of unfasted ewe liveweight during period 1 and was highest in the ewes on the 550 sward. During subsequent periods daily DMI represented a similar proportion of bodyweight. These values are well above the 3% maximal bodyweight figure suggested by Evans (1960), but are less than the maximum DMI of 5.2 - 6.6% of bodyweight (Peart 1967) or the 4.7% recorded by Barnicoat et al. (1949).

# Daily energy intake

The metabolisable energy (ME) intakes of single- and twin-rearing ewes, derived from predicted DMI and <u>in vitro</u> herbage DOMD values, are shown with recommended requirements for ewes at maintenance during lactation (Geenty and Rattray 1987) in Table 11.18.

		Pe	eriod of lactatio	n:		
Sward	L 27-32		L 59-6	52	L 66-70	
	Single	Twin	Single	Twin	Single	Twin
550	21.38	21.48	19.15	19.29	20.01	20.84
1150	22.28	23.94	24.48	24.96	24.39	26.51
1400	23.95	24.05	25.31	24.71	25.09	23.37
1550	23.82	24.29	26.81	26.76	23.10	24.69
2000	25.40	24.42	28.81	27.61	25.47	24.40
Recommend	ed ME <sup>a</sup>					
50 kg ewe	26.5	31.0			20.5	22.0
55 kg ewe	28.0	32.0			21.5	23.0
60 kg ewe	29.0	33.0			22.0	24.0

Table 11.18Effect of sward treatment on estimated metabolisable energy intakes(MJME/ewe/d) of single- and twin-rearing ewes during lactation, experiment 13.

<sup>a</sup> Geenty and Rattray (1987).

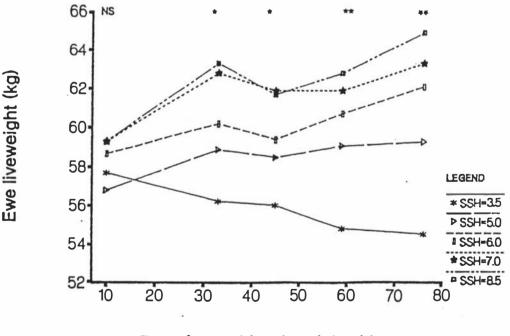
At weck 4 of lactation intakes of all ewes were below the recommended requirement. However, at this stage ewes were losing liveweight (Table 11.11). Gecnty and Rattray (1987) estimated that requirements can be reduced by 1.7 MJME/d for every 100 g of liveweight loss, but increase by 6.5 MJME/d for the same amount of gain. By week 9 of lactation, and with liveweight change considered, estimated energy intakes were more comparable to the recommended levels. The mid-lactation figures compare favourably with the respective ME intakes of 32.3 and 33.8 MJME/d for c.80 kg single- and twin-rearing Masham ewes at pasture (Newton and Orr 1981).

No measure of energy expended while harvesting pasture was obtained but visual observation showed that ewes on the 550 sward consistently spent more time grazing than ewes on the remaining treatments. Coop and Drew (1963) estimated that, relative to <u>ad libitum</u> grazing, 30-60% extra energy was required where ewes were grazing very short pastures and grazing time exceeded 6-8 hours. This was reflected in higher intakes, but lower production, by ewes continuously grazed on a low sward height compared to those on longer pastures. In a more precise study with ewes suckling twin lambs, Penning and Hopper (1985) found that ewes at a sward surface height of 3.0 cm, spent significantly longer times grazing each day (770 minutes) than ewes on 6 cm (637 minutes), 9 cm (562 minutes) or 12 cm (553 minutes) pastures. The rate of pasture consumption by the ewes on the 3 cm sward was therefore about 50% of those on the longer pastures (which were similar to one another). The biting rate of twin-rearing ewes also increased when lower daily herbage allowances were offered under rotational grazing (Penning et al. 1986). Bircham (1981) considered that the inflexion point beyond which grazing time began to decline was when extended tiller lengths were less than 4-5 cm (i.e. about mid-way between the 550 and 1150 swards). In both the trial of Penning and Hooper (1985) and that of

Bircham (1981) bite size, rather than biting rate, was affected by herbage mass. It can therefore be inferred that relative to those on the other swards, ewes on the 550 sward expended more energy grazing, so diverting energy which could otherwise have been used for liveweight gain, wool growth or milk production. Thus productive performance of the 550 sward ewes, as in the trial of Coop and Drew (1963), was lower than suggested by daily OMI or energy intakes.

# Ewe Liveweight and Body Condition Changes

The small numbers of ewes in each treatment group, and the considerable variation in their individual productivity (and initial body condition) made it difficult to clearly identify the effects of rearing rank on liveweight change. The results suggest that these were relatively small but this is contrary to research where large numbers of ewes have been involved (e.g. McEwen et al. 1983). However, changes in ewe liveweight during weeks 5 and 6 of lactation indicate that even generous pasture allowances, and associated high levels of intake, were inadequate to maintain liveweight in single- and twin-rearing ewes (Figure 11.6). A similar effect, usually greatest in twin-rearing ewes, has been noted by other researchers (Gibb and Treacher 1978; Peart 1968). Body reserves are therefore drawn upon to meet the additional requirements for lactation.



Days from mid-point of lambing

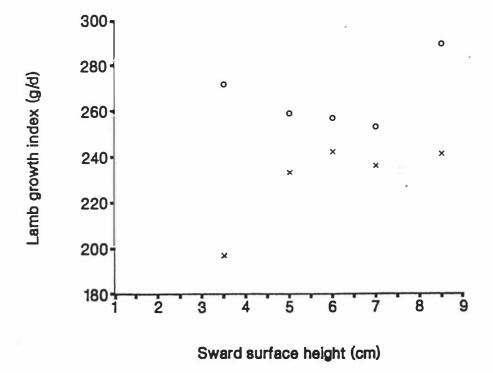
Figure 11.6 Effect of sward surface height on group mean changes in ewe liveweight during lactation, experiment 13.

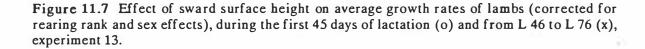
Although no initial records of body condition or backfat depth were obtained, relative changes in condition between L 33 and L 76 suggest that ewes, especially on the 550 sward, lost body condition most rapidly during the first four weeks after lambing. The tendancy for more rapid loss of body condition in the twin-rearing ewes was probably associated with their higher levels of milk production (Barnicoat et al. 1949). However by mid-lactation (L 45) energy demand for milk production normally starts to decline as lambs begin to consume significant amounts of herbage (Gibb and Treacher 1978; Penning and Gibb 1979; Gibb et al. 1981; Owens 1984). The maintenance of high intakes through to the ninth week of lactation therefore enabled the ewes on the 1150 and longer swards to regain liveweight. This was associated with a reduction in the rate of loss of body condition, and in some cases regaining of body condition on these swards.

#### Lamb Production

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Lamb growth rates were unaffected by a wide range in pasture conditions (Figure 11.7), especially during the first 45 days of lactation, presumably because ewes mobilised body reserves to maintain milk production.





Increasing the availability of pasture to the twin-rearing ewes did not enable them to increase the growth rates of their lambs to the level of the single-reared lambs during the first six weeks of lactation. Growth rates of twin lambs were apparently constrained by the level of milk production of their mothers, since lamb rumen function and ability to digest herbage does not develop until 3-4 weeks of age (Brown 1964). Although daily milk production, which is primarily influenced by post-lambing nutrition (Peart 1970; Rattray et al. 1975: Geenty and Sykes 1986) and to a lesser extent ewe condition (Treacher and Gibb 1980), is approximately 30% higher in twin-rearing than single-rearing ewes at the peak of lactation, the volume of milk available to each twin lambs is still less. Peak lactation is likely to have been achieved two weeks after set stocking on the respective treatments (Barnicoat et al. 1957: Peart 1967; Gibb and Treacher 1980; Geenty and Sykes 1986). The similarity of lamb growth rates across treatments during the first six weeks of lactation implies that differences in ewe milk production were small. To achieve this ewes on the 550 sward must have mobilised additional body reserves to maintain milk production. Ewe body condition at the commencement of the trial (L 10) was probably similar across treatments because ewes were randomly selected from the same population and grazed together until set stocking on the treatment paddocks. Other researchers, including Coop et al. (1972), Clarke (1978), Clarke et al. (1986) and Penning et al. (1986), have demonstrated the ability of ewes to buffer lamb production under conditions of low feeding by reducing liveweight and body condition.

The effects of pasture availability on lamb growth grates were apparent on the 550 sward during the second half of lactation (i.e. from L 46 to L 76). During this period ewes on the 550 sward and twin-rearing ewes on the remaining treatments had less body condition to mobilise (Table 11.13). In addition, the effect of milk production on lamb growth would have been smaller because approximately two-thirds of total milk production is produced by week six of lactation (Barnicoat et al. 1949; Peart 1967, 1968). This suggests that differences in lamb growth rate from L 46 to L 76 arose primarily from variation in herbage intakes by the lambs. Lambs would have begun to compete with their dams for forage first on the 550 sward from 3 to 4 weeks of age (Penning and Gibb 1979; Penning et al. 1986). Although the smaller physical dimensions of the lamb mouth may have enabled them to graze more effectively than the ewes on the short pastures of the 550 sward (Gibb and Treacher 1976; Wadsworth 1979), their total herbage and milk intake apparently still provided less energy for growth than was the case for lambs on the longer swards. This indicates, that for optimum growth rates, lambs should have access to pastures with a sward surface height exceeding 4.0 cm from 3-4 weeks of age.

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#### **Ewe Wool Production**

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The negligible effect of increasing sward surface height on wool production of ewes of different rearing rank during lactation is consistent with other research (Corbett 1979). In a long term genotype-environment study of factors influencing wool production of Romney ewes, Newman (1988) established that annual greasy fleeceweight was affected by -0.03 to 0.06 kg in ewes rearing twin lambs relative to those with singles. Differences in clean wool production were smaller and ranged from 0.00 to -0.12 kg across years. These differences were related to seasonal conditions, the largest differences occurring in years with poor levels of pasture production. A 0.22 kg difference in the annual production of greasy wool by single- and twinrearing Romney ewes was measured by Stevens and Wright (1951), but only 0.13 kg of this difference originated during the period of lactation. Sumner et al. (1989) recorded a 6% (0.01 kg) and 12% (0.05 kg) decrease in annual wool production in single- and twin-rearing ewes, respectively, compared to dry ewes. In the current experiment depressions of 0.05 and 0.16 kg in total greasy fleeceweight of twin-rearing rearing two year old and mixed-aged ewes respectively were measured relative to the performance of comparable single-rearing ewes. However, the midside wool growth data suggest that relatively little of this difference originated during the lactation period. Oddy (1985) established, in an indoor trial with Merino ewes, that the depression in wool production during lactation, which was relatively small between single- and twin-rearing ewes (3-8%), was directly related to the level of milk production. Reduced wool growth is therefore greatest where the lactation period is long and is associated with poor levels of nutrition. Thus McEwen et al. (1983) showed that greasy wool production during lactation was 0.16 to 0.45 kg less in twin-rearing ewes than in single- rearing ewes depending on lambing and weaning dates. Although variation in wool production between ewes was high, the depression in intakes of ewes on the 550 sward could not have been sufficiently long or severe to be reflected in reduced wool growth. Similarly the relatively low midside wool growth rates could be explained by little or no wool accumulation during the first six weeks of lactation, a phenomenon observed by Geenty and Sykes (1986) in Dorset ewes on both high and low planes of nutrition.

The significantly higher clean wool production of the two year old compared to the mixed aged ewes corresponds to results reported by Sumner and Wickham (1969), Hight et al. (1976), Bigham et al. (1978) and Newman (1988). In the study of Newman (1988) two year old ewes produced 0.42 to 1.24 kg more clean wool annually than 5 year old ewes grazed in the same flock over six seasons, although some of this effect was due to a slightly longer shearing interval in the younger ewes. Increasing feed availability did not result in increased overall staple strength of the wool grown during the trial period, but staple strength was lower in the twin-rearing ewes on the 550, 1150 and 1400 swards. In the last two cases it is difficult to explain this effect in terms of the intakes achieved. Staple strength of ewe wool can vary markedly between seasons according to feeding level, although the effects of lactation are not necessarily greater in twin- than in single-rearing ewes (Newman 1988). Staple strength was greater in the two year old than in the mixed aged ewes, a result which is at variance with those of Bigham et al. (1983) who reported greater fleece tenderness in younger ewes.

The excellent colour (Y-Z) of both the midside and contralateral samples can be explained by the time of wool harvesting. Wool grown from early spring to November has the lowest level of discolouration of annual growth (Sumner 1985). The whiter fleece of the older ewes probably relates to staple length, with the longer staple of the December-shorn mixed age ewes affording greater protection from the environment than the shorter stapled wools of the March-shorn two year old ewes.

# Lamb Wool Production

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Very little data on lamb wool production in English long wool type breeds has been published. It is therefore difficult to evaluate the nil response of lambs to sward height and the inconsistency of wool production between lambs reared as singles or twins. When wool growth is considered in relation to the patterns of lamb liveweight gain, it seems reasonable that differences in wool production would also be small and largely reflect the milking ability of their dams, at least during the first 6 weeks of lactation.

# Practical Application of Trial Results

The feed intake response to sward surface height indicates that the most critical aspect of grazing management for the lactating ewe rearing either single or twin lambs is to provide pastures with an average green leaf height of 5 cm (EPM compressed height 4.0 to 5.0 cm) or a sward mass of approximately 1000 kg OM/ha (1100 kg DM/ha). Hodgson et al. (1986) suggested similar target sward surface heights of 4 to 5 cm for medium, and 5 to 6 cm for high, growth rates respectively in lactating ewes and their lambs under British conditions. The advantage of communicating in terms of sward height is that it is a measure which is readily understood and applied by farmers. In New Zealand considerable confusion amongst farmers has arisen through the use of the term DM mass (or green leaf mass), because of regional differences in sward density and composition, and variation in the techniques employed to determine pasture mass (Thompson 1986). In addition the vertical distribution of plants is often

more closely related to animal intakes, through its effect on bite size, than is residual herbage mass (Hodgson 1981, 1982).

In practice farmers often have difficulty providing (and maintaining) a sward of 5 cm at lambing (Parker 1984a). The lamb and wool production responses measured from L 0 to L 45, and the earlier results of Coop et al. (1972), indicate that farmers can afford to restrict lactating ewes for short periods without jeopardising production. This period should be no more than three weeks, as effects on production reflect the extent of underfeeding (Coop et al. 1972) and initial ewe condition. During this time ewes should preferably not be grazed below a sward surface height of 3.0 cm (600-700 kg DM/ha). A useful management strategy may be to progressively set stock ewes at lambing, by initially allocating ewes at higher than average stocking rates to a proportion of the area designated for the ewes during lactation. Pasture growth on the nongrazed area could then continue to accumulate until the required target levels for grazing were achieved. This strategy would be more effective where ewes had been drafted into lambing groups on the basis of crayon marks from tupping, because the nutritional requirement for animals within groups would be similar. In addition, if nitrogen fertiliser was to be applied, planning would allow nitrogen application to the later grazed area, providing more time for spring soil temperatures to increase, but still allowing a 3-4 week interval between fertiliser application and grazing (Field and Ball 1978).

Once the ewes have been allocated to pastures with sward surface heights exceeding 5.0 cm it is important that changes in height continue to be monitored. Where lambing date is reasonably matched with the onset of spring growth, the major management problem during the second half of lactation will be to control pasture growth in order to maintain a leafy sward with high digestibility (Sheath and Bircham 1983). Increasing sward pasture height results in higher dead material contents (and therefore wastage of pasture grown) and reduced clover content, as demonstrated by the 1550 and 2000 swards in this study. Both of these factors are associated with poor post-weaning lamb performance (Rae et al. 1963; Lewis and Cullen 1973). Sward surface height should therefore preferably be maintained within the range 4 to 7 cm during lactation. This can be achieved by measures such as adjusting stocking rate, conservation of hay or silage, chemical control to reduce seedhead emergence Brookes and Holmes (1985) or grass growth (Williams and Palmer 1969), and integration of cattle with ewes (McCall et al. 1986). The requirements for integrated grazing of lactating ewes with cattle need better definition (Hodgson et al. 1985), but a recent experiment has shown that c.300 kg male cattle will grow at 0.9, 1.5 and 1.6 kg/day when continuously grazed on compressed sward heights of 4, 8 and 12 cm respectively during spring (Michel 1989). Liveweight gains in yearling cattle of around 1.0 kg per day could be expected if integrated grazing with ewes and lambs, to maintain pasture height between 5.0 and 7.0 cm, was adopted. An autumn experiment with the same cattle recorded liveweight responses of 0.0, 0.6 and 1.3 kg/d for swards of similar height (Hirschberg et al. 1990) demonstrating that the relationship between sward height and production may change

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during the year. This is also reflected in the sward height targets set by Hodgson et al. (1986) for different seasons and physiological states for sheep, and by the contrasting spring and autumn lamb performance data for different sward heights recorded by Hight and Sinclair (1965), and Lewis and Cullen (1973) under New Zealand conditions. This indicates that sward surface height management guidelines for the lactation period cannot necessarily be applied to other times of the year. Further research is required to establish appropriate guidelines for ewes under continuous grazing at other times of the year but in the meantime the concurrence between the present experiment and British results for the same period suggests that until such local standards have been established, the recommendations of Hodgson et al. (1986) could be adopted by New Zealand sheep farmers.

# CONCLUSIONS

The results of this experiment suggest that New Zealand sheep farmers would gain little benefit in terms of herbage intake and production responses from separate grazing of ewes of different rearing rank over the lactation period. However, the results apply to only one set of conditions and further work is required to establish the effects of low ewe condition at lambing, and perhaps a lower sward height, on productive performance of the ewe. These conditions are relatively common on New Zealand sheep farms at lambing (McEwen et al. 1983; Parker and Townsley 1986). The non-nutritional benefits of differential grazing management at lambing and during lactation, including better utilisation of shelter, improved efficiency of selection of flock replacements and earlier drafting of heavier single lambs (Garrick 1984) also need to be considered. For example, Bowman et al. (1989) were able to demonstrate by simulation modelling that positive cash benefits resulted from scanning Merino wool producing flocks despite a low incidence of twinning (1.3 to 7.9%). Benefits increased with improved flock fecundity. Under New Zealand conditions twinning rates in commercial flocks are much higher (Kelly 1982; Rohloff et al. 1982; Davis et al. 1983) and potential benefits of pregnancy diagnosis are greater (Blair 1986).

The greatest advantage of differential management may be improved lamb survival. Scales et al. (1988) concluded from a lamb mortality study that differential nutrition of single- and multiple-bearing ewes during the final six weeks of lactation would improve survival of twin lambs. Although differential management would result in only a small improvement in the birthweight of lambs born as twins (Russel et al. 1967; Clarke 1978; Smeaton 1983), the higher body condition of the ewe at lambing would provide a number of advantages. First, the ewe would be better equipped to cope with the rigors of parturition; second, the onset of milk production and particularly the secretion of colostrum would be earlier (McCance and Alexander 1959; Khalaf et al. 1979; Earle and Male 1988); and third, the separated ewes could be allocated to paddocks with the best shelter (Bird et al. 1984b) and on hill country to lambing

paddocks with gentler slopes (McMillan and Knight 1985). The results from experiment 11 (Chapter Ten) suggest that the feed intakes of ewes bearing twins may have been disadvantaged through competition with single-bearing ewes. Hadjipieris and Holmes (1966) showed that this response may occur because the volume of the conceptus limits rumen space if diet quality is low. Differential management would allow twin-bearing ewes to be provided with higher quality feed during the final trimester of pregnancy, while poorer quality pasture, carried over from late autumn-early winter, could be offered to single-bearing ewes. Although such reallocation of resources to favour twin-bearing ewes could reduce the performance of single-bearing ewes, the overall flock performance and farmer returns should increase (Parker 1984b; Blair 1986; Bowman et al. 1989).

The major recommendation from this experiment is that farmers should aim to provide singleand twin-rearing ewes with pastures having a sward surface height of 5 to 7 cm during lactation. This will maximise both lamb and ewe performance. Provision of longer pastures will not improve herbage intakes, but is likely to jeopardise subsequent pasture quality and hence sheep performance.

# CHAPTER TWELVE GENERAL DISCUSSION

#### INTRODUCTION

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Despite the momentous changes made to the agricultural sector of the New Zealand economy since 1984, provision of pasture remains the major on-farm cost of pastoral-based livestock systems. In fact, removal of institutional support for agriculture has heightened the importance of producing and utilising pasture by low cost (low input) systems, which increasingly must be perceived to be environmentally friendly. In the introduction to this study, potential gains in efficiency resulting from improved utilisation of pasture were identified. It was suggested that sheep farm production and financial returns could be increased by modifying grazing management of the ewe during late pregnancy and lactation, to better fit management to the different pregnancy and rearing status of ewes. Although the opportunity to improve the efficiency of this aspect of pasture allocation on sheep farms had been recognised since the early 1950's, progress in developing new management systems for the ewe was restricted by the absence of equipment that provided for the accurate diagnosis of pregnancy status in the ewc and by a lack of data defining relationships between pasture availability, feed intake, and ewe and lamb productivity. By the mid-1980's a low cost, transportable and accurate pregnancy diagnosis technique based on realtime ultrasound scanning had been developed (see p. 13). However, research into grazing management of the ewe continued to be hampered by the absence of an easily applied technique for measuring feed intake with little disturbance to normal grazing behaviour. The purpose of this thesis was to address the latter issue, first by validating the use of new CRC technology for measuring feed intake by sheep, second by measurement of feed intake in ewes of different pregnancy and rearing status, and third by incorporating the intake data and associated productivity responses into a model to design improved grazing systems for the ewe. In this Chapter progress in achieving these objectives is reviewed and areas requiring further study are identified.

# VALIDATION OF INTRARUMINAL CRC FOR USE IN SHEEP

The validation experiments conducted during this study have demonstrated that CRC release  $Cr_2O_3$ into the rumen in a uniform manner once initiation of matrix extrusion has been completed 2 to 3 days after capsule insertion. The subsequent period of linear release was found to be dependent on orifice diameter, matrix composition and length of the pressed tablet core, confirming the results reported by Laby et al. (1984) and Ellis et al. (1988). In comparison to manufacturer-controlled characteristics of capsules, environmental factors, within and outside the sheep, had relatively small effects on the rate or linearity of  $Cr_2O_3$  release. Thus release rates were only about 4% lower if daily feed intake was at 0.7 M compared to an <u>ad libitum</u> level (non-significant). Plunger travel, and hence  $Cr_2O_3$ release rate, was reduced if intakes were maintained at <0.6 M for 4 to 6 days but recovered to their **previous** level when feed intakes were raised to a maintenance or higher level. Feed intakes below 0.6 M for this period of time are generally not required for grazing experiments but may be encountered in dry rangeland situations where daily intakes are dependent on the availability of browsing shrubs and low quality native pasture. In these circumstances it is recommended that an alternative to CRC for estimating faecal output be used. Release rates were not affected by different pasture species consumed fresh at equivalent levels of feed intake, but showed a tendency to increase by 1.5 to 2.0% if feeds which create 'coarse' rumen contents (e.g. hay) were eaten, probably because abrasion of matrix at the capsule orifice is increased.

The experiments in which capsules were recovered from the rumen by slaughter indicated that variation in  $Cr_2O_3$  release rate between sheep grazing in the same environment was low (CV 2.0 to 6.5%). Variation in release rate between CRC within animals was usually lower.

The largest difference in release rate was between capsules in rumen-fistulated and non-fistulated sheep. Although only one experiment was conducted with rumen-fistulated and intact sheep grazed together (Chapter Eight), several other indirect comparisons between the two types of animal were made incidental to other studies. In these cases both types of sheep grazed similar pastures and were fitted with CRC from the same manufacturing batch. Plunger travel was consistently about 10% lower in rumen-fistulated than in non-fistulated sheep. This difference can probably be attributed to temperature fluctuations associated with the removal of the capsule from the rumen for measurement. Different gaseous conditions in a fistulated compared to an intact rumen are also likely to have been a contributing factor. In contrast, Laby et al. (1984) implied that there were no differences between f istulated and non-fistulated sheep while Ellis et al. (1982) reported a more rapid release of Cr<sub>2</sub>O<sub>3</sub> from CRC (with a cast core matrix) in rumen-fistulated cattle compared to that in CRC recovered from slaughtered steers. It is important that the relationship between plunger travel in fistulated and intact animals be precisely defined because rumen-fistulated animals provide the most simple method of estimating the Cr<sub>2</sub>O<sub>3</sub> release rate from CRC used for trial purposes. If the difference was constant, rumen-fistulated sheep could be grazed with, or on similar pastures to, the experimental animals to provide estimates of release rates. More information on CRC performance will become available in the next few years as the capsules are used at different research stations. This will indicate the extent to which plunger travel measurements in one environment (e.g. those taken at a CRC testing site) can be extrapolated across other environments (e.g. different trial locations).

Alternatively the rate of  $Cr_2O_3$  release can be determined to  $\pm 0.5$  d by measuring the endpoint of matrix extrusion (Ellis et al. 1988), but this requires additional animal handling and labour, and increases analytical expenses. A group mean endpoint, which provides the appropriate release rate for deriving faecal output of treatment groups, can be obtained by sampling from the sward each morning and evening over the final 4 to 5 days of matrix release (e.g. experiment 13). As a result of the programme reported here research has commenced at Massey University on the use of cupric sulphide (CuS) as a secondary faecal marker, included in the final tablet of the matrix core, to clearly distinguish the end of  $Cr_2O_3$  release (W.J.Parker, unpublished data). Cupric sulphide meets the

requirements specified by Kobt and Luckey (1972) for faecal markers and its concentration in the faeces can be determined by atomic absorption spectrophotometry after digestion by the methods used for  $Cr_2O_3$ . It should be noted that the insertion of a second capsule to release CuS (or an alternative secondary marker) jointly with a capsule releasing  $Cr_2O_3$  would allow two or more treatment groups of animals grazing together to be identified when faeces are sampled from the sward. A dual marker system has particular relevance when minimal disturbance of grazing is desirable, individual animal data are not required or problems with animal handling (e.g. ewes with young lambs at foot) prevent sampling per rectum.

It is concluded that, because local environmental factors and the management system adopted for experimental animals can influence the rate of  $Cr_2O_3$  release, the release rate data provided by the capsule manufacturers (derived from rumen-fistulated wethers at Massey University and at the CSIRO research station at Armidale) should be used only as a guideline. Wherever possible,  $Cr_2O_3$  release rates should be established for each experiment to ensure that reliable estimates of faecal output are obtained.

The 18 day period of linear release after attainment of steady state concentrations of  $Cr_2O_3$  in the faeces (7 to 8 days after capsule dosing) provides the opportunity to apply experimental designs involving cross-overs or multiple feeding levels with the commercial sheep capsule (i.e. that containing a 3.0 cm pressed tablet core). This opportunity is greater in non-commercial CRC which have a longer linear release phase either because more  $Cr_2O_3$  tablets are included in the barrel (e.g. experiment 7) or because a smaller orifice reduces the rate of matrix extrusion (e.g. experiment 1). It was estimated from the results of experiment 8 (Chapter Seven) that for relatively small changes in intake (<0.4 M) a 3 day period would be usually be adequate for  $Cr_2O_3$  to adjust to a new steady state but for larger changes 5 or more days may be necessary. It was not possible to accurately define the adjustment period for larger changes in feed intake because each feeding level in that trial was of only 6 days duration. Better definition of adjustment periods for different levels of intake of a range of feed types (including changes to a different feed type) is required.

The validation experiments reported in this thesis were mainly conducted with sheep managed under continuous grazing to maintain a uniform (or minimum) residual pasture height or mass. Under these conditions, fluctuations in daily intake are probably reduced and between-day variation in faecal chromium concentration minimised relative to rotational grazing management (Raymond and Minson 1955). However, rotational grazing of sheep is widely practiced by sheep farmers, especially from weaning until 2 to 3 weeks before the start of lambing, and researchers will want to apply CRC under these conditions. The effects of alternative rotational grazing regimens on faecal chromium concentrations should therefore be quantified. This should also include the measurement of carry-over effects on faecal chromium levels between grazing intervals, particularly in situations where intakes range from <u>ad libitum</u> to sub-maintenance levels within each grazing interval.

Under indoor conditions a correlation of 0.90 to 0.99 between daily faecal output derived by  $Cr_2O_3$  dilution and faecal output measured by total collection for individual sheep was obtained (experiment 6 and Appendix VI). This compares favourably with the correlation of 0.83 reported by Lee et al. (1988). Under outdoor conditions the correlation between predicted mean faecal output and total collection over a 3-day period was 0.87 (experiment 9). Ellis and Rodden (1987) reported a correlation of 0.99 between predicted and actual daily faecal output of sheep fitted with CRC where faeces of three sheep had been collected off the sward after each defaecation over a period of 72 hours. However, estimates of individual animal intakes are less reliable because small changes in the digestibility of feed consumed may exert substantial effects on intake (see equation 1.1, p. 20). The importance of this effect is illustrated by the data shown in Table 12.1 (derived from Appendix VI). Three measures of DMI were obtained: the <u>actual</u> DMI (DMIA); intake predicted from faecal output estimated by CRC  $Cr_2O_3$  dilution and the animal's <u>own</u> digestibility (DMIO); and the intake predicted from estimated faecal output using the group <u>mean</u> digestibility (DMIM).

Table 12.1 Liveweight (LW), dry matter digestibility (DMD) and actual or predicted intakes of sheep fed ryegrass/white clover pasture.

Shecp	LW (kg)	DMD (%)	DMIA		DMIO		DMIM		
			g/d	Rank	g/d	Rank	g/d	Rank	
1	43.0	73.05	1357	1	1291	1	1263	1	
2	45.5	70.68	1494	2	1549	3	1641	4	
3	48.0	71.36	1598	3	1778	4	1833	5	
4	45.0	70.08	1633	4	1501	2	1611	3	
5	49.0	77.54	1649	5	1858	6	1488	2	
6	49.0	72.21	1786	6	1841	5	1856	6	
Mean	46.6	72.48	1589		1636		1615		

Intakes predicted from faecal output and the animal's own digestibility (DMIO) ranged from 94 to 116% of the actual intake (DMIA) and the correlation between the two measures was relatively low  $(r^2=0.69)$ . There was considerable variation between animals in <u>in vivo</u> digestibility (CV=3.7%). As a result intake estimated from faecal output and mean digestibility (DMIM) had a still lower correlation with actual intake  $(r^2 = 0.55)$ . It is this latter figure (DMIM) which most closely approximates estimates that would be obtained in the field (i.e. those based on estimated faecal output and an assumed digestibility). However, in the field situation, between-animal variation in digestibility would likely be increased (due to greater opportunity for diet selection). This would be most significant under ad libitum grazing conditions. Thus the poor correlation between actual and estimated intakes is likely to be exacerbated in the field. It can be concluded (Parker et al. 1990a, Appendix VII) that CRC technology will not be suitable for accurate estimation of individual animal intakes until improved herbage digestibility measurement techniques are developed. However, in a group situation, errors due to faecal sampling, variable release of Cr2O3 from the CRC, chromium assay variation and between-animal variation in digestibility are assumed to be randomly distributed across the groups being studied. Differences in diet selection between groups should be monitored particularly carefully when the groups are grazed on dissimilar swards (e.g. when comparing feed intakes on alternative pasture species).

The greatest advantage of CRC technology is its reduced animal handling requirements and increased flexibility of faecal sampling routines. This means that large groups of animals can be studied (e.g. Barlow et al. 1988; Lee et al. 1990; experiment 13), increasing the chances of detecting differences between groups of animals (Ulyatt 1972). For example, a 3 to 4% difference in group mean intake could be detected with group sizes of 50 animals, providing errors in estimating faecal output and herbage digestibility were such that the CV in predicted feed intake between and within groups was 10% (see Parker et al. 1990a, Appendix VII). Alternatively replicated treatments with smaller group sizes can be adopted to improve the chances of detecting treatment differences in group mean feed intake (Cruickshank et al. 1987).

Unsatisfactory estimation of faecal output and feed intake by CRC has been reported by Lee et al. (1990) and Glimp (1990). Lee et al. (1990) attributed the poor result to the fact that faecal sampling commenced 6 days after capsule administration when steady state levels of  $Cr_2O_3$  had not yet been attained in the faeces. Estimates of faecal output improved for a second three day sampling period commencing on d 16. Poor results in the trial by Glimp (1990) are probably related to faecal sampling when  $Cr_2O_3$  had not recovered to a steady state after a transition between feeding levels (i.e. no adjustment period was allowed for), sampling after d 26 (when the release phase was likely to have been completed for some of the CRC) and to application of the release rates specified by the manufacturer (which, as noted earlier, can only be used as a guide) for estimating faecal output. Errors will also occur if sampling periods are not sufficiently long to account for variation in faecal output between days by individual animals (Lee et al. 1990). A minimum of three samples per animal (one sample per day) is recommended where daily intakes are relatively uniform (e.g. under continuous grazing at a fixed sward height), but the number of samples should be increased to 5 or more where variation in daily intake is greater (e.g as animals graze down through a sward over a 3 to 7 day period).

## Improvements to the Chromium Assay

The reliable assay of faecal  $Cr_2O_3$  is critical to the success of CRC (and related methods) for estimating faecal output. Generally the phosphoric manganese acid - potassium bromate digestion procedure and AA determination of chromium in solution used in this study worked well. It is essential that pool samples be included with each batch to monitor intra- and inter-assay variation (typical CV between 2.5 and 5.0%) and that a standard solution be aspirated into the AA flame every 5 to 10 readings to control variation in spectrophotometer performance. Adoption of a disposable glassware system (Costigan and Ellis 1987), which will dispense with the need to quantitatively transfer digest to 50 cm<sup>3</sup> volumetric flasks and from volumetric flasks to vials for reading by AA, is being investigated. This change will reduce labour requirements (by c. 40%) and eliminate error associated with transfer of digest. Problems with foaming and loss of material during digestion occurred when the faeces of sheep on legume-dominant diets (> 90% daily intake) were analysed. Completing the second stage of the assay at  $140^{\circ}$ C (rather than > 190°C) reduces foaming, but lower temperatures may affect the level of chromium recovery (see p. 37). The relative chromium concentration in pool samples assayed under the two temperature regimens will indicate the extent to which reduced chromium recovery may have occurred. Alternatively, the addition of an anti-foaming agent (e.g. that used for control of bloat in cattle) may be appropriate.

Williams et al. (1962) reported that the endpoint of digestion could be detected by a change in the colour of the solution to deep purple and the cessation of effervescence. The colour indicator was unreliable for faeces from animals consuming some types of feed. In the case of pasture hay, for example, a clear-yellow solution at the end of digestion was sometimes obtained, but this did not have an apparent effect on AA readings. The inconsistency of the colour change indicator may cause difficulties where chromium in solution is to be determined by colourimetery.

Further research is necessary to define minimum times and temperatures for each stage of the digestion (currently c. 20 minutes for heating to  $140^{\circ}$ C after the addition of the acid mix and c. 50 minutes for heating to >190°C after addition of potassium bromate). Williams et al. (1962), in contrast to Le Du and Penning (1982) and Costigan and Ellis (1987), did not specify digestion times but reported almost complete recovery of spiked Cr<sub>2</sub>O<sub>3</sub> in faecal material when both digest reagents were added together. Improved definition of this aspect of the assay may reduce the number of steps involved and the time required taken to complete digestion.

# APPLICATION OF CRC TO THE MEASUREMENT OF HERBAGE INTAKE BY EWES

The application of CRC to herbage intake studies of ewes of different rearing status proved to be straight-forward once the appropriate sampling regimens for CRC had been defined. The initial absence of this information was reflected in the incomplete results obtained for the pilot study of feed intake by ewes during pregnancy and lactation (i.e. Chapter Ten).

The major finding of the comparison of single- and twin-rearing ewes during lactation was that intakes were maximised for both groups at a sward height of about 5 cm (1000 to 1200 kg DM/ha) on mixed ryegrass, browntop and white clover sheep pastures. The practical implications of for farmers have already been discussed (see p.182) and need not be repeated here. There was a strong indication, from the patterns of change in ewe liveweight and condition score, that the relatively small effect of the low (2.5 to 3.5 cm) sward height on lamb production was due to the ewes utilising body reserves. Interactions between ewe condition (liveweight) at lambing and subsequent effects of nutrition on lamb, milk and wool production have been reported in a number of studies (e.g. Coop 1950; Papadopoulos and Robinson 1957; Peart 1968; Maxwell et al. 1979; Smeaton et al. 1985; Geenty and Sykes 1986). However, none of these studies provided ewe feed intake data for a range of swards heights during both late pregnancy and lactation. A follow-up study using single- and twin-bearing ewes with low or medium body condition (i.e. CS 1.5 and 3.0) from 4 to 6 weeks pre-partum is recommended. The maximum sward height should be 6.0 cm, since intakes are apparently not increased on swards exceeding this height when ewes are continuously grazed (Hodgson et al. 1986; experiment 13). A very low (1.5 to 2.0 cm) sward height could be included during the lactation period because ewe intakes were not significantly constrained at a sward height of c. 3.0 cm in experiment 13. Estimates of herbage intake should be supplemented with measurement of the time spent grazing, resting and ruminating at each sward height (c.g. Penning et al. 1986). Defining grazing behaviour in this manner will help to quantify maintenance requirements for ewes at the different sward heights.

The above trial would enable the relative intakes of single- and twin-bearing ewes at different body conditions during late pregnancy to be assessed. Except for the indoor feeding experiments by Hadjipieris and Holmes (1966) and Foot and Russel (1979), and a modelling study by Forbes (1977), little information appears to have been published on this aspect of ewe nutrition during pregnancy. The results from the pilot study (experiment 12) indicate that twin-bearing ewes are likely to be disadvantaged when grazed with single-bearing ewes during late pregnancy. If intakes of multiple-bearing ewes are constrained by foetal development reducing rumen volume, differential mangement to provide these ewes with highly digestible pasture or a high energy supplement (e.g. Earle and Male 1988) may be worthwhile.

## **MODELLING ALTERNATIVE SHEEP PRODUCTION SYSTEMS**

Less attention was given to modelling studies because the feed intake and production data obtained from experiment 13 suggested that farmers would gain little benefit from separate grazing of singleand twin-rearing ewes during lactation. In addition, results from both experiment 12 and experiment 13 indicated that further information describing the interactions between ewe pregnancy/rearing status, feed supply and body condition was required to develop an effective grazing management model for the ewe from late pregnancy until weaning. However, a model currently being developed for analysing alternative wool production systems (Gray 1990) would be suitable for evaluating the effects of partitioning pasture between ewes of different production status. The wool production model will incorporate data from field studies using CRC management procedures reported in this thesis to determine feed intakes of shorn and unshorn ewes at different stages of production (W.J. Parker, S.T. Morris, D.I. Gray and S.N. McCutcheon, unpublished data). Providing the model with feed intake data enables ewe liveweight change, lamb growth rates and wool production (lamb and ewe) to be simulated for ewes of different pregnancy and rearing status. From this information the relative profitability of alternative grazing systems can be derived using an attached gross margin model.

# CONCLUSIONS

Controlled release capsules, which provide for the continuous and uniform delivery of the indigestible marker  $Cr_2O_3$  into rumen, are well suited to the estimation of mean intake by groups of animals. Costs for materials and labour are lower with CRC technology than with twice-daily drenching of chromic oxide gelatin capsules or  $Cr_2O_3$ -impregnated paper (Corbett et al. 1960). Alternative indirect faecal markers, such as n-alkanes (Mayes et al. 1986) and ytterbium chloride (Prigge et al. 1981), either have higher material costs or require more expensive laboratory analysis than  $Cr_2O_3$ . Compared to  $Cr_2O_3$ , natural faecal markers such as acid insoluble ash (Van Keulen and Young 1977) generally do not yield better estimates of feed intake and are difficult to accurately measure in the feed consumed (Meijs 1981). The advantages of a single CRC application providing a sampling period of up to 18 days and low diurnal variation of  $Cr_2O_3$  in the facces will enhance the use of  $Cr_2O_3$  for estimation of feed intake. This will result in a substantial improvement to current knowledge of feed intake by ruminants at pasture.

A number of factors, particularly variation in capsule release rates and in the animal's own ability to select or digest its diet, result in substantial errors when attempts are made to estimate individual intakes. In practice, however, livestock at pasture are usually managed as groups with pasture allocated to stock classes according to their relative feed requirements and likely financial returns. In this context chromic oxide CRC have the potential to significantly improve current knowledge of the relationships between pasture allowance, animal intakes and production, particularly under continuous grazing conditions. For example, the intakes measured for the single- and twin-rearing ewes grazed at different pasture heights indicated that the relationship between intake and pasture height (mass) was non-linear with an inflexion point at 5 to 6 cm, confirming the findings of earlier British studies (Bircham 1981; Milne et al. 1981). In contrast, Gibb and Treacher (1978) and Rattray ct al. (1982b) have reported linear and curvilinear relationships between herbage allowance and intake by lactating ewes that have been shifted to fresh pasture every 1 to 3 days. The sward conditions that result in optimum sheep performance under rotational and continuous grazing management, during lactation and other periods of the year, will be able to be defined more easily using CRC technology in conjunction with grazing behaviour studies. This information is important for farmers who are currently dependent on feed requirement tables that have been derived mainly from feed intake measurements made indoors or by the pasture difference technique. In the next decade it can be expected that CRC will make an important contribution to updating feed requirement tables to account for different grazing management systems and to the development of feeding standards for classes of livestock (e.g. deer) where very little feed intake data is available at present.



### **APPENDIX I**

# SPLIT-PLOT ANALYSIS : AN EXAMPLE

A number of experiments reported in this thesis required the analysis of data in which several measurements had been made on the same variable through time (e.g. faecal output of individual animals over several days, sequential measurements of CRC plunger travel). In some cases the data could have been analysed separately for each time interval but this would have provided no tests of the change of treatment effects with time (Rowell and Walters 1976). The effects of time (or several measurements on the same variable at one time) can be considered by repeated measures analyses or by split-plot analysis (Gill and Hafs 1971; Rowell and Walters 1976; Gill 1988). Multivariate repeated measures analysis is restricted where there are more repeated samples taken on animals than there were animals to begin with<sup>1</sup> (Cole and Grizzle 1966) and may introduce interpretational difficulties relative to the split-plot approach (Rowell and Walters 1976). The split-plot method was best suited to the experimental designs adopted in this research programme. The following example for the analysis of plunger travel, measured 7 times (c) for 15 CRC, fitted in groups of 3 (b) within 5 rumen-fistulated wethers (a) (i.e. experiment 1), demonstrates the general form of the split-plot models adopted.

Model	dſ	п	Expected Mean Square <sup>f</sup>
Total	abc	105	
Mean		1	
Sheep(s)	a-1	4	$V_e + 3V_{txs} + 7V_{CRCxs} + 21V_s$
CRC	b-1	2	V <sub>e</sub> +5V <sub>CRC x t</sub> +7V <sub>CRC x s</sub> +35V <sub>CRC</sub>
CRC x sheep	(b-1)(a-1)	8	Ve <sup>+7V</sup> CRC x s
Time (t)	(c-1)	6	$V_e + 5V_{CRCxt} + 3V_{txs} + Q(t)$
Time x sheep	(c-1)(a-1)	24	$V_e + 3V_{txs}$
Time x CRC	(c-1)(b-1)	12	$V_e + 5V_{CRC \times t}$
Time x CRC x sheep	(c-1)(b-1)(a-1)	48	Ve

<sup>f</sup>Variance components are designated as follows:  $V_e$  = variance (error);  $V_{t x s}$  = variance (time (sheep));  $V_{CRC x s}$  = variance (CRC(sheep));  $V_{CRC x t}$  = variance (CRC x time); Q(t) = variance time as a quadratic function.

<sup>1</sup>This problem was encountered in the analysis of faecal output data for experiment 5 (Chapter Five). To complete the analysis the observations were broken down into meaningful sub-series of repeated measurements (McClelland 1986).

F-tests:

- 1. Differences in CRC between sheep =  $\underline{CRC}$ Sheep x CRC
- 2. Differences in CRC plunger travel with time between sheep = <u>Time x CRC</u> Time x CRC x sheep
- Differences in plunger travel with time between CRC within the same sheep
   <u>Time x Sheep</u>
   Time x CRC x sheep

The effect of time could be fitted in the model as a polynomial of the sixth order to go through seven points. Partitioning time (6 df) into linear (1 df), quadratic (1 df) and "lack of quadratic fit" (4 df) components enabled the nature of plunger travel with time to be tested. Thus the linear term tested the average rate of plunger travel, the quadratic term tested for changes in the rate of plunger travel (e.g. rate diminishing or increasing) and the "lack of quadratic fit" tested whether there were other causes for plunger movement to deviate from normal quadratic travel.

# **APPENDIX II**

# THE MEASUREMENT OF INTRARUMINAL CRC PLUNGER TRAVEL BY REALTIME ULTRASOUND SCANNING

### BACKGROUND

A critical factor in the application of chromium CRC in outdoor herbage intake experiments is the accurate determination of daily chromium release rates. If fistulated animals are used, CRC can be recovered from the rumen at regular intervals and plunger displacement measured. However, fistulated sheep are expensive to prepare and maintain, and may not be representative of an intact animal. In intact animals plunger travel can be estimated either by serial slaughter or by estimating the time of matrix expiration through changes in faecal chromium concentration (Ellis et al. 1988).

Serial slaughter is practical if animals have a low commercial value and adequate processing facilities are available but any measurements on these animals are restricted to those which can be obtained prior to slaughter. An alternative is to graze additional animals for slaughter with the treatment animals.

Rectum grab sampling to monitor changes in faecal chromium concentration near the expected date of matrix expiration can provide estimates of the endpoint of chromium delivery to within about 0.5 days of actual expiration (Ellis et al. 1988). However, rectum sampling requires a high labour input, creates significant animal disturbance and has relatively high laboratory costs if large numbers of animals are involved in the trial. In addition 100% sampling of animals at each collection is often not achieved. A less intensive approach using this method, but only providing group mean plunger travel, would be to obtain sward samples from ring sites twice daily over the final 4 to 5 days of the expected CRC release phase.

Other possibilities for the indirect measurement of plunger travel are X-ray fluoroscopy and realtime ultrasound scanning. Both methods are non-destructive and allow repeated measures on the same animal (Wilkins et al. 1984). X-ray fluoroscopy has been used to monitor the movement of radioactively labelled CRC through the oesophagus after drenching and subsequently around the rumen and reticulum by Ellis and Taylor (1987). The clear radiographs obtained and the ability to freeze frames of the CRC on a connected video monitor indicated that plunger travel could be measured by this means providing a radioactively labelled spacer was placed at the end of the  $Cr_2O_3$  matrix. However, because X-ray equipment is usually stationary (portable machines developed for pregnancy diagnosis in sheep are

exceptions (Rizzoli et al. 1976; Beach 1984), expensive to operate and pose a health danger, it could not be used routinely for grazing trials involving CRC.

In contrast, realtime ultrasound scanners are portable, safe and inexpensive to operate. They are being used more widely for pregnancy diagnosis of animals, as well as for other veterinary diagnostic purposes (e.g. Carter 1986). Ultrasound may therefore provide a simple and quick means of measuring CRC plunger displacement in individual sheep under field conditions. An investigation of ultrasound scanning for that purpose is reported in this Appendix.

## MATERIALS AND METHOD

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Captec chromium CRC (65%  $Cr_2O_3$  matrix, 3.0cm core, 9.00 mm orifice) were modified to improve ultrasound detection in the rumen by including a steel washer to mark the end of the  $Cr_2O_3$  matrix and by wiring a steel rod (2.5 mm diameter) to the barrel. The latter provided a fixed reference against which the relative position of the washer could be measured.

Single CRC, with a nylon string attached to facilitate recovery, were placed inside a plastic container of water or inside the rumen of a fistulated sheep freshly removed from grazing at pasture. Wool around the fistula and down to the belly was clipped within 3 to 4 mm with an electric handpiece.

A realtime body scanner (Shimadzu, Japan) with a 3.5 MHz external probe was used to scan for the CRC. The transducer and the sheep's skin were coated with soya bean oil to provide a contact medium (Carter 1986). Scanning took place while the sheep was restrained in a standing position.

### **RESULTS AND DISCUSSION**

A clear outline of the CRC suspended in water was obtained when the CRC was placed within 10 cm of the side of the container wall. At a high resolution (2 to 14 cm sound wave penetration) only about 40% of the CRC could be viewed but with a horizontal orientation the reference steel rod and the internal washer could be detected. At a low resolution (0 to 20 cm sound wave penetration) all of the CRC could be seen and only the steel rod could be identified.

No image of the CRC placed in the rumen could be developed, despite the CRC being held against the rumen wall adjacent to the scanner probe. The relatively dense and fibrous nature of the rumen contents (pasture and soil) apparently reflected the sound waves to a similar degree as the CRC. When ultrasound is used for pregnancy diagnosis the sound waves penetrate the ewe's body and pass through the embryonic fluid until new layers of tissue (the foetus) are contacted. Sound waves reflected off the foetus generate a distinctive picture on the scanner's video monitor (Beach 1984). The accuracy of pregnancy diagnosis is improved if the ewe has a low gutfill and an empty bladder because this makes the position of the uterus more distinctive. Detection of CRC in the rumen of sheep might therefore be improved during the spring when pasture has a low dry matter content and less fibre, and when animals have been removed from pasture 24 hours prior to scanning. For many experiments both of these options are impractical.

### CONCLUSION

The real time ultrasound technology used in this trial did not provide a means of measuring the plunger displacement of intraruminal CRC in sheep grazing pasture because the rumen contents reflected a high proportion of sound waves. Even if rumen fill was very low in dry matter content detection would be limited to those CRC which were within 10 cm of the transducer. A proportion of CRC would therefore not be seen in adult sheep (50 to 70 kg liveweight) because the rumen may be up to 25 cm wide when fully distended with herbage. In addition CRC are large in comparison to a 40 to 70 day old lamb foetus and an image of the entire CRC can only be obtained on-screen if the maximum scanning field with an associated low resolution is used. More powerful ultrasound scanners may provide greater penetration of sound waves into the rumen but will not overcome the problem of reflectance by the rumen contents.

## **APPENDIX III**

# HERBAGE INTAKES OF EWES AT FLUSHING AND DURING THE FIRST TRIMESTER OF PREGNANCY

#### INTRODUCTION

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The normal practice on New Zealand sheep farms is to increase ewe feed allowances during a 3-6 week period prior to and during mating to obtain a flushing response in ovulation rate (Smith et al. 1983). These above-maintenance (M) feeding levels are commonly continued through until the end of mating (typically 51 days), when allowances are progressively reduced over a 7 to 10 day period to 0.8 to 1.0 M (Parker 1984a). However, if farm pasture cover is low the farmer may conserve additional pasture for winter by progressively removing ewes crayon-marked as being mated from the flock at 8 to 10 day intervals and assigning these ewes to a lower feeding regimen. Thus to effectively manage their flock during this important period, farmers need to understand the relationship between the amount of herbage offered and herbage intake of ewes. This appendix reports on an experiment where the herbage intakes of ewes fitted with chromium CRC, and grazed under different pasture conditions during flushing -and early pregnancy, were estimated<sup>1</sup>.

## MATERIALS AND METHODS

Twenty ewes, aged eighteen months and 10 mixed aged (MA)), from a larger flock of 235 first cross Border Leicester-Romney ewes involved in a mating management trial (Walsh 1989), were drenched with a single commerical sheep chromium CRC on March 16, 1988. All ewes had previously been fitted with controlled internal drug releasers (CIDR's, Type G, AHI, Hamilton) containing progesterone six days previously (d 0). Faecal collection (per rectum) commenced on d 21 and continued at 1000 h daily for the ensuing 5 days (period 1). This first faecal collection period was used to determine the herbage intakes of ewes during flushing and the first week of mating. Rams were introduced at a ratio of 1:13 for a 3 day synchronised mating from d 13.

<sup>1</sup>This experiment was conducted in conjunction with a masterate study and is more comprehensively reported by Walsh (1989).

On day 2 post-mating (d 17) the flock, including the "chromium" ewes, was divided into two equal sized groups (balanced for age) and introduced either to <u>ad libitum</u> (c. 1.8 M) or restricted (c. 1.0 M) feeding allowances. A second 6 day period of faecal collections from the two groups of ewes with chromium CRC (period 2) commenced 7 days after allocation to these different herbage allowances (i.e. d 23 to d 28). To coincide with measurement of reproductive parameters, rectum grab samples were obtained at 0900 h or 1400 h during this collection period.

Ewes were rotationally grazed throughout the experiment with the frequency of shifts being determined by residual grazing height. This was 4.0 cm during period 1, and 3.5 and 6.1 cm for the low and high pasture allowance groups respectively during period 2. Pasture height was measured by taking 50 Ellinbank Pasture Meter (EPM; Earle and McGowan 1979) readings every 3 days in paddocks grazed by the ewes. Pasture height was calibrated with pasture mass by taking 0.18 m<sup>2</sup> quadrat cuts from each paddock (total cuts = 80) at the commencement of each intake period. Separate herbage samples were randomly collected across each of the swards by hand-plucking to determine their botanical composition.

The <u>in vitro</u> digestibility of herbage was estimated from hand-plucked samples collected from the sward on each day of rectum grab sampling for each grazing treatment. Hand selection of herbage was based on observations of ewe grazing behaviour. The hand-plucked samples were sealed in plastic bags and immediately stored in crushed ice. The frozen samples for each treatment were subsequently freeze dried, ground through a 1.00 mm sieve and composited on an equal dry weight basis (1 g ground herbage/d) for <u>in vitro</u> digestibility determination using the assay of Roughan and Holland (1977). Samples were assayed in duplicate against 6 standards of known <u>in vivo</u> digestibility (collected from wether sheep fed indoors) covering the expected range of digestibility.

Ewe liveweights off pasture were recorded on days 0, 17, 21 and 28.

For both faecal collection periods, rectum grab samples (typically 0.5 to 3.0 g DM/ewe/d) were bulked intact on an equal dry weight basis per day (0.5 g/ewe/d) for individual sheep within periods for chromium analysis using the method described in Chapter Two. Herbage intakes were calculated as detailed in Chapter Three.

Group mean faecal output and feed intake were compared within periods by Student's t-test (SPSSX 1983).

### **RESULTS AND DISCUSSION**

Sward conditions and feed quality during the two intake periods are summarised in Table III.1.

 Table III.1
 Characteristics of pasture swards grazed by ewes during flushing (period 1) and early pregnancy (period 2).

	Period 1	Perio	d 2
Pasture Parameter	Both	Group 1	Group 2
	groups	(Low)	(High)
Average pasture residual (kg DM/ha)	2543	1441	2695
Average pasture height (cm)	5.8	3.5	6.1
	(3.5-7.5) <sup>a</sup>	(3.0-4.0)	(5.0-6.5)
Pasture composition (% DM)			
Grasses	72.7	67.8	76.6
Clover	2.4	0.6	3.8
Weeds	0.8	0.6	1.0
Dead	24.2	31.0	18.7
n vitro Digestibility (%)			
Dry matter (DMD)	67.35	59.43	72.97
Organic matter (OMD)	72.94	66.83	76.88
D-value (DOMD)	63.01	57.25	66.91

<sup>a</sup>Range in average height of pastures grazed during each period.

Predicted faecal output and organic matter intakes (OMI) of ewes are presented in Table III Feed intakes were similar during flushing/mating (period 1) when both groups of ewes were grazed together, but increased significantly (P < 0.01) in the high allowance group during period 2 to 1.67 kg DM/ewe/day. This is equivalent to 1.7 times the maintenance requirement of a 55 kg ewe (Rattray 1986). At this level of intake and herbage DOMD, liveweight gains of around 100g/d could be expected.

Interestingly, predicted faecal output was higher in the low allowance ewes during period 2. This can be attributed to the combined effects of the lower digestibility of the herbage consumed (P < 0.01; Table III.1) and the increased ingestion of soil (reflected by a 4.93% higher faecal ash content of the low allowance ewes). Despite the high dead matter content of the herbage offered to these ewes (Table III.1), their daily mean intake of 1.27 kg DM/d and the DOMD of the herbage means that energy intakes would have been sufficient to maintain

liveweight.

	Perio	od 1 (P1)	Perio	d 2(P2)	Sign	nificanc
	Group 1	Group 2	Group 1	Group 2	<b>P1</b>	<b>P</b> 2
Herbage allowance	Ned-Low	Med-Low	Lov-Med	High		1
Ewe liveweight (kg)	58.96±1.72	57.93±2.24	56.08±1.39	58.32±2.30	NS	NS
Faecal chromium (ppm)	6.71 <u>+</u> 0.31	6.70+0.27	5.39 <u>+</u> 0.20	6.25 <u>+</u> 0.24	NS	*
Faecal Ash (% DM)	19.76 <u>+</u> 0.01	19.95 <u>+</u> 0.01	22.81 <u>+</u> 0.01	17.87 <u>+</u> 0.01	NS	+
faeces output						
(g DN/d)	396 <u>+</u> 18	395 <u>+</u> 17	489 <u>+</u> 17	423 <u>+</u> 17	NS	**
(g 0H/d)	316 <u>+</u> 12	314 <u>+</u> 12	375 <u>+</u> 16	346 <u>+</u> 15	NS	NS
DOMI (g ON/d)	1168 <u>+</u> 48	1163±44	1131 <u>+</u> 45	1500 <u>+</u> 63	NS	***
(g OM/kg(0.75)	54.9 <u>+</u> 1.4	55.8 <u>+</u> 2.6	54.9 <u>+</u> 1.6	70.2 <u>+</u> 2.5	NS	+++
DMI (kg DM/d)	1.30±0.05	1.29±0.05	1.25±0.05	1.66±0.7	NS	
Energy intake						
(MJHE/d) <sup>b</sup>	13.35	13.25	11.66	18.10		
Maint, intake						
(x M/d) <sup>C</sup>	1.2	1.2	1.0	1.6		

Table 111.2 : Ewe liveweight, predicted faecal output and voluntary herbage intakes (mean  $\pm$  sem) of ewes during flushing (period 1) and early pregnancy (period 2).

<sup>a</sup>Significance of Student's t-test between groups.

<sup>b</sup>Energy intake = (DOMD x 16.3 x DMI); (DOMD values are from Table 1).

<sup>c</sup>Maint. intake = energy intake as a proportion of daily maintenance requirements (11 MJME/ewe/d).

These apparent mean daily intakes indicate that the experimental objective of feeding ewes at 1.0 M and 1.8 M in the post-mating period was almost achieved. It is not possible to accurately verify changes in liveweight because measurement periods were relatively short (10 d), and ewes were always weighed fresh off pasture at intervals which did not correspond exactly with those of the feed intake periods. Differences in liveweight are therefore likely to have largely reflected changes in gutfill, which could have been as high as 11% of the total liveweight in the high allowance ewes, rather than changes in the weight of body tissue.

### CONCLUSIONS

The results of this experiment indicate that, relative to published estimates, chromium released from CRC can provide acceptable estimates of herbage intakes by ewes at pasture. However, the combined effects of gutfill and the short periods of study meant that changes in liveweight could not be reconciled with the predicted pattern of intake. Collection of fasted liveweights poses a practical difficulty because animal performance and faecal chromium concentrations are compromised by removing animals from pasture.

The ewe intake data and subsequent lambing results for these ewes (Walsh 1979) show that farmers could reduce herbage allowances for ewes soon after the introduction of rams, if pasture is in short supply, without compromising lambing performance. Even if pasture heights are maintained at to 2.5 to 3.0 cm (500 to 600 kg green DM/ha) under set stocking, ewes will be able to consume sufficient herbage to meet maintenance requirements.

## **APPENDIX IV**

## **EXPERIMENTAL PROCEDURES**

### WOOL MEASUREMENT METHODS

### Greasy and Clean Midside Sample Weights

Midside samples were conditioned for at least 48 hours in a humidity room at 20°C and 65% relative humidity (RH), then weighed greasy. For scouring, samples were placed in individually labelled terylene mesh bags and submerged sequentially in four bowls of inorganic solvents (Table IV.1). Samples were placed in each solvent for 3 minutes. Squeeze rollers removed excessive liquid between bowl transfers. After the final bowl, the wool was spun dry and dried further in a forced draught at 82°C before being returned to the conditioning room for 48 hours. The clean weight was then recorded and yield (%) expressed as the ratio of clean to greasy weights.

Bowl	Tcmp( <sup>0</sup> C)	Detergent (ml) <sup>a</sup>	$Na_2 CO_3(g)$	Na HCO <sub>3</sub> 9(g)	рН
1	55	8	51		9.5
2	51	23	-	227	8.2
3	46	19	-	-	8.1
4	cold	rinse	-	-	7.6

 Table IV.1 Inorganic solvents used in wool scouring process (after Elgabbas 1986).

<sup>a</sup>A technical grade of nonyl phenol condensed with ethylene oxide.

### Colour

Wool colour was measured on two 3 g sub-samples taken from each clean and carded sample using a Hunterlab D 25 D2M Colorimeter. Red (X), green (Y) and blue (Z) reflectances were measured. Each sub-sample was measured on two faces and the average of four readings was recorded for each value. Y - Z values, indicating the degree of yellow discolouration, were also calculated.

#### Tensile Strength

Staple samples from the greasy contralateral mid-side sample were conditioned in a controlled humidity room at 20 °C and 65% RH for at least 24 hours. They were then tested on a "Hounsfield Tensometer" (Ross 1960). After weighing, the staple butt was securely clamped in a stationary set of jaws to 1.0 cm and a test length of 7-8 cm (placing the wool grown over the experimental period approximately in the centre) was measured to a set of movable jaw clamps. Tension was applied by means of a hand-wound worm thread until the staple length began to sever. The load at which the break began to occur was noted. The fibres between the jaws were then cut free and weighed to the nearest mg. This was converted to a clean weight by multiplying the greasy weight by the percentage clean yield recorded on a <sup>sub</sup>-sample of wool from the same mid-side area. Five staples for each ewe were tested in this manner. Tensile strength was expressed, on a clean wool weight basis, as the mean kg force per gram per m of length (N/ktex), using the following relationships:

N | ktex = kg/g x (9.80665/l) where 1 N = 9.80665 kg 1 ktex = 1 g of fibre, 1 m in length l = (100 cm/length of test staple (cm))Thus if l = 8.0 cm, there are 12.5 ktex/g of fibre.

# BOTANICAL COMPOSITION OF DIET SAMPLES COLLECTED FROM OESOPHAGEAL FISTULATES

The point-analysis procedure used for determining botanical composition of oesophageal fistulate extrusa samples was as follows (after Clarke and Hodgson 1986).

- 1. Extrusa sample (frozen or fresh) was rinsed with tap water through a fine sieve to remove saliva and chlorophyll colouration. The small amount of sample lost during rinsing would be unidentifiable.
- A 3-5 g sample was placed onto a white dissecting tray with a marked grid of 100 points (10 cm x 10 cm). Sufficient water to float the sample evenly across the tray was added.
- Material at each point intersect was identified and a count for botanical components recorded.
   If material was layered the "first hit" material was counted.
- 4. Botanical composition of diet was expressed as the proportion (%) of components by count.

To convert to a quantitative weight value the area and the relationship between area and mass would also need to be determined. The technique is most accurate where the proportion of inflorescent and dead material is low.

## ESTIMATION OF CHROMIUM RELEASE RATE, CHAPTER ELEVEN

Two sources of data were available to estimate the average daily rate of chromium release from CRC in experiment 13 (Chapter Eleven). The first was from serial slaughter measurements of plunger travel in CRC (from the same manufacturing batch), administered to ewes continuously grazed at three pasture heights on swards adjacent to the trial area (see Chapter Eight for full details) and the second from the pattern of chromium disappearance in faecal samples collected from ring sites on each of the swards over the expected endpoint of CRC life (d 25 to d 33 after administration (Table IV.2). The endpoint day was interpreted as being the final day at which chromium remained at a concentration similar to those recorded on days 20-25. Since the ewes were on a uniform herbage allowance, steady diminishment or a sudden fluctuation in chromium levels were most likely to be attributable to the expiration of the CRC matrix. Previous trials involving recovery of CRC by serial slaughter or from rumen-fistulated wethers (e.g. Chapter Six) indicated that linearity of chromium release was maintained until 3.0 to 4.0 mm (i.e. part of the fifth and final tablet) of the original 30 mm of matrix remained. Thus, the endpoint occurred after delivery of 26 to 27 mm of  $Cr_2O_3$  matrix, and the average rate of release could be calculated as being approximately equal to 26.5/n (where n = number of days after CRC insertion endpoint occurred). The rate of chromium release would be marginally higher than this value because steady state levels of release are not attained until approximately 3 d after the initiation of the extrusion process.

Capsulc	Residual herbage height (cm)	Estimated endpoint (d)	Plunger travel (mm/d)	Chromium release (mg Cr/d)
CRC 1	2.4 - 2.7	28 - 29	0.93 <sup>a</sup>	132
	4.0 - 4.7	28 - 29	0.93	132
	5.8 - 6.7	27 - 28	0.96	137
	6.7 - 7.8	27 - 28	0.96	137
	8.3 - 9.7	26	1.02	145
CRC 2	2.1 - 2.7	28	0.95	135
	3.7 - 4.4	26 - 27	1.00	142
	4.7 - 6.0	26 - 27	1.00	142
	5.9 - 6.6	26 - 27	1.00	142
	6.0 - 8.0	26	1.02	145
Expt 9	1.6 - 2.6	-	0.95 <sup>b</sup>	136
	3.5 - 4.6	-	0.98	139
	> 5.5		0.99	142

Table IV.2 Estimated endpoints of linear release of  $Cr_2O_3$ , average rates of plunger travel and associated daily outputs of Cr for CRC administered on L 10 (CRC 1) and L 45 (CRC 2). Corresponding data obtained by serial slaughter in experiment 9 (Chapter Eight) are also shown.

<sup>a</sup> Rate of travel required to extrude 26.5 mm of matrix by estimated endpoint.

<sup>b</sup> Estimated by linear regression for d 4-24.

The endpoint data provide confirmation that average chromium release rates were similar to those recorded by serial slaughter in experiment 9. The 4% difference between the highest and lowest rate of release in experiment 9 (142 vs 136 mg Cr/d) was significant (P < 0.05) and, although the endpoint data suggest that the same trend in release rate and herbage allowance occurred in experiment 13, there is insufficient evidence to indicate that differential rates of chromium release should be applied to derive herbage intakes. Furthermore the lowest pasture height in experiment 13 was considerably less severe than that of experiment 9. A single rate of 139 mg Cr/d was therefore applied across treatments. The

Table IV.3. This indicates ceteris parabus the same percentage differences in release rate are

maintained through to the estimate of DMI.

Table IV.3 Derivation of herbage intakes for a sheep faeces sample with 0.25 mg Cr/g DM, and assuming different rates of CRC chromium release, an OMD of 80% and plant ash content of 15%.

	Chromium release (mg Cr/d)		
	136	139	142
Faecal DM (g)	544	556	568
Faecal ash (%)	20	20	20
Faecal soil DM (g)	23	24	24
Faecal soil OM (g)	2	2	2
Faecal plant OM (g)	433	442	453
DOMI (g OM/d)	2165	2210	2272
DMI (kg DM/d)	2.49	2.54	2.62

### **CORRECTION FOR SOIL CONTAMINATION IN SHEEP FAECES**

The daily intake of animals grazing at pasture includes a soil component (Healy 1968). This may be significant during wet conditions when pasture material is soiled, when animals are grazed to low pasture residuals (such as during drought conditions or under restricted feeding levels during the winter) and when there are high levels of worm castings on the soil surface. Faecal DM therefore comprises both soil and plant components which can be further subdivided into organic matter (OM) and ash fractions (Scoffield 1970).

Faecal DM = Plant OM + Plant ash + Soil OM + Soil ash

y = Po + Pa + So + Sa

To estimate daily intakes of herbage OM (or DM) from faecal output, correction must be made for the soil component (So + Sa) which is essentially indigestible. Failure to correct for soil contamination will result in herbage intakes being overestimated.

A simple method of measuring soil in faeces is to weigh the residue following extraction of the ashed faeces with hot 6N HCL (Healy and Ludwig 1965). Precision at low levels of soil contamination is poor because of variation in the acid-soluble fraction from plant sources and because some of the soil may be soluble in acid. A more accurate method is to measure the titanium content in the faeces using X-ray fluorescence (Healy 1968). The concentrations of titanium in herbage is approximately 0.01 of that in soil; variation in faecal titanium therefore primarily reflects changes in soil intake. Soil titanium may vary between the site of soil sampling and soil types, so that separate calibration standards for experiments are necessary.

An alternative approach is to estimate soil contamination indirectly from faecal ash (or OM content) although variations in soil type (Healy 1969) and pasture composition (Nes 1975) will influence results to some extent. From three grazing experiments (two with dairy cows and one with sheep), Healy (1968) derived the linear relationship for faeces with soil contents ranging from 10 to 75%:

 $y = 1.83 x - 33.6 (\pm 4.7)$ where x = ash content of faeces (%).

At low levels of soil contamination the prediction is reportedly less precise because the relationship is curvilinear.

Nes (1975) established that the soil contamination of herbage could be derived from the following formula rather than by using the more laborious titanium method:

Errors of 2.4 to 3.4% in predicting herbage soil contamination using this formula were mainly associated with variation in plant ash percentages. For clean white clover and clean ryegrass this varied from 7.50 to 9.62% and from 7.28 to 8.60% respectively during the year.

The equations of Healy and Nes were tested against actual soil faeces data collected by Scoffield (1970), from sheep grazing ryegrass - white clover and cocksfoot - white clover swards established on Ohakea and Tokomaru silt loam soil types. These soils had an average loss on ignition (LOI; Healy 1969) of 8% during 24 hour ashing at 550°C. Faecal soil content (% DM) was calculated from titanium concentrations measured by X-ray influorescence. A third linear regression equation (NEW) relating soil content directly to faecal OM content (rather than ash) was developed for 60 faeces samples from the Scoffield data set using the SPSSX regression routine. Soil contents of these samples ranged from 1 to 71% and yielded the equation:

```
Y_{NEW} = 91.978 (\pm 0.972) - 1.096 (\pm 0.017) x, n=60, r=0.993, *** where x = faecal OM content (%)
and <math>Y_{NEW} = percentage soil in faeces DM.
```

Soil contents estimated by the Healy, Nes and NEW equations are compared with actual values in Table IV.4. Predictions by the Healy and Nes equations were significantly less reliable, particularly at high levels of soil contamination (r = 0.799 and 0.709 respectively).

	Actual F	acces Va	lues		Pr	edicted	Soil Conte	ents	
Faeces	FOM	Soil	Soil OM	-	New	H	lealy		Nes
(gDM)	(%)	(%)	(g)	(%)	(g OM)	(%)	(gOM)	(%)	(gOM)
302	80	4	1	4	2	3	1	13	4
261	60	27	6	26	5	39	10	37	8
420	50	39	26	37	25	57	40	41	32
375	40	47	14	49	14	62	23	77	18
548	31	59	26	58	25	92	40	72	32
556	22	72	32	68	30	109	49	83	37
1586	13	70	89	78	99	125	159	94	119

**Table IV.4** Predicted and actual soil contamination of sheep faeces samples collected by Scoffield (1970).

The NEW linear regression equation is developed from data for sheep faeces only and from sheep grazing pastures on the same soil types as the studies described in this thesis. It therefore provides an appropriate indirect method for correcting for soil intake by sheep in the experiments reported.

# PASTURE AND EWE FEED INTAKE DATA (EXPERIMENT 13)

			Harve	st date		
ward Component	Sward	L 2 <sup>a</sup>	L 28	L41	L 56	L 69
yegrass	550	80.25	69.53	80.86	69.32	76.77
.) - 5:	1150	89.31	76.79	86.12	71.54	81.25
	1400	85.55	74.54	79.76	75.61	81.53
	1550	84.34	73.35	75.20	82.41	76.15
	2000	82.98	76.81	80.58	75.36	74.52
ver	550	11.73	15.24	7.18	13.29	9.26
	1150	7.93	9.52	2.21	15.43	5.21
	1400	3.91	3.53	1.65	3.01	3.77
	1550	5.22	2.51	3.00	2.85	1.53
	2000	5.32	2.66	3.97	1.23	2.53
d	550	2.88	1.93	5.02	10.87	5.39
	1150	0.34	0.89	1.26	6.38	3.47
	1400	1.56	1.66	0.24	0.58	1.69
	1550	2.01	1.59	0.82	1.01	1.75
	2000	0.71	2.42	0.00	0.21	2.00
d	550	5.14	13.30	6.94	6.52	8.59
	1150	2.41	12.80	10.41	6.65	10.07
	1400	8.98	18.89	18.35	20.81	13.00
	1550	8.43	22.55	20.98	13.74	20.57
	2000	10.99	18.12	15.45	23.20	20.95

Table V.1 Botanical composition (%) of pasture samples collected manually from swards at the time o each pasture mass determination, experiment 13.

<sup>a</sup>August 31,1988

Parameter	Sward		Extrusa Colle	ection	
		L 24-28 <sup>a</sup>	L 52-53	L 68-69	Mean±sem
Ash (%)	550	26.90	22.84	20.22	24.21±1.47
	1150	16.87	18.79	15.24	16.97±0.77
	1400	19.18	19.06	15.99	$18.07 \pm 0.70$
	1550	18.99	15.44	14.66	$16.36 \pm 1.25$
	2000	14.24	16.13	15.56	$15.31 \pm 0.66$
DMD (%)	550	77.97	72.86	77.26	75.68±1.11
	1150	78.18	79.22	79.03	78.81±0.27
	1400	78.52	77.93	77.12	77.86±0.35
	1550	78.21	78.45	78.74	$78.47 \pm 0.42$
	2000	76.19	78.50	76.97	$77.22 \pm 0.44$
OMD (%)	550	82.19	79.03	80.75	80.63±0.67
	1150	80.80	82.39	81.86	$81.68 \pm 0.47$
	1400	81.82	81.62	80.98	81.47±0.47
	1550	80.74	81.33	81.54	81.21±0.37
	2000	79.50	81.39	80.28	80.39±0.36
DOMD (%)	550	65.29	64.16	67.52	65.13±0.70
	1150	69.61	70.17	71.65	$70.48 \pm 0.40$
	1400	69.45	69.16	70.20	69.60±0.42
	2000	69.56	70.59	69.59	69.92±0.37

Table V.2 Oesophageal fistulate extrusa ash contents and in vitro digestibility coefficients for three collection periods, experiment 13.

<sup>a</sup>September 12-16, 1988. <sup>b</sup>All values are the mean of collections made from three oesophageally fistulated wethers, except for the third collection from paddock 1 which is for a single animal.

Parameter	Sward		Extrusa Colle	ection	
1 4		L 24-28 <sup>a</sup>	L 52-53	L 68-69	Mean±sem
Grass	5507	7.80		80.83	77.31±1.50
01233	1150	80.49	75.59	80.92	79.00±1.71
	1400	81.08	75.89	73.17	76.71±2.32
	1550	84.31	78.51	78.15	80.32±2.00
	2000	77.12	70.87	65.81	71.27±3.27
Clover	550	8.55		5.00	67.8±1.78
_10vCl	1150	4.07	18.11	12.21	$11.46 \pm 4.07$
	1400	0.90	8.04	2.44	3.79±2.17
	1550	1.96	3.31	2.52	$2.60 \pm 0.39$
	2000	0.85	3.94	4.27	$3.02 \pm 1.09$
Vood	550			2.50	$2.50 \pm 0.00$
Weed	1150		1.57		$0.52 \pm 0.00$
	1400	-	1.57	-	
	1550		-	-	
	2000	-	5.51	-	$1.84 \pm 0.00$
Dead	550	13.68		11.67	$12.68 \pm 1.00$
Jeau	1150	15.45	4.72	6.87	9.01±3.28
	1400	18.02	16.07	22.76	$18.95 \pm 1.99$
	1550	13.73	18.18	19.33	$17.08 \pm 1.71$
	2000	22.03	19.69	29.91	$23.88 \pm 3.09$

**Table V.3** Sward botanical composition, by point analysis, of bulked extrusa samples collected from oesophageal fistulated wethers, experiment 13.

	Period	1 (L 28-32)	Period	2 (L 59-62)	Period	3 (L 66-69)
Treatment	Single	Twin	Single	Twin	Single	Twin
OMI (kg OM/cwc/	d)					
550	$1.74 \pm 0.12$	$1.82 \pm 0.13$	$1.55 \pm 0.09$	$1.56 \pm 0.12$	$1.56 \pm 0.11$	1.64 ± 0.07
1150	$1.74 \pm 0.09$	$1.86 \pm 0.04$	$1.92 \pm 0.08$	1.96 ± 0.11	1.87±0.09	$1.04 \pm 0.16$
1400	$1.84 \pm 0.08$	1.84 ± 0.09	$1.97 \pm 0.07$	1.95 ± 0.09	$1.92 \pm 0.08$	$1.79 \pm 0.04$
1550	$1.89 \pm 0.07$	1.93 ± 0.09	$2.09 \pm 0.14$	2.09 ± 0.09	$1.77 \pm 0.06$	$1.90 \pm 0.11$
2000	$1.92 \pm 0.07$	$1.82 \pm 0.07$	$2.00 \pm 0.08$	$1.95 \pm 0.11$	2.03 ± 0.09	$1.95 \pm 0.14$
DMI (kg DM/cwc/	d)					
550	$2.15 \pm 0.14$	$2.17 \pm 0.15$	$1.83 \pm 0.09$	$1.84 \pm 0.14$	$1.82 \pm 0.12$	$1.90 \pm 0.09$
1150	$1.96 \pm 0.10$	$2.11 \pm 0.05$	$2.14 \pm 0.09$	$2.18 \pm 0.10$	$2.09 \pm 0.10$	2.27 ± 0.18
1400	$2.12 \pm 0.07$	$2.12 \pm 0.10$	$2.24 \pm 0.07$	$2.19 \pm 0.10$	$2.20 \pm 0.09$	$2.04 \pm 0.04$
1550	$2.14 \pm 0.08$	$2.18 \pm 0.10$	$2.32 \pm 0.16$	$2.32 \pm 0.10$	$1.98 \pm 0.07$	$2.11 \pm 0.12$
2000	$2.13 \pm 0.08$	$2.15 \pm 0.08$	$2.44 \pm 0.09$	$2.41 \pm 0.11$	$2.24 \pm 0.10$	$2.16 \pm 0.16$
(g OM/kg cwc LW/	d)					
550	31.20	33.59	$28.38 \pm 1.74$	28.37 ± 1.73	29.13 ± 1.55	29.39±1.24
1150	28.12	32.20	31.49 ± 1.65	34.30 ± 2.26	29.90±1.55	35.16 ± 2.4
1400	28.73	32.57	31.33 ± 1.63	34.29 ± 1.27	30.05 ± 2.03	31.31 ± 1.0
1550	29.64	31.48	34.30±0.74	34.76 ± 0.74	28.54 ± 1.41	31.26 ± 2.3
2000	28.97	30.95	33.44 ± 1.31	37.18 ± 1.42	29.21 ± 1.76	33.79 ± 2.60
Overall mean	30.	71	32.78	3	30.	77
Sb	2.	88	5.37	,	5.	03
Sw	1.	68	3.76	1	4.4	42
Effects						
Rearing rank				i.		
Ewe age	NA	<b>\</b>				
Freatment	N	8	NS		P.	NS .
Freatment x rank	N		NS		ĩ	NS .
Period (P2 vs P3)		NA				

Table V.4 Effect of sward surface height on herbage intakes (mean  $\pm$  sem) of ewes rearing either single or twin lambs at three stages of lactation (ANOVA split-plot analysis refers to OMI/kg LW ewe/d; all other differences were non-significant).

## **APPENDIX VI**

# PREDICTION OF FEED INTAKE OF INDOOR-FED SHEEP USING INTRARUMINAL CHROMIUM CRC

### INTRODUCTION

This appendix describes an indoor feed digestibility trial in which sheep were fed cut pasture <u>ad</u> <u>libitum</u>. Chromium CRC were inserted into each sheep because feed intake and total faeces output were already being measured to determine <u>in vivo</u> digestibility (Nasution 1990). This study therefore provided the opportunity to evaluate the precision with which feed intakes could be estimated from the concentration of CRC chromium in the faeces.

## MATERIALS AND METHODS

Six 20-month old Romney rams were housed in metabolism crates at Massey University's Animal Physiology Unit in late November 1988. The mean  $(\pm \text{ scm})$  livewcight of the rams at this time was 44.9 ± 1.0 kg. A single chromium CRC (65% Cr<sub>2</sub>O<sub>3</sub> matrix, 9.00 mm orifice diameter, QS wing design) was administered orally by a Captec capsule dosing gun (Captec (NZ) Ltd, Auckland) to each ram four days later (d 0 of CRC life). Fresh pasture, cut each day at 0830 and 1530 h with a drum mower (Vicon, New Zealand) and collected in a silage wagon (Strautman, West Germany), was offered ad libitum to the rams for a 7 day pre-treatment period. Pastures were harvested from a mixed ryegrass - white clover "dairy cattle" sward with an average composition of 45% grass, 6% clover, 40% weed and 9% dead material. A 10 day period of measuring intakes and faecal output commenced on d 3 of CRC life. The wet weight of feed offered and refused was recorded for each ram daily. Duplicate sub-samples of the feed offered (for <u>am</u> and <u>pm</u> harvests) and of refusals (for individual sheep) were oven-dried at 80<sup>o</sup>C to determine their respective dry matter contents (DM %). DM intake (DMI, g/d) was calculated as the difference between DM offered and DM refused. The fresh weight of faeces, collected in a urine separator tray fitted to the metabolism crate, was recorded at 0900 h each day. After thorough mixing a duplicate sub-sample (c. 100 g) of the faeces was taken and ovendried at 80°C for a minimum of 72 h. A sample of the dried faeces (c. 60 g) for each days output was retained for chromium analysis. The rams were weighed and returned to pasture on d 19.

<sup>&</sup>lt;sup>1</sup>This experiment was conducted in association with the MAgrSc programme of Nusantara Nasution.

Faeces were analysed for their chromium content using the method described in Chapter Two. The intra-assay coefficient of variation (CV) was 2.9% and the inter-assay CV 3.7%. Chromium recovery was calculated by multiplying the concentration of chromium in the faeces ( $\mu$ g Cr/g DM) by the faecal output (g/DM/d) of each ram and expressing this as a proportion of the expected average daily payout of 140 mg Cr from the CRC. This payout was based on the rates of chromium release measured by the serial slaughter of 14 month old rams fitted with CRC of the same specifications as those used in the present experiment. Faecal samples for each sheep were prepared in two ways for the chromium analysis; first as intact faeces on a daily (DAILY) basis (1 g/DM/d) and second by bulking (BULK) faeces (10 g DM/d) over 5 day sub-periods (period 1 = d 3-7; period 2 = d 8-12). The bulked sample was ground through a 1.00 mm sieve (Cranston, England) and duplicate sub-samples (1 g/DM) were taken for chromium analysis.

DMI for individual sheep were predicted from the faecal output estimated by chromium dilution and the <u>in vivo</u> dry matter digestibility (DMD) value of the feed (Raymond and Minson 1955).

Group mean predicted faecal outputs and DMI were compared with actual values by paired ttests.

### RESULTS

Recovery of chromium was significantly (P < 0.001) lower in period 1 than in period 2 (Table VI.1). This was because chromium recoveries in excess of 90% of the expected payout were not achieved in three of the sheep until d 5 to 7. These sheep, according to their daily pattern of DMI, took longer to adapt to the indoor feeding conditions of the trial. A group mean chromium recovery of 90% was achieved by the fifth day after CRC insertion (Figure VI.1).

Period 1			Pe	riod 2
Sheep	Daily	Bulk	Daily	Bulk
1	66.5	79.6	107.0	97.5
2	66.0	81.8	91.4	113.50
3	93.3	96.0	109.1	109.4
4	66.0	68.2	99.1	93.6
5	93.13	100.4	100.5	88.5
6	78.4	84.6	100.5	100.1
Mean	78.4	84.6	100.5	100.1
sem	4.6	2.4	3.7	3.9

**Table VI.1** Recovery of chromium (% of expected) from faeces using either daily (DAILY) or bulked (BULK) sampling of faeces

Mean recoveries of chromium during period 2 for both methods of sampling were not significantly different from 100%. Differences in the rate of CRC chromium release between sheep, as well as sampling and assay errors, contributed to recoveries for individual sheep being greater or less than 100%.

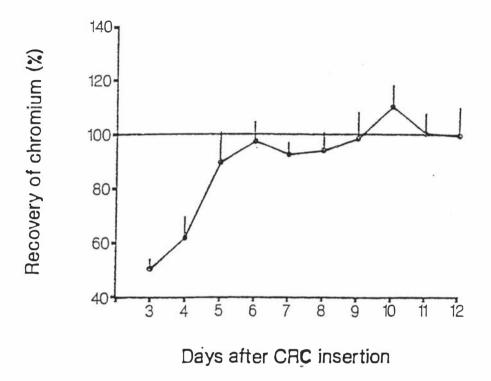


Figure V1.1 Mean pattern of faecal chromium recovery from indoor-fed rams fitted with chromium CRC. Bars indicate sem.

DMI increased by an average of 307 g/d between periods 1 and 2, indicating that intakes had not fully stabilised by the start of intake measurements (Table VI.2). Average faecal ouput also increased during period 2 to 433 g/DM/d. The in vivo DMD of the pasture,  $73.12\pm1.98\%$  and  $72.48\pm1.54\%$  respectively for periods 1 and 2, was therefore consistent during the digestibility study. Sheep liveweights increased by an average of 3.1 kg (172 g/d; P<0.1) during the 18 days of indoor feeding. This also contributed to the increased DMI during period 2.

Method	Period	FO		DMI		Sign <sup>a</sup>	
		Predicted	Actual	Predicted	Actual	FO	DMI
Daily	1	458±19	340±13	1667±67	1280±45	***	***
(n = 3())	2	445±17	433±17	1615±62	1587±36	NS	NS
Bulkcd	1	407±29	340±29	1479±105	1280±45	*	+
(n = 6)	2	437±33	433±22	1589±120	1587±60	NS	NS

Table VI.2 Group mean ( $\pm$  sem) and actual faecal output (FO, g DM/d) and DMI (g/d) of rams fed cut pasture <u>ad libitum</u> indoors for two methods of faecal sampling.

<sup>a</sup>Significance of paired t-test between predicted and actual values.

Group mean faecal outputs and DMI predicted from faecal chromium concentrations did not differ significantly from actual values during period 2 for either of the sampling methods (Table VI.2). Predicted values were much poorer for period 1 because of the lower levels of chromium recovery.

#### DISCUSSION

This indoor feeding trial has shown that the group mean feed intake of sheep can be reliably estimated from the concentration of marker chromium delivered from intraruminal CRC, provided sufficient time after administration of CRC is allowed for chromium to reach steady state concentrations in the faeces. Although chromium first appears in the faeces within 24 hours of CRC insertion a further period of 4 to 7 days is required before recoveries in excess of 90% of the expected average daily payout are achieved. The attainment of steady state conditions is highly variable because it is dependent on factors such as the pattern of chromium release, the amount of chromium administered, feed characteristics and animal health (Raymond and Minson 1955; Pigden and Brisson 1956; Lambourne 1957b). It can be expected that equilibrium will be achieved most rapidly if rumen mean retention time is low and intakes are high (Raymond and Minson 1955; Faichney 1983).

The general recommendation of the manufacturer of chromium CRC is that sampling for intake estimation can commence after d 5 (CAPTEC Technical Supplement for Sheep CRC, 1988). However, this experiment has shown that steady state conditions will not be achieved within this time frame for some sheep. It would therefore be prudent in most situations to delay the first sampling of faeces until day 8 after CRC insertion to ensure that steady state conditions are achieved in all animals (Figure VI.1). This would still allow time for two 5-day faecal sampling periods before the CRC linear release phase expired 25 to 28 days after their insertion. Alternatively, changes in faecal chromium concentration could be monitored on a daily basis for individual animals from d 5. This would substantially increase the costs associated with the assay of chromium in trials where a large number of animals were involved. This experiment has shown that, where daily monitoring is not required, grinding faeces bulked across days and taking sub-samples from this for chromium analysis will provide a reliable representation of group mean faecal output. Lambourne (1957b) reported a similar result for bulking faeces of wethers drenched with  $Cr_2O_3$ , thus confirming the results reported in Chapter Six.

This experiment offers further evidence of the potential of chromium CRC for the study of herbage intakes by ruminants (Ellis et al. 1981; Laby et al. 1984). However, between animal variation in CRC (CV 5-8%), together with the inability to measure the true digestibility of feed consumed by individual animals, precludes their use for estimating intakes of individual animals.

## APPENDIX VII

## PUBLICATIONS

- Parker, W.J.; McCutcheon, S.N., Carr, D.H. 1989: Effect of herbage type and level of intake on the release of chromic oxide from intraruminal controlled release capsules in sheep. <u>New Zealand journal of agricultural research 32</u>: 537-546.
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- Parker, W.J.; McCutcheon, S.N. 1990: Measurement of faecal output by sheep grazing at pasture using intraruminal chromium controlled release capsules. <u>Proceedings of the</u> <u>fifth AAAP Conference (Taipei)</u> 3: 92.
- Parker, W.J.; Morris, S.T.; Garrick, D.J.; Vincent, G.L.; McCutcheon, S.N. 1990: Intraruminal chromium controlled release capsules for measuring herbage intakes in ruminants. <u>Proceedings of the New Zealand Society of Animal Production 50</u> (in press).

Intraruminal chromium controlled release capsules for measuring herbage intake in ruminants

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Abstract Intraruminal chromium controlled release capsules (CRC, Captec (NZ) Ltd, Auckland) provide for the linear release of  $Cr_2O_3$  over c. 25 days in sheep and c. 20 days in cattle. Uniform release of Cr2O3 is achieved 2 to 3 days after oral administration of the CRC but steady state levels of Cr<sub>2</sub>O<sub>3</sub> in the faeces are usually not achieved until day 7 or 8 in sheep and day 5 or 6 in cattle. Where cross-over or multiple feeding level experimental designs are applied, time should be allowed for Cr<sub>2</sub>O<sub>3</sub> to adjust to a new steady state in the faeces before sampling for each new treatment. Low diurnal variation of Cr2O3 in the faeces, due to the continuous mode of marker release in the rumen, allows flexible rectal sampling regimens to be applied. Sward sampling reduces disturbance of animal grazing to a minimal level since Cr<sub>2</sub>O<sub>3</sub> is delivered by a single CRC application. The effects of level of feed intake and feed type on the rate of Cr2O3 release are usually small, but release rates may be higher in rumen-fistulated than in non-fistulated animals and may differ for capsules of the same type applied to different ruminant species. Reduced animal handling, flexible faecal sampling times and lower costs (for Cr2O3 and labour) with CRC technology, compared to daily drenching of Cr<sub>2</sub>O<sub>3</sub>, enable the number of experimental animals to be increased. This improves the likelihood of detecting differences in mean intake between groups. Individual animal intakes will not be reliably estimated with chromic oxide CRC until the digestibility of herbage consumed by individual animals can be measured more accurately.

Keywords ruminants; herbage intake; chromic oxide; controlled release capsule.

### INTRODUCTION

Research into grazing management of ruminants has traditionally been restricted by the absence of techniques which allow the measurement of feed intake with little disturbance to the animal's normal grazing behaviour. Most herbage intake studies in New Zealand have been based on the pasture difference technique (e.g. Rattray et al. 1982), total collection of faeces (e.g. Ulyatt et al. 1974) or the use of chromic oxide ( $Cr_2O_3$ ) as a faecal marker (e.g. Carruthers and Bryant 1983). The widespread application of these methods has been hampered by their high labour requirements, variable accuracy and, in the case of faecal markers, the absence of systems allowing their uniform release into the rumen (Meijs 1981). The purpose of this paper is to describe the application of a new technology, intraruminal chromium controlled release capsules (CRC), to the indirect measurement of feed intake in ruminants.

## DEVELOPMENT AND APPLICATIONS OF CHROMIC OXIDE CRC

The prototype CRC, also referred to as the "controlled release device" (CRD), for delivering  $Cr_2O_3$  into the rumen of sheep (Harrison et al. 1981) was developed from a variable-geometry slow release device patented by Dr R.H. Laby of the CSIRO in 1969. The capsules have a plastic barrel into which a matrix of  $Cr_2O_3$  sucrose mono-stearate (65:35 w/w) is inserted (usually as a series of tablets). This is followed by a plastic plunger and a compressed steel spring. An orifice of variable size (depending on required release rate characteristics) exposes the matrix to the rumen contents, forming a gel which is extruded through the force of the spring. A plastic strip, folded against the barrel for dosing, opens into a T-configuration (wings) to retain the CRC in the rumen (Lehane 1982). In rumen-fistulated animals the capsules may be suspended in the rumen by a nylon filament attached to the fistula. Chromic oxide capsules for sheep with a 25 day linear release of 180-210 mg  $Cr_2O_3/d$  (Captec (NZ) Ltd. Auckland) were first marketed in 1987 (Ellis and Rodden 1987). Cattle CRC, with a 20 day linear release of 1650-1800 mg  $Cr_2O_3/d$  (Hirschberg et al. 1990), were made available the following year.

Tests of alternative CRC designs and their performance in sheep have been reported by Ellis et al. (1981), Harrison et al. (1982) and Laby et al. (1984). Similar studies for cattle-sized CRC and their application in feed intake experiments have been published by Ellis et al. (1982), Bird et al. (1984), Grainger et al. (1987), Graham (1988) and Barlow et al. (1988). The first use of CRC (sheep type) in fallow deer was described by Kelly et al. (1985). These were later used by Parker and Ataja (1990) to measure faecal output by 8-month old red deer stags.

### ADMINISTRATION OF CRC

CRC are administered orally to sheep by a dosing-gun (Captec, Laverton, Australia) which releases the capsule at the back of the tongue. The swallowing motion of the animal then carries the capsule into the rumen. A Captec balling-gun is used to dose cattle with CRC in the same manner. Alternatively capsules can be placed down the oesophagus within a lubricated flexible rubber tube and released at the thoracic inlet. For both methods, administration occurs with the animal in a standing position and the wings of the CRC are temporarily restrained against the capsule barrel by a tape which dissolves after 30-40 seconds in the presence of rumen fluids. The animal should be observed for at least this period of time after administration to ensure that the capsule has been retained in the rumen. The 'back of the tongue' dosing procedure is more rapid and usually less stressful to the animal, but can result in jaw and teeth damage to the capsules if they are dislodged from the applicator in the mouth or are regurgitated from the oesophagus before swallowing to the rumen has been completed. Damaged capsules (<3%)should be discarded if the barrel is punctured or if indentations in the plastic would restrict plunger movement. Releasing the capsule at the thoracic inlet provides greater protection to the capsule, makes it less prone to regurgitation and is better suited to animals such as adult deer in which the larynx is relatively larger and located further down the throat than in sheep or cattle. Treating animals which are difficult to handle (e.g. adult deer, some classes of cattle) with a low dosage of a sedative (e.g. 2% Rompun, Bayer Ltd. Petone) assists CRC administration. Regurgitation of CRC rarely occurs from sheep but a mismatch of CRC size and liveweight can

result in the loss of capsules from cattle. Assigning numbered CRC to individual animals assists the management of this problem.

## TIME OF FAECAL SAMPLING

Release of the  $Cr_2O_3$  tablet matrix is initiated at the orifice of the capsule through wetting by rumen fluids. The extruding matrix is brushed off and mixed with the contents of the rumen and reticulum by their normal muscular activity. A uniform rate of release is generally achieved 2 to 3 days after administration but steady state levels of  $Cr_2O_3$ , at maintenance levels of intake, are usually not achieved in the faeces until day 7 or 8 in sheep (Parker et al. 1989; Lee et al. 1990) and day 5 or 6 in cattle (Graham 1988; Nasution 1990). Kassano (1988) recommended a preliminary period of not less than 7 days before faecal collection in dairy calves (55-70 kg) dosed with sheep CRC. The attainment of steady state conditions in sheep is dependent on the level of feed intake and feed type, and is less rapid in rumen-fistulated animals than in intact animals (Parker et al. 1989; Parker 1990).

The 12 and 18 day periods of linear release of  $Cr_2O_3$  after attainment of steady state concentrations, in the faeces of cattle and sheep respectively, provide the opportunity to apply experimental designs involving cross-overs or multiple feeding levels. However, before sampling for the new treatment commences, time should be allowed for  $Cr_2O_3$  to adjust to a new steady state level in the faeces. For relatively small changes in sheep feed intakes (c. < 0.4 maintenance (M)) a period of 3 days is usually adequate (Parker 1990) but, for larger changes 5 or more days may be necessary. This has implications for rotational grazing treatments where feed intakes can vary considerably between days. It is likely that the procedure adopted by Raymond and Minson (1955), of averaging the chromium concentration of samples taken across the grazing interval, should also be applied to CRC.

The continuous mode of Cr<sub>2</sub>O<sub>3</sub> release from CRC reduces within-day (diurnal) variation in faecal chromium concentration in sheep to about one third of the level achieved by twice-daily

drenching of  $Cr_2O_3$  in gelatin capsules (coefficient of variation (CV) 6.2 vs 20.1 %; Ellis et al. 1981). In more recent studies both Parker et al. (1989) and Lee et al. (1990) have reported nonsignificant levels of diurnal variation (CV 4-8%) in sheep dosed with CRC despite different patterns of feed intake. Similarly low levels of diurnal variation in the  $Cr_2O_3$  content of faeces from cattle treated with CRC have been measured by Grainger et al. (1987) and Nasution (1990).

Low within-day variation allows flexible faecal collection regimes. This is demonstrated by the estimates of faecal output for ram lambs when faecal samples were obtained per rectum at different times of the day (Table 1). Rectum samples obtained once daily over three days provided estimates similar to those derived from twice-daily grab samples and total bagged collections.

<Table 1>

The uniform release of  $Cr_2O_3$  following a single CRC application allows faecal samples to be collected from the sward if minimum disturbance of grazing is desirable, individual animal data are not required or problems with animal handling (e.g. ewes with young lambs at foot) prevent sampling per rectum. We have compared estimates of faecal output by sward ring sampling (Raymond and Minson 1955) over an 8 day period with total collection of faeces and sampling per rectum from 5 ram lambs dosed with CRC and grazed to maintain a uniform pasture height. Total collections were obtained on alternate days to sward and rectum samples. Differences between the mean (±sd) faecal output of  $363\pm26$ ,  $366\pm27$  and  $355\pm47$  g dry matter (DM)/d for total collection, sward ring sampling and rectum grab sampling respectively, were not significant.

A further advantage of the uniform mixing of  $Cr_2O_3$  in the rumen and low diurnal variation is that faecal samples can be analysed by chemical digestion without prior grinding. For example, estimates of mean (±sem) intake of ram hogeets, fed lucerne chaff <u>ad libitum</u> indoors, were

1165±78 g DM/d for faeces assayed after being bulked intact on an equal weight basis across 5 days, 1221±81 g DM/d for faeces bulked across 5 days and ground through a 1.00 mm mesh before assay and 1281±97 g DM/d for faeces assayed intact on a daily basis for the same period. These values were not significantly different from the actual mean intake of 1195±88 g DM/d (Parker 1990).

## FACTORS AFFECTING THE RATE OF CHROMIC OXIDE RELEASE

The rate of release of  $Cr_2O_3$  is primarily controlled by orifice diameter, matrix composition, plunger spring strength (Laby et al. 1984) and the length of  $Cr_2O_3$  matrix (Ellis et al. 1988). With a 65%  $Cr_2O_3$  matrix and a 9.00 mm orifice diameter, release rates of 160-175 and 190-210 mg  $Cr_2O_3/d$  respectively are achieved from CRC with a 6.0 cm or 3.0 cm length of pressed tablet matrix. This provides for  $Cr_2O_3$  release over 30 and 75 days respectively (Parker 1990). With a 50%  $Cr_2O_3$  matrix, 7.00 mm orifice diameter and 6.0 cm of matrix, a 100 day release period is obtained (Parker et al. 1989). Uniformity of release is also maintained by the passage of rumen gases into the capsule barrel by osmosis to relieve the vacuum formed as the matrix diminishes (Laby 1986). Thus movement of the matrix can actually be reversed if rumen dysfunction occurs.

Studies of plunger travel in CRC recovered from ewes by serial slaughter have shown that release rates are reduced if very low levels of intake (<0.6 M) are imposed for 4-7 days (Parker 1990). Plunger travel returned to the expected rate when intakes were restored to maintenance levels or above. In a second slaughter trial, rates of plunger travel were not significantly different in ewes set stocked to maintain a low, medium or high residual pasture mass (c. 500, 1000 and > 1250 kg DM/ha respectively), although a slight trend to increased release rates with higher pasture availability was evident. This confirmed the findings of Laby et al. (1984) and Parker et al. (1989) that consistency of plunger travel is maintained across a wide range of feed types and feed intakes. However, release rates will increase slightly if feeds which create a more abrasive rumen environment are consumed (Parker et al. 1989). The CV in CRC release rates between sheep is typically 3 to 6% while variation between CRC within animals is low (CV 1-3%)(Parker et al. 1984) and Parker et al.

al. 1989,1990).

Trials at Massey University have shown that plunger travel in rumen-fistulated sheep is 10-13% slower than in non-fistulated animals. This difference can probably be attributed to temperature fluctuations associated with the removal of the capsule from the rumen for measurement. Different gaseous conditions in a fistulated compared with an intact rumen are also likely to be a contributing factor. In contrast, Laby et al. (1984) implied that there were no differences between fistulated and non-fistulated sheep. Until further research defining the differences between the two rumen types is completed, plunger travel measurements from fistulated animals can only be used as a guide to the daily output of  $Cr_2O_3$  in intact animals. Actual  $Cr_2O_3$  release can be determined by recovering CRC from animals through slaughter at appropriate times but this is often impractical and expensive. Alternatively, release rate can be estimated by monitoring the time of  $Cr_2O_3$  disappearance from the faeces, i.e. "endpoint" determination (Ellis et al. 1988). Experiments with CRC containing a second marker in the final tablet of the matrix to more distinctly label the end of  $Cr_2O_3$  release are in progress. CRC with a second marker are also being investigated to provide differentiation between the faeces collected, by sward sampling, from two or more treatment groups of animals grazed together.

At present little is known about the relative release rate characteristics of CRC in different animal species. We have shown for sheep and deer grazed separately but on similar pastures that average (±sem) plunger travel rates of sheep CRC, recovered by slaughter, are lower in deer than in sheep ( $0.78\pm0.01 \text{ vs } 0.95\pm0.04 \text{ mm/d}$ ). Preliminary results also suggest that plunger travel of sheep CRC may be more rapid in a cattle rumen. Caution therefore needs to be exercised when applying Cr<sub>2</sub>O<sub>3</sub> release rates across ruminant species as described by Kelly et al. (1985).

### CONCLUSIONS

The reduced animal handling and flexible faecal sampling regimes associated with CRC usage allow the number of experimental animals to be increased compared to previous methods of Cr<sub>2</sub>O<sub>3</sub> administration (e.g. Barlow et al. 1988; Lee et al. 1990). Costs are also lower with CRC technology than with twice-daily drenching of chromic oxide gelatin capsules or Cr<sub>2</sub>O<sub>3</sub>impregnated paper (Corbett et al. 1960). For example, a single sheep CRC provided Cr2O3 for 25 days at a cost of \$NZ 12.50 in 1990 compared to c.\$NZ 50 for two gelatin capsules per day over the same period. This does not take into account the additional labour costs for daily drenching of gelatin capsules. Parker et al. (1990) estimated that, under ideal conditions and with group sizes exceeding 50 animals, differences in intake between groups of <5% could be detected. Cruickshank et al. (1987) calculated that a 10% difference in mean intakes could be detected at P < 0.10 with group sizes of 6 animals using estimates of faecal output from animals dosed with CRC. Reliable estimates of individual animal intakes are unlikely to be measured with CRC technology until the digestibility of herbage consumed by individual animals can be determined more accurately (Parker et al. 1990). In practice, however, livestock at pasture are usually managed as groups and in this respect chromic oxide CRC have the potential to make a significant contribution to improving our understanding of the relationships between pasture, animal intakes and production.

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Routine	Samples <sup>a</sup>	Period 1	Period 2	
Actual	3	352±13	355±9	
AM-PM	6	360±13 (102.3)	334±15 (94.1)	
AM-PM-AM	3	348±12 (98.9)	341±16 (96.1)	
PM-AM-PM	3	373±14+b (106.0)	326±12** (91.8)	
AM only	3	356±12 (101.1)	346±17 (97.4)	
PM only	3	363±15 (103.1)	322±18** (90.7)	

Table 1 Actual and predicted group mean faecal outputs (g DM/d) of 5 ram lambs for alternative 3-day rectum grab sampling routines. Figures in brackets are predicted values expressed as a percentage of actual values obtained by total collection of faeces.

<sup>a</sup>Number of samples collected per animal.

<sup>b</sup>Significance of paired t-tests between predicted and actual (bagged) faecal output: + P < 0.01, \* P < 0.05, \*\* P < 0.01.

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