A COMPARISON OF IN-FIELD TECHNIQUES FOR ESTIMATING THE FEED INTAKE OF YOUNG BOER GOATS ON A *LEUCAENA LEUCOCEPHALA* /GRASS HAY DIET

Brigid Aileen Letty
BSc. Agric

Submitted in partial fulfilment of the requirements for the degree of
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In the Department of
Range and Forage Resources
University of Natal
P/Bag X01
Scottsville
3209

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DECLARATION OF ORIGINALITY

I, Brigid Aileen Letty, declare that the research reported in this thesis comprises my own original work except for the assistance acknowledged, or where due reference is made in the text. This thesis has not been submitted for any degree or examination at any other university.

B.A. LETTY

ABSTRACT

Two methods of estimating the intake of a 25% leucaena : 75% grass hay diet by young male Boer goats were assessed. (a) The purine derivative technique which uses the urinary excretion of purine derivatives (expressed relative to creatinine concentration in the same sample) as an index of feed intake, and (b) the conventional marker method, utilizing chromic oxide (Cr_2O_3) contained in gelatin capsules and dosed twice daily, as the marker.

Following a prerun the two techniques were compared in three runs of an indoor experiment. In each run 10 goats were randomly allocated to five feeding levels (500 to 1100 g fodder d^{-1} on air dried basis). A preliminary and an adaptation period during which goats were dosed with the Cr_2O_3 and fed their daily feed allowance, was followed by a 4 day collection period during which spot samples of urine were collected and analysed for allantoin and creatinine (allantoin being used instead of total PDs) and faecal samples were collected for chromium analysis and percentage dry matter determination. For the first two runs, two grab samples per day for each goat were bulked and analysed for chromium content. For the last run, the total daily faecal collection was subsampled and analysed for chromium. Work was done in metabolic crates to determine the effect of time of collection on the ratio of allantoin : creatinine (A/C) in spot urine samples and it was found to non-significant ($P>0.05$).

Linear regressions of:
(a) feed intake expressed per unit metabolic mass ($g.d^{-1}.LW^{-0.75}$ (I_mmass)) against A/C ratio;
(b) faecal output ($g\ d^{-1}$) against feed intake ($g\ d^{-1}$); and
(c) faecal chromium concentration ($mg\ kg^{-1}$) against faecal output ($g\ d^{-1}$) were fitted to the data.

During the prerun, only regression (a) was fitted and was non-significant ($P>0.05$), showing no trend at all. For the first true run, the regression of I_mmass against A/C ratio was significant and the correlation was high ($P \leq 0.001$, R^2_A 0.715, $n = 10$) but for the second and third runs, the correlations only became significant when the apparent outliers were discarded from the data. (Run 2: $P \leq 0.001$, R^2_A 0.824, $n = 8$; Run 3: $P \leq 0.05$, R^2_A 0.430, $n = 9$).

It was concluded that the relation between I_mmass and A/C ratio is not well enough defined to be used for predictive purposes.

When regression (b) was investigated, all the runs produced significant results ($P \leq 0.001$, $P \leq 0.01$, $P \leq 0.001$ for runs 1, 2 and 3 respectively) however the correlations were not as high as expected (R^2_A being 0.714, 0.565 and 0.863 respectively). For the regression of faecal Cr concentration against faecal output (regression c), all runs showed significant relations ($P \leq 0.001$, $P \leq 0.0001$, $P \leq 0.001$ for runs 1, 2 and 3 respectively) and the correlations were high (R^2_A being 0.836, 0.837 and 0.912 respectively).

The data from the three runs were pooled and single equations established for regressions (b) and (c) to allow for the prediction of intake from faecal chromium concentration.
Faecal output = feed intake * 0.448 + 19.341 ($P \leq 0.001$, r 0.853, R^2_A 0.718, SE 25.664, n = 30)
Faecal chromium concentration = faecal output * -241.547 + 1.315E+05 ($P \leq 0.001$, r 0.904, R^2_A 0.811, SE 5603.788, n = 30).

In vitro figures were determined for a range of leucaena : hay mixes but no apparent trend was found between percentage leucaena in the mix and the digestibility of the mix. These results compared favourably with *in vivo* results obtained for a 25% leucaena : 75% hay mix.

Neither technique proved entirely satisfactory, but the external marker method was found to be more effective than the purine derivative technique. More work is required especially with respect to the latter method.

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CHAPTER 1

Introduction and literature review

It has been said that the quantity of feed consumed by an animal contributes more to its level of productivity than the quality of the feed itself. When pasture yields are low or quality is poor producers can feed supplements to increase total feed intake (Minson 1990).

Leucaena leucocephala (Leucaena) is a high protein, leguminous shrub which can be used to supplement grasses with high rates of growth but low quality which is often the case with natural pastures (Jones 1979, Mtenga & Shoo 1990, Adejumo & Ademosun 1991). 'Tree legumes can play an important role in maximising utilisation of grass forages and improving animal performance especially since small quantities of such legumes are required' (van Eys *et al.* 1986). Leucaena is fast growing when established and is able to tolerate adverse moisture conditions because of the deep root system (Adejumo & Ademosum 1991). Toxins associated with the ruminal breakdown of leucaena prevent high levels of supplementation but it is suggested that growing animals on diets containing less than 30% leucaena should not be affected by the toxicity (Jones 1979). If the animals contain certain rumen bacteria which degrade these toxins, then they are not adversely affected by high levels of leucaena in the diet (Jones & Megarrity 1986).

In many shrubland situations, initial overgrazing by cattle and sheep has rendered the vegetation suitable for goats only. This is because goats select a wide range of plant species and appear to be able to utilise the nutrients contained in high fibre feedstuffs better than sheep or cattle (Devendra 1978). They are cheaper to rear than cattle, require less space and shelter and their meat can be handled easily by small families and communities with no access to refrigeration (Adejumo & Ademosun 1991). Goats are often found thriving on marginal land which, because of soil, terrain or climatic conditions is unfit for crop cultivation, or in semi-arid areas where resources for stock-watering are inadequate for large stock and feed quality is generally low (Devendra 1978).

Much of South Africa is marginal with respect to annual rainfall and is not suitable for cultivation

and much has been degraded by inappropriate land use. A goat/grass/leucaena production system is well suited to these areas.

1.1 Why is there a need to measure intake?

According to Gordon (1995), the principal reasons for measuring herbage intake of free-ranging animals are (1) to understand the relation between resource distribution, sward structure and herbage intake and (2) to explain between-animal variation in intake and performance in relation to animal nutritional status, grazing regime and management practices.

1.2 Methods currently used for measuring intake

Intake measurements are considered to be either short-term or long-term. Short-term intake, measured as grams minute^{-1}, consists of a combination of bite-size and short-term rate of biting. Over the period of a day, animals spend a certain amount of time not actually feeding. Intake over the period of a day is called the long-term intake rate and is measured in kg day^{-1}. It is usually the long-term rate which is related to animal productivity (Gordon 1995). Long-term intake can be calculated from short-term intake if the actual number of hours per day spent grazing is determined. This, however, is a time-consuming method which requires spending the entire day with the animals and is therefore not suitable for production-scale field research. Short-term rates of intake were not of importance to this study and shall be given little mention from here on.

1.2.1 Conventional methods of determining feed intake

In the past, animal-based techniques for measuring intake (**I**) have relied on the measurement of faecal output (**F**) and diet digestibility (**D**) (Mayes *et al.* 1995). The formula describing this relation is:

$$I = \frac{F}{(1 - D)} \qquad \text{----------(1.1)}$$

1.2.1.1 Determining faecal output

Total collection methods for measuring faecal output are labourious and often difficult in the field as the animals must be adapted to harnesses and frequent handling. It is also necessary to ensure that the apparatus remains in place so that there is no loss of faeces (Doyle *et al.* 1994).

Faecal output can be estimated from the dilution in the faeces of an indigestible substance (marker) given to a test animal (Dove & Mayes 1991). Marker methods which give biased estimates of faecal output will affect intake estimates accordingly (Musimba *et al.* 1987). An external marker is one which is not inherently present in the feed but is either added to the feed or administered orally or intraruminally to the animal. It may be given continuously at a constant rate or as a single dose. According to Kobt & Luckey (1972, cited by Merchen 1988), an 'ideal' marker must be inert with no toxic effects, it must be neither absorbed nor metabolised in the digestive tract. It must have no appreciable bulk and must mix intimately with and remain uniformly distributed in the digesta. It must have no influence on gastro-intestinal secretions, digestion, absorption, or normal motility and it must not affect the micro flora. It must have physicochemical properties that are readily discernible throughout the digestive tract, making precise quantitative measurements possible.

Faecal output can then be determined using the formula:

$$F = \frac{(M_d * R)}{M_f} \qquad \text{----------(1.2)}$$

where

R is recovery rate (assumed to be 1.00 for Cr_2O_3);

F is faecal output

M_f is the concentration of an external marker in the faeces and

M_d is the daily dose of marker administered.

1.2.1.1.1 Metal oxides

Several insoluble metal oxides have been used in nutritional studies. Chromic oxide (Cr_2O_3), which shall be discussed in more detail, has been a popular marker for estimating faecal output (Raleigh *et al.* 1980 cited by Hollingsworth *et al.* 1995). Titanium dioxide (TiO_2) has also been used to estimate faecal output, though to a lesser extent. Since chromium (III) compounds have been shown to have carcinogenic properties, TiO_2 may well replace Cr_2O_3 as the most commonly used metal oxide marker.

In most of the early studies, chromic oxide was suspended in oil in gelatin capsules which were then dosed on a daily basis. It has the major advantage of being completely recoverable in faeces. One of its disadvantages, however, is that it travels through the gut independent of both liquid and solid phases, and forms a sediment in the reticulo-rumen, causing it to be transferred sporadically into the lower gastro-intestinal tract so that excretion is subject to diurnal variation (Merchen 1988). Langlands (1975) also reported on variations in excretion due to incomplete mixing of digesta and marker giving rise to biased estimates of faecal output. Forage source appears to affect the rate of marker excretion, while within a forage type, higher levels of intake lead to faster rates of excretion. Good quality forage results in quicker appearance of the marker and an earlier and higher excretion peak, compared with marker excretion of an animal on a fibrous diet. These excretion pulses can be summed to predict what the marker excretion patterns will look like under different dosing regimes.

Various dosing strategies have been investigated in an effort to reduce this variation in excretion of Cr_2O_3 over the day. Once and twice daily being the most common as more frequent dosing would not be practical due to the disturbance of the animal's grazing habits. It is most suitable to sample at dosing times to minimise handling and disturbance of grazing behaviour.

According to Lambourne (1957) once daily dosing and sampling is unsatisfactory since samples collected at the dosing time do not reflect the true concentration (Figure 4.01). Sampling twice daily would improve the estimate however if animals are to be handled twice daily then it would make sense to dose twice daily.

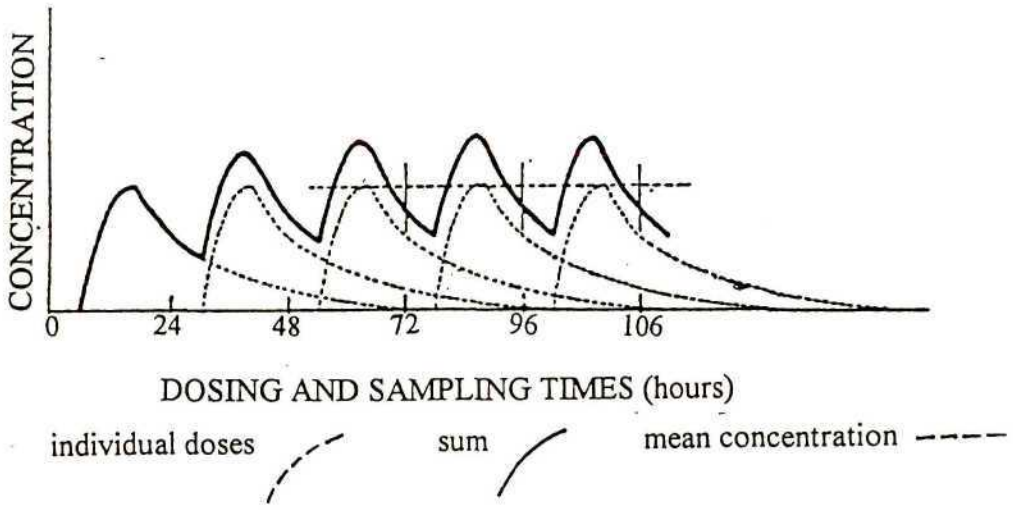

Figure 1.01 Marker excretion patterns predicted for a once daily dosing and sampling program (Lambourne 1957).

Twice daily dosing and sampling at 12 hourly intervals results in samples being taken from peaks regardless of changes in rates of marker excretion, so marker concentrations and faecal output would be biased. Dosing and sampling twice daily at uneven intervals results in marker content of samples being fairly evenly distributed around the mean value. 7/17 hour interval dosing and sampling (Figure 4.02) is satisfactory, but it should be noted that an error of 10-12% can be expected if a twice daily dosing and sampling program together with a bulking procedure is employed. Bulking samples for each animal over the collection period will give a better estimate than a single day's sample, due to the differences in concentration for any particular animal between days (Lambourne 1957; Redmon *et al.* 1995)

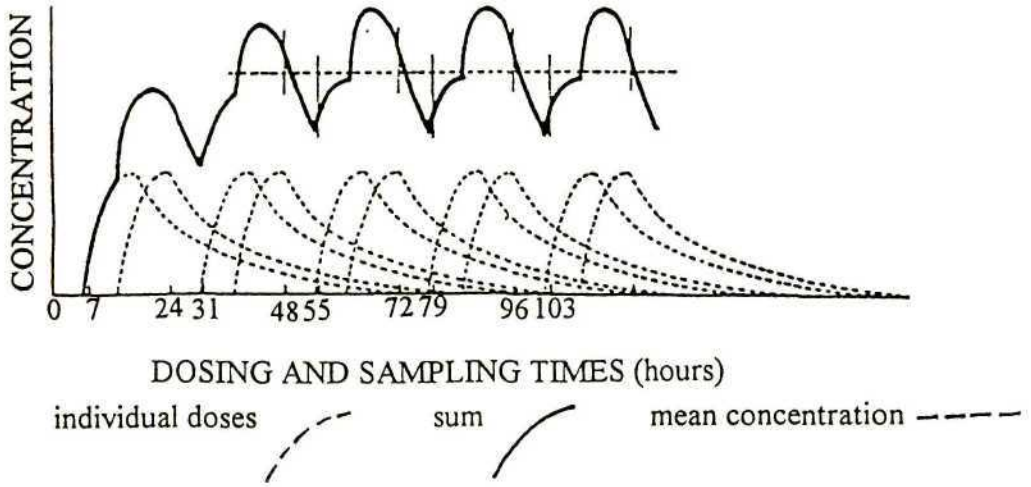

Figure 1.02 Likely excretion patterns for a 7 and 17 hour interval dosing and sampling program (Lambourne 1957).

Since the accuracy of intake estimates is limited to a greater extent by the accuracy of digestibility than faecal output measurements (Gordon 1995), an error of approximately 10% should be satisfactory. Parker *et al.* (1990) also felt that it is unlikely that reliable estimates of intake for individual animals will be possible because of the difficulty in estimating the digestibility of the diet of the animal.

The chromic oxide marker technique was revised in an attempt to reduce this diurnal variation in marker excretion. It was thought that by making the passage of the marker through the gut more regular, which would result from more even mixing with the contents of the reticulo-rumen, the sampling technique would become more reliable (Corbett *et al.* 1960). Paper impregnated with Cr_2O_3 was investigated and was found to achieve this. Langlands *et al.* (1963) also found that the paper yielded better estimates of faecal output than capsules. Although it did not eliminate diurnal variation and still did not allow for a once daily dosing and sampling regime, it did yield more stable errors of estimates. It is unlikely that any technique can totally eliminate this variation since there is diurnal variation in forage intake and faecal output (Minson 1990). The mean bias of any of these techniques can be determined by harnessing a subsample of animals for total collection (Langlands *et al.* 1963; Hollingsworth *et al.*1995).

Further revision of the marker technique lead to the development of controlled-release devices (CRDs). It was thought that these might simplify the marker dilution method for calculation of faeces output (Corbett 1980). These devices consist of a plastic barrel containing a series of matrix tablets holding Cr_2O_3. A plastic plunger and a compressed spring within the barrel force the matrix, which forms a matrix on exposure to rumen contents, out of the orifice at the end of the barrel. Controlled-release devices are designed to deliver Cr_2O_3 at a constant, predictable rate over a specified time period (Parker *et al.* 1990). This method of administering Cr_2O_3 has been found to reduce diurnal variation to approximately one third of the level achieved by twice daily dosing with gelatin capsules (Ellis *et al.* 1981 cited by Parker *et al.* 1990). This confirms the work of Lambourne (1957) and Corbett *et al.* (1960) who showed that more frequent dosing and an increase in the length of time that the Cr_2O_3 is retained in the rumen reduces the variation in the rates of faecal chromium excretion. The reduced within-day variation allows for a more flexible sampling program (Parker *et al.* 1990). Once-only administration and less frequent grab

sampling reduces the amount of animal disturbance and the reduction in handling allows for use of more experimental animals (Costigan & Ellis 1990) especially on extensive or difficult terrain. Uniform release rates and accurate specified time to expiry are requirements of a controlled release device, however a specified release rate cannot be expected to apply under all conditions because some feeds create a more abrasive rumen environment which releases more marker from the device. Feeds creating an abrasive rumen environment may increase release rates by 2 to 6% (Parker *et al.* 1989). Luginbuhl *et al.* (1994) also found that the type of diet affected the rate of release of marker from the controlled release capsules. It has been suggested that total collection on a subset of animals can be implemented to correct estimates derived by the marker device for each forage source (Hollingsworth *et al.* 1995). Alternatively, specified release rates can be corrected by determining the number of days to expiry in order to eliminate error introduced by variation in period over which marker is excreted (Parker *et al.* 1990). Luginbuhl *et al.* (1994), however, concluded that controlled-release capsules should be used with caution in research. Doyle *et al.* (1994) concluded from their study that controlled-release devices give reasonable estimates of faecal output for groups of animals, but felt that total collection might in fact be easier and more accurate. Santos & Petit (1996) evaluating Captec controlled release devices found that although the release rate differed quite substantially from that specified by the manufacture, but found overall that the devices produced reasonable estimates of intake ($r = 0.97$; $P = 0.03$). They did admit that the type of feed affects the accuracy of estimates considerably, the CRDs being unsatisfactory for use with subtropical forages (Luginbuhl *et al.* 1994) or pelleted diets (Hatfield *et al.* 1991). It was Lambourne (1957) who said that a technique for predicting feed intake should be satisfactory over a wide range of feed intakes and feed quality since individuals vary both in appetite and selectivity. It would appear that the use of controlled release devices does not allow for this.

According to Buntinx *et al.* (1990), controlled release capsules do not reduce diurnal variation sufficiently to allow once daily grab sampling to accurately predict faecal output. Thus if animals need to be handled twice daily anyway, then this method may not be much of an improvement over twice daily dosing of capsules. Luginbuhl *et al.* (1994) found that varied sampling schemes did not overcome the deficiencies of the CRDs. Momont *et al.* (1991) however, concluded that once daily grab sampling for a five day period was in fact adequate (supported by Brandberry *et*

al. 1991; Santos & Petit 1996) and also reported that no substantial differences in release rate were found across the levels of forage DM intake and supplementation schemes used.

Many of the benefits of the controlled release devices were not of use in the current trial since the goats were brought inside every evening so that dosing and sampling twice daily was satisfactory. The variation in success achieved with the controlled release devices lead to the decision to use the gelatin capsules in the current trial.

1.2.1.1.2 Rare earth elements

The rare earth elements have received attention as markers for use in digestibility-related studies. They have been said to possess strong adsorption properties for particulate matter (Ellis & Huston 1968) which could minimise the diurnal variations associated with the use of discreet doses of marker. This is not in agreement with Prigge *et al.* (1981) who found no appreciable decrease in diurnal variation associated with the use of ytterbium chloride compared with Cr_2O_3. Teeter *et al.* (1984) also reported that there had been diurnal variation in the excretion of the marker but it was thought to be due to changes in the number of binding sites which occurred with changes in forage maturity. Krysl *et al.* (1985) used compartmental modelling techniques applied to faecal marker excretion curves from a pulse dose of rare earth markers to determine faecal output. Although results were favourable, there are limited data available regarding the validity of using this technique under free-ranging conditions (Krysl *et al.* 1988). Ytterbium (Yb) has been found to overestimate faecal output and also organic feed intake by as much as 40% (Musimba *et al.* 1987). It was concluded by Krysl *et al.* (1988) that since the ytterbium-labelled forage and dysprosium-labelled faeces gave such variable results under different dietary conditions, they may not offer any advantage over more widely used markers such as chromic oxide. Another factor, against their use, is that the detection of the rare earth elements, with the exception of Yb, requires specialised, and therefore expensive, analyses.

Estell *et al.* (1990) used a controlled release Yb bolus and found that irrespective of the method of calculating the daily dose, or the faecal sampling method used, estimates were variable and greater than total collection values. Thus it would appear that there is no great advantage in using

a rare earth element rather than chromic oxide as a marker for determining faecal output.

1.2.1.2 Determining the digestibility of the diet

Grazing or browsing animals have the opportunity to select a diet of different quality to that of the total vegetation available (Mayes *et al.* 1995). Generally, the accuracy of estimates of intake is limited to a greater extent by the accuracy of determining digestibility of the diet than determining faecal output. Digestibility of the feed consumed can be determined using either *in vivo* or *in vitro* methods.

1.2.1.2.1 *In vivo* methods

In vivo methods involve the live animal and provide a standard against which the accuracy of indirect methods can be assessed. The apparent digestibility of forage is the proportional difference between the quantities consumed and excreted in faeces. During a digestibility trial, the animals are housed in metabolic crates for ease of handling and collections and forage is given in exact amounts for a period long enough to allow a "steady state" of faecal excretion to be reached, and then faeces excreted during a measured interval of time are collected (Minson 1990).

Another *in vivo* method of determining herbage digestibility which is available, requires rumen fistulation. Small nylon bags containing a sample of feed are placed into the rumen through a fistula. They are then collected in the faeces. The digestibility of the feed can then be calculated from the undigested residue in the bag (Minson 1983).

For many years, internal markers (markers inherently present in the herbage) have been used to determine digestibility according to the equation:

$$Digestibility = 1 - \frac{herbage\ marker\ concentration}{faecal\ marker\ concentration} \qquad \text{----------(1.3)}$$

Lignin, silica, indigestible acid-detergent fibre and acid insoluble ash are internal markers which have been used (Gordon 1995). However, because they are not discreet chemical entities, what is measured in the faeces, may not be the same substance as that in the diet. Results are good in some situations but poor in others (Dove & Coombe 1992 cited by Mayes *et al.* 1995).

Corwin & Forbes (1951) investigated the feasibility of using added dye as a reference material for determining the digestibility of a ration fed to lambs. Anthraquinone violet was found to be a suitable dye. Recovery in faeces was satisfactory (96.4 to 106.0 %). Diurnal variation in excretion was recorded, with noon faecal collections containing a greater dye concentration than either the night or morning collections (animals dosed twice daily with gelatin capsules). Thus a large number of samples collected at different times throughout the day would be necessary and this is not suitable for field conditions.

1.2.1.2.2 *In vitro* methods

In vitro methods of determining digestibility were developed since internal markers were found not to be adequate for all situations. These techniques involve incubation of a sample of forage in buffered rumen liquor, followed by acid pepsin digestion (Tilley & Terry 1963). Generally reliable results are obtained, however there are a number of disadvantages. *In vitro* methods do not allow for variation between animals in terms both of diet selected and rely on the assumption that digestibility is unaffected by level of intake or the feeding of supplements (Mayes *et al.* 1995). It is also difficult to obtain a sample which is representative of that selected by the animals. Oesophageally fistulated animals have been used to obtain samples for estimation of the digestibility of the diet (Forbes & Beattie 1987).

1.2.2 Faecal nitrogen as an index of intake

The relation between the digestibility of feed organic matter and the concentration of nitrogen in faecal organic matter has been used quite widely to provide regression equations (Arnold & Dudzinsky 1963). Lancaster (1949) cited by Arnold & Dudzinski (1963) found that over a range of forages, the amount of faecal nitrogen excreted per unit of feed intake was relatively constant.

He suggested that the relation might be used to estimate the intake of pasture. Faecal nitrogen (FN) has been used extensively on single species pastures, but requires calibration for different sward types (eg. multi-species pastures such as veld), species of animal and animals of different physiological state. Both *in vitro* and faecal indices suffer from the fact that on high quality pastures, errors on estimates of digestibility can be multiplied three to five times when estimating intake (Gordon 1995). It would seem that the equations would have to be altered according to the physiological state of the animals and the stage of maturity of the forage. In work done by Birrell (1980), it was confirmed that FN indices based on feeding trials of green herbage are unreliable for predicting digestibility of green herbage in the field. The FN technique is known to overestimate digestibility when available herbage is sparse, and underestimate it when available forage is plentiful (Langlands 1969).

1.2.3 Predicting intake by means of rumen evacuation

Mendoza *et al.* (1995) attempted to predict intake from the amount of indigestible fibre contributing to ruminal contents. A model which assumed that the intake and passage of indigestible fraction digesta are in a steady state was utilised. Following an adaptation period, ruminal contents were removed via a rumen cannula, mixed and a sample analysed for indigestible acid detergent fibre (I-ADF). Predicted values were found to under estimate feed intake and the conclusion of the study was that intake cannot be predicted from the I-ADF ruminal pool.

1.2.4 The double alkane method

The use of alkane signatures involves the use of both an internal and external marker. The cuticular waxes of forage plants contain long-chain n-alkanes with odd carbon chain lengths in the range C_{25} to C_{35}, which are relatively indigestible, and can be used as internal markers. The predominance of odd-chain alkanes in herbage and the fact that even-chain alkanes of similar chain length are relatively inexpensive, lead to the development of the dual-marker technique for estimating herbage intake (Mayes *et al.* 1986a). This technique, in contrast to the widely used chromic oxide technique, does not require an independent assessment of digestibility. Few of the methods for measuring the coefficient of dry matter digestibility make allowances for differences

in digestive efficiency caused by levels of feeding, parasites or age and species of the animal (Minson 1990) thus a technique which does not require determination of digestibility would be of great benefit and would allow for factors affecting the digestibility of the consumed forage (Dove *et al.* 1989).

Rather than using faecal levels of a natural alkane to estimate herbage digestibility and dosed alkanes to estimate faecal output, an adjacent pair of alkanes (1 dosed, 1 naturally occurring) can be used to calculate herbage intake. When a mixed diet (roughage and concentrates) is involved, and the concentrates offered also contain alkanes, the following equation is used to calculate herbage intake (Mayes *et al.* 1986a):

$$\textit{Herbage intake } (kg\ DM\ d^{-1}) = \frac{(F_i/F_j)(D_j + I_c * C_j) - I_c * C_i}{H_i - (F_i/F_j) * H_j} \qquad \text{----------(1.4)}$$

Where

F_i, H_i are faecal and herbage concentrations of the odd-chain alkanes;

F_j, H_j are faecal and herbage concentrations of the even-chain alkanes;

D_j is the daily dose of even-chain alkane;

I_c is the daily intake of concentrates;

C_i is concentration of odd-chain alkanes in concentrates; and

C_j is concentration of even-chain alkanes in concentrates.

The incomplete recoveries of naturally occurring alkanes (due to absorption of hydrocarbons from the digestive tract) leads to an underestimation of dry matter digestibility, but this error is overcome if daily faecal production is measured by feeding a known quantity of an even-chain alkane of similar recovery as the natural alkane, as in this case the incomplete recoveries cancel out. Thus the alkane technique also does not rely on the quantitative recovery of the dosed alkane used as the external marker (Vulich *et al.* 1991).

From the work of Mayes *et al.* (1986b) it was concluded that C_{32} as the dosed alkane and C_{33} as the internal marker, provided best estimates of herbage intake since the recovery increases with increasing alkane chain length and C_{32} is present in herbage at fairly low concentrations.

In common with external markers such as chromic oxide (Langlands 1975), diurnal variations in faecal excretion of alkanes could increase errors in herbage intake estimation as the composition of faecal grab samples is assumed to be representative of that of the total faecal output over the collection period. Diurnal variation can result from diurnal variation in intake, from the marker dosing scheme and from events in the digestive tract. It would appear that at least six days dosing is required before commencing faecal sampling (Mayes *et al.* 1986a). This external marker may be fed each day or released from a sustained release capsule in the rumen (Laredo *et al.* 1991).

The results of the experiments carried out by Vulich *et al.* (1991) comparing two methods of dosing (gelatin capsules versus impregnated paper pellets) support the results reported by Mayes *et al.* (1986a), that the n-alkane technique can provide an accurate and precise estimate of herbage intake. The general validity of the technique was also supported by the estimates of repeatability determined, as no significant differences were detected between the repeatability estimates of actual intake for any of the herbage intake estimators (alkane pair combinations). According to Vulich *et al.* (1991), the method for preparation and administration of n-alkanes needs to be simplified to facilitate large scale experimentation. Recent results (Dove & Mayes 1991) indicate that the application of intra-ruminal slow-release technology for delivering the dosed alkanes will result in even greater accuracy. This development will improve the usefulness of the technique in free-range applications.

1.2.5 Purine derivative technique for predicting feed intake

This is a technique that utilises a urinary metabolite as an index of feed intake.The major advantage of this technique is that, as for the dual alkane method, it does not require a digestibility value.

Ruminal microbes form the main source of protein to the ruminant (Chen *et al.* 1992b). Organic matter (OM) consumed by the ruminant undergoes ruminal fermentation which is coupled proportionally with the synthesis of microbial protein (Osuji *et al.* 1993) so that the output of microbial protein from the rumen is said to provide a measure of the amount of rumen-fermentable organic matter consumed by the animal. The nucleic content of microbial mass is

correlated with that of microbial protein (Chen *et al.* 1992a). Purines are a group of simple nitrogenous organic molecules including adenine and guanine which are constituents of nucleic acids (Tootill 1980). Thus the excretion of purine derivatives (PD), the end product of nucleic acid catabolism, should be correlated with the amount of microbial protein produced. Purine components of nucleic acids (NA) are absorbed from the small intestine, they enter the body pool, are metabolised and excreted in the urine mainly as allantoin ($C_4H_6N_4O_3$) (Antoniewicz *et al.* 1980). Microbial protein supply estimated from PD excretion refers to the amount entering the small intestine (Chen *et al.* 1992c).

Of the purine nitrogen (N) excreted in urine, 70 to 90% is in the form of allantoin (Morris & Ray 1939 cited by Lindberg 1985). Allantoin (5-ureidohydantoin) is the end product of purine metabolism in mammals and results from the oxidation of uric acid (Lewis 1993). The overall conversion of nucleic acid-N into allantoin-N is affected by nucleic acid digestibility, rate of purine base uptake and the extent of their catabolism (Antoniewicz *et al.* 1980). Chen *et al.* (1992a) working with steers, found that allantoin was a constant fraction of total urinary PD excreted.

Therefore the excretion of purine derivatives in the urine provides a measure of the supply of microbial nucleic acids, and thus could be a possible indicator of microbial protein production and indirectly the amount of organic matter consumed since, according to Osuji *et al.* (1993) the intake of rumen-fermentable OM can be predicted given the daily ruminal output of Microbial N.

Although purine derivatives are mainly derived from the internal digestion and absorption of rumen microbial nucleic acids (due to the large proportion of nucleic acid in microbial material), changes in the levels of endogenous allantoin excretion due to the nutritional and physiological status of the animal can introduce error (Antoniewicz *et al.* 1980). Daily excretion is directly related to the daily amount of purines absorbed (Chen *et al.* 1992a). If microbial supply is to be calculated from PD excretion, it is necessary to assume that the ratio of purine-N : total-N in mixed microbial cells is constant and this requires validation.

Han *et al.* (1992) predicted allantoin-N from the ratio of allantoin : creatinine (A/C) in the urine, the average creatinine excretion and the live weight of the animal. It was found that predictions

scatter closely around the line of unity so it should be possible to use spot samples. According to Balcells *et al.* (1991), the use of urinary PD as an index of net microbial synthesis requires a better understanding of the recovery of purines as urinary catabolites, and the contribution of the endogenous fraction to total urinary losses. Balcells *et al.* (1991) did, however, report that under most practical conditions, microbial purines will exceed the requirements for endogenous losses and thus urinary allantoin could constitute a suitable index to estimate, accurately, microbial production.

According to Chen *et al.* (1992b), although the daily excretion of PD is largely determined by the absorption of microbial purines, the relation is not linear because some of the absorbed purines are utilised by the animal's tissues, thus replacing *de novo* purine synthesis which reduces the net endogenous contribution to total excretion. Nutrient supply determines the extent of *de novo* synthesis and the salvage of nucleic acids. It is possible that when the protein in the diet exceeds the requirements of the animal, a smaller proportion of the microbial nucleic acid derivatives is used by the animal tissues for nucleic acid synthesis (Puchala & Kulasek 1992).

The urinary concentration of allantoin, as with all other substances excreted in urine, varies according to the volume of urine produced, making it difficult to quantify. The concentration is a function of dietary and environmental factors. It is therefore necessary to express it as a ratio of an independent substance (Chen *et al.* 1992a). Creatinine, another substance excreted in urine, is a product of the breakdown of phosphocreatinine which is the form in which energy is stored in muscle. It is converted to creatinine at a fairly constant rate and distributed throughout the body water when energy is needed (Finco 1980, cited by Lindberg 1985). Creatinine is excreted in proportion to the live weight of the animal within a wide range of body weights (Brody 1964). Daily excretion is constant and Chen *et al.* (1992a) found that the concentration of creatinine in urine showed similar changes in time to those seen for PD so that when allantoin was expressed as molar proportions of creatinine in the same sample, the influence of time was effectively reduced. Ratios were found to be almost constant over the day even for once daily feeding regimes. This should make it possible to use a spot-sampling technique under field conditions (Chen *et al.* 1992a). According to Mayes *et al.* (1995), this method gives larger errors than total urine collection methods.

The disadvantage of using the allantoin : creatinine (A/C) ratio is the possible variability in creatinine excretion (between animals and between days) which would add to the variability of the ratio (Chen *et al.* 1992a). It was found that variation in the ratio was reduced when a twice daily feeding regime was used instead of once daily, but the variation was always quantatively small. This does mean that under *ad lib* feeding conditions, diurnal variation would be even less.

Han *et al.* (1992) stated that it should be possible to predict urinary PD excretion and indirectly rumen microbial protein production from the PD/creatinine ratio in spot samples. It was also found that the excretion of PD (both allantoin and total PD) was linearly related to the intake of digestible organic matter (DOM) within a range of 718 to 1060 g DOM per day. The regression obtained from their study was Allantoin-N = 1.205±0.07* DOM - 136.709±37.399 ($n = 16$, $p < 0.0001$, rsd = 22.97). It was found that recalculation on the basis of metabolic mass did improve the correlation. This is not in agreement with the work of Chen *et al* (1992a) who defined A/C ratio as [allantoin (mmol l^{-1}) / creatinine (mmol l^{-1}) * LW $^{0.75}$ (kg)], making this correction to facilitate comparisons between animals based on metabolic mass as well as between periods.

Chen *et al.* (1992a) reported that the magnitude of the least significant differences indicates that the use of PD in spot samples as an index of microbial supply is only suitable for detecting relatively large differences. It was also found that for single-time measurements, a better estimate of the daily mean A/C ratio can be obtained using the mean of samples taken at the same time on consecutive days than the mean of several samples taken at different times within the same day.

In the work done by Lindberg (1985) with milk goats weighing 35.5 to 58.5 kg, when daily allantoin excretion was predicted from a spot sample according to equation (3.1), allantoin was found to be linearly related to intake of digestible organic matter (DOM) according to the equation:

Allantoin = 3.40 DOM - 442.75 ($n=67$, $r=0.83$, $cv=14.76$, $P<0.0001$).

$$PD = LW * C_{LW} * \frac{PD}{C} \qquad \text{----------(3.1)}$$

where

LW is live weight in kg;

C_{LW} is creatinine excretion in mg kg^{-1} LW (11.0±0.20 mg kg^{-1} being average value); and

PD/C is the ratio of PD to creatinine in the urine (both measured in mg/100ml).

In work done by (Chen *et al.* 1992b) with sheep, it was found that the supply of microbial protein is not solely determined by the amount of feed consumed because the efficiency of conversion is not constant. Excretion of PD per unit of intake was found to increase with higher levels of intake. The physical bulk of the feed relative to rumen fill can result in a 2 to 3 fold difference in the microbial protein supply per unit of diet as a result of differences in digesta passage rates. Thus increasing intake by making use of a supplement will increase microbial protein outflow. The supply of microbial protein to sheep was found to also be affected by the physical bulk of the feed since conditions resulting in low digesta passage rates, due to low intake levels will result in an increase in the outflow of microbial protein if bulk is increased with roughage. It has also been found that the type of diet fed can affect the rate of microbial protein synthesis estimated using the microbial nucleic acid method because of the differences in the N concentration and metabolisable energy. A high protein, high energy diet was found to give the highest rate of synthesis while the low protein, low energy diet gave the lowest (Puchala & Kulasek 1992). Allantoin excretion in sheep was found to be reduced when they were fed a diet with a high concentration of structural carbohydrates. This could be due to the reduced digestibility associated with increased passage rate. It has also been found that for animals of different masses receiving the same amount of feed, allantoin excretion is inversely related to live weight (Chen *et al.* 1992b). According to Mayes *et al.* (1995), a simple relation between PD excretion and digestible organic matter intake cannot be assumed since large differences in the efficiency of microbial synthesis can occur for fresh herbage, hay and silage (Minson 1990). The synthesis of microbial crude protein is energy dependent and the efficiency of conversion of dietary to microbial crude protein depends on the rate of energy release compared with that of amino acids

and ammonia during forage degradation in the rumen (Hogan 1982 cited by Minson 1990). It was concluded by Mayes *et al.* (1995) that PD excretion can only be used as an index of feed intake if specific relations are established for particular ruminants ingesting particular diets. This was contradicted by Lindberg (1985) who found that there were no significant differences in urinary allantoin excretion due to the diets fed . In this case diets consisted of roughage and concentrate which was either unsupplemented or supplemented with urea, fish meal or soy-bean meal.

Balcells *et al.* (1991) reported that since Chen et al. (1990) had recorded variation in plasma allantoin concentration (which is not subjected to the variation in concentration as is urinary allantoin) the use of allantoin : creatinine ratio in spot samples should be considered with caution. It was however acknowledged that the results of Antoniewicz *et al.* (1980) suggest that this variability is not reflected in the urinary concentrations.

The PD technique appeared to have much potential for estimating intake despite these limitations that were identified.

1.3 Objectives of the study

The main objective of the study was to evaluate methods of measuring feed intake that could be used under conditions where animals are free-ranging on a leucaena/veld pasture.

1.4 Justification for selection of which techniques to be evaluated

Since goats grazing any pasture other than a single-species pasture will be able to feed selectively, a method of estimating intake which does not require determination of digestibility would be most satisfactory. Should a conventional method be adopted, it would be necessary to apply some average digestibility figure to all individuals in the group. The animals have the freedom to select their level of intake and dietary composition, and apart from plant species, they can also select different plant parts which may affect the digestibility of the diet.

The double alkane method seems to overcome this problem, however it is not suitable for use with leucaena. A minimum of 50 mg alkane kg^{-1} DM is necessary for this type of study (Laredo *et al.* 1991), however, leucaena leaves contain very low levels of alkanes. Investigations carried out during my study supported the literature. The most abundant odd-chain alkane (C_{29}) was found to be present at approximately 40.5 mg kg^{-1} DM. It is likely that the alkane profile for the leucaena would have been masked by that of other plant species contributing to the diet of the goats. The double alkane technique was therefore discarded.

In a study conducted by Birrell (1980), it was found that faecal nitrogen indices based on pen feeding trials of green herbage are unreliable for predicting digestibility of green herbage in the field. It has been found that for any particular pasture, intake is related to FN in a significant manner, however in a multi-species pasture, the animals are able to select varying diets and this relation is known to not be a constant (Fels *et al.* (1959) cited by Arnold & Dudzinski (1963)). Thus it would be difficult to make comparisons between animals, even though they may be grazing the same pasture. Techniques for determining digestibility which require fistulation and cannulation were avoided since there is a possibility that these animals behave differently to those which have not been operated on. The rumen evacuation technique requires rumen cannulation, and these animals' grazing habitats will be affected by emptying of the rumen. Thus it was decided that this technique was also unsuitable for the current study.

The purine derivative (PD) method seemed the most suitable method of measuring intake under the prevailing conditions since it did not require a measure of digestibility, nor did it require surgical preparation of the animals. A number of researchers reported very satisfactory results using this technique (eg. Lindberg 1985, Osuji *et al.* 1993).

The conventional marker technique for estimating intake was also evaluated under the current set of conditions. Although a number of shortfalls have been identified with this technique, it is still frequently-used.

1.5 Thought process behind design

It was decided that the techniques had to be evaluated under controlled conditions. In order to use the purine derivative technique it was necessary to establish regressions of intake against urinary allantoin concentration to use under field conditions. Initially it was intended to obtain 24 hour urine collections so that total allantoin excretion (or average daily allantoin excretion) could be regressed against feed intake. When difficulties in making these 24 hour collections arose and the situation was reviewed, it was decided that a spot-sampling technique might in fact be more useful. Under free range conditions, it would be impracticable to obtain total urine collections, so a spot-sampling technique would have to be adopted. It would therefore be more appropriate to develop regression equations using allantoin concentrations in spot samples than average daily allantoin excretion.

Since it was not always possible to collect spot samples from all the goats at the same time and since it was thought that diurnal excretory patterns might vary between goats, the need arose for the metabolic crate trial in order to establish whether the time of collection did in fact affect urinary purine derivative concentration. The metabolic crate allowed for far more controlled conditions than occurred in the pens.

With each run conducted in the pens, another ten goats were selected randomly from the flock such that none of the goats were used in more than one run. Since the goats were all similar in age, all having been weaned together, the average live mass increased from one run to the next. The prerun was conducted in December, the first run in January, the second run in April and the third run in July.

The evaluation of the conventional marker method using chromic oxide for estimating feed intake was conducted concurrently with that of the PD technique. This method is known to be satisfactory, although it is accepted that some error in the estimation of faecal output is inevitable (Lambourne 1957; Vogel *et al.* 1985, cited by Redmon *et al.* 1995).

1.6 Location of experiment

The study was undertaken at the Ukulinga Research Farm (URF) outside Pietermaritzburg (30°24'S, 29°24'E; 700 m a.s.l.), KwaZulu-Natal, South Africa. Mean annual maximum and minimum temperatures are 25.7 and 8.9°C respectively. Light to moderate frosts occur occasionally in winter. The pens were situated in an open-sided shed, while the metabolic crates were housed within the sheep-shearing shed.

CHAPTER 2

Evaluation of the purine derivative technique

2.1 Technique investigated in pens

2.1.1 *Objective*

To develop regression equations describing the relation between feed intake and the ratio of allantoin to creatinine in spot samples of urine in order to allow the prediction of intake from a spot urine sample of a grazing animal.

2.1.2 Method and materials

2.1.2.1 Prerun to the evaluation of the purine derivative technique

At the start of the experiment a prerun was conducted to establish the most practical method of evaluating the purine derivative technique for predicting feed intake under the current conditions. Ten young male Boer goats with average mass 21.1 ± 1.997 kg were randomly allocated to 5 feeding levels of a 25% leucaena : 75% hay diet . These levels were selected to cover a range from restricted intake to *ad lib* intake, while meeting maintenance requirements. In order to simulate more natural conditions than can be obtained in metabolic crates, the goats were housed on the ground in pens approximately 1 by 2 metres in size. The two components of the diet were fed in separate bins and fresh water was freely available.

A preliminary adaptation period of 2 weeks preceded the 4 day collection period. During this time the animals adjusted to frequent handling and confinement in pens. They received their daily feed allowance during this period so that the excretion patterns of urinary substances would have stabilised by the start of the collection period. During the prerun the total feed allocation was given at the 07h30 feed.

I originally intended to measure total daily allantoin excretion by collecting all urine produced in a 24 hour period and then analyse a subsample. The apparatus for urine collection consisted of a piece of tyre tube secured around the animal's belly and a length of flexible tubing attached via a tractor tube valve to a urine collection bag. The animals were not in raised pens so the urine did not drain freely into the collection bags, but remained in the tubes, some running back into the tubes when the animals were lying down. It was thus necessary to adopt a collection procedure that did not involve acid preservation since this might have burnt the goats and caused urinary tract infections. The collection apparatus proved to be unsatisfactory in the pens where the goats had freedom of movement and total urine collection was difficult because the apparatus did not remain in place. On the occasions that I lost a collection, I analysed a spot urine sample. Samples were diluted 1 in 5 with clean water to prevent the precipitation of uric acid. I was unable to analyse samples on the day of collection so samples were frozen (Puchala & Kulasek 1992) until analysis, which occurred within a week. Urine samples were analysed for allantoin and creatinine. Both analyses involved spectrophotometry, see appendices 1.01 and 1.02 for techniques).

Since
daily allantoin excretion directly related to feed intake; and
daily allantoin excretion = LW * C_{LW} *A/C;
now assuming C_{LW} is constant for this study
(C_{LW} being creatinine excretion (mg kg^{-1} LW)); then
Daily allantoin excretion is directly related to LW * A/C which is directly related to feed intake; therefore,
feed intake/ LW is directly related to A/C
and if LW is expressed as metabolic mass, then
Intake / metabolic mass (I_mmass) is directly related to A/C

This relation then needed to be defined.

Orts (g d^{-1} on a dry matter (DM) basis) were collected daily to allow for calculation of intake (g d^{-1} on a dry matter basis). Subsamples of feed and orts were dried in the oven at 60 °C for 48 hours to determine percentage DM. Since intake by grazing animals must vary with some

function of body weight, a good criterion for expressing intake might be its relation to maintenance in order to adjust for differences among animals (Moore & Mott 1973, cited by Cordova *et al.* 1978). The expression of intake (g d^{-1} on a dry matter basis) per unit metabolic mass ($LW^{0.75}$) seems adequate for most situations (Cordova *et al.* 1978). Following laboratory analysis, intake (I_mmass) was plotted against average A/C ratio for each goat. The correlations between intake and A/C ratio were not improved by expressing feed intake as an absolute daily intake (g d^{-1}). This was investigated in case it was found to produce better regressions than those obtained when intake was expressed relative to metabolic mass.

Linear regressions were investigated using the advanced regression facility of Quattro Pro 6.01 for Windows. The Genstat 5 Statistical Package (release 3.1 Copyright 1993, Lawes Agricultural Trust, Rothamsted Experimental Station) was used for all other statistical analyses.

2.1.2.2 First phase of the evaluation

As for the prerun, the first run was conducted in the loose pens. The ten goats with an average live weight of 24.95± 2.35 kg were randomly allocated to the five feeding levels fed their daily allowance in two meals at 07h30 and 15h30. The tyre apparatus used in the prerun was discarded and a simpler method was sought to obtain the spot samples. Plastic bags tied around the goats' bellies just prior to feeding times proved satisfactory. When urine was produced (this was normally achieved for all animals within 90 minutes) it was immediately removed, acidified (ph<3) and frozen for later analysis. During this run the animals were also dosed with chromic oxide capsules so that the external marker technique could also be evaluated. The method was as for the prerun apart from the mode of collection of the urine. (Note: as an inert marker, Cr_2O_3 does not impact on the purine production of the rumen bacteria)

2.1.2.3 Second phase of the evaluation

This evaluation was conducted in January using the modified method used in the first run . The new set of goats used for this run had an average live mass of 32.49 ±1.23 kg and were randomly allocated to the five feeding levels.

2.1.2.4 Third phase of the evaluation

The ten goats used in this run had an average live mass of 34.52±3.37 kg. The method was as for run 2 apart from the storage and time of analysis of the samples. I attempted to improve upon the results obtained for the first two evaluations of the purine derivative technique by modifying the storage procedures. Every effort was made to analyse the samples on the day of collection so that samples had to merely be refrigerated rather than frozen following acidification and dilution.

*See appendix 4 for data for each of the runs

2.1.3 Results and discussion

2.1.3.1 Prerun

When I_mmass was plotted against the allantoin : creatinine (A/C) ratio (Figure 2.01), no trends were apparent. According to the literature, a positive linear correlation should have existed but none was found here (Appendix 2.01). There was no apparent correlation ($P \geq 0.05$). Although the prerun produced poor results, it did achieve its purpose. That being to establish problem areas and find means of overcoming these problems.

Inadequate preservation of urine probably accounts for the poor results obtained for the prerun. Since I wished to avoid using acid, and did not collect samples immediately, I should perhaps have employed some other technique such as keeping the collection container in ice in order to prevent deterioration of the sample through bacterial action. Han *et al.* (1992) collected urine into a vinyl bag submerged in ice and analysed urine daily. By being inconsistent in my sampling strategy, samples were subjected to different lengths of time at air temperature prior to refrigeration and possibly to varying degrees of bacterial deterioration. The spot sampling technique which I employed in subsequent runs did overcome this problem since the samples were collected promptly and refrigerated. The method of feeding was also a source of error since feeding the whole daily allowance at 07h30 resulted not only in a wastage of feed through soiling, but those goats on the lower feeding levels had finished their feed by 12h00, while those on the higher feeding levels fed throughout the day . This might have introduced error into the results if the different feeding patterns had affected the PD excretion curves of the goats differently (however, according to Chen *et al.* 1992a, the effect of feeding frequency on diurnal variation in urinary purine derivatives is very slight). Another problem encountered during the prerun was the occurrence of diarrhea in goats 9 and 10 which were treated with sulmethotrim. This drug acts by affecting the purine synthesis of the bacteria causing the infection. This could possibly have affected normally-occurring rumen bacteria too, which would have affected the efficiency of microbial protein production.

The laboratory analysis for allantoin concentration (Appendix 1.01) is not complicated however

it does require specific attention to time period between stages. At the time of the prerun, I was unable to obtain consistent results from repeats. Generally it was found that the first run of analyses needed to be repeated since the dilutions were often incorrect and the results fell outside of the standard curve. I could manage one more run within the day, but further repetition required overnight storage of samples which may have lead to the poor repeatability because, according to the literature, it would appear that urine is highly sensitive to storage methods.

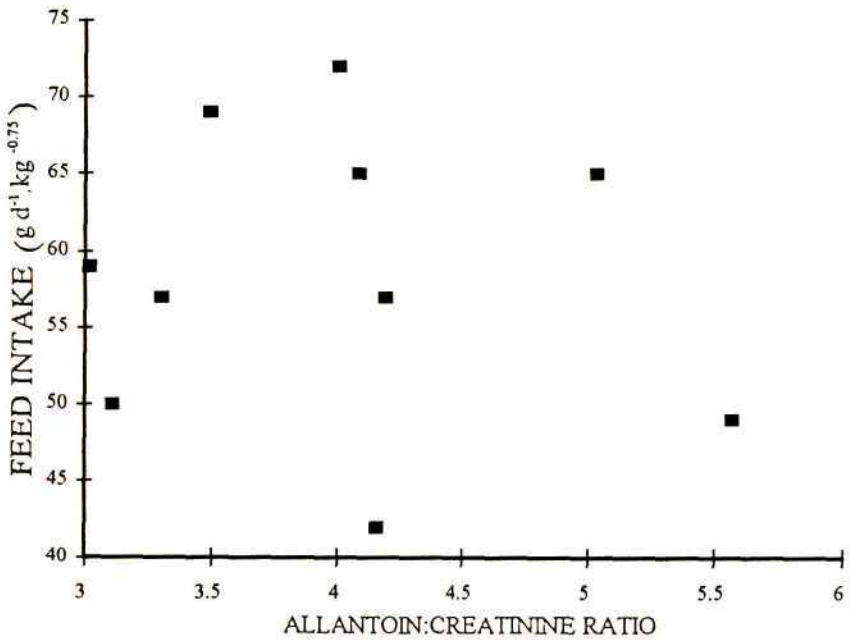

Figure 2.01 Average intake (expressed relative to metabolic mass) plotted against average urinary allantoin : creatinine ratio in spot samples collected from the ten goats during the prerun.

2.1.3.2 First evaluation

When I_mmass was plotted against the A/C ratio averaged over the collection period for the first run (Figure 2.02), a positive correlation was identified and the relation was found to be significant ($p \leq 0.001$; Appendix 2.02). The regression equation obtained for the first run from the statistical analysis is:

$$I\_mmass = 12.637 * A/C \text{ ratio} + 15.383$$

($P \leq 0.001$, $r = 0.864$, $R^2_A = 0.715$, $SE = 5.910$, $n = 10$)

On the basis of the results from this evaluation of the PD technique, it would seem that intake can be reliably predicted from the average allantoin : creatinine ratio of spot samples of urine collected once daily on four consecutive days.

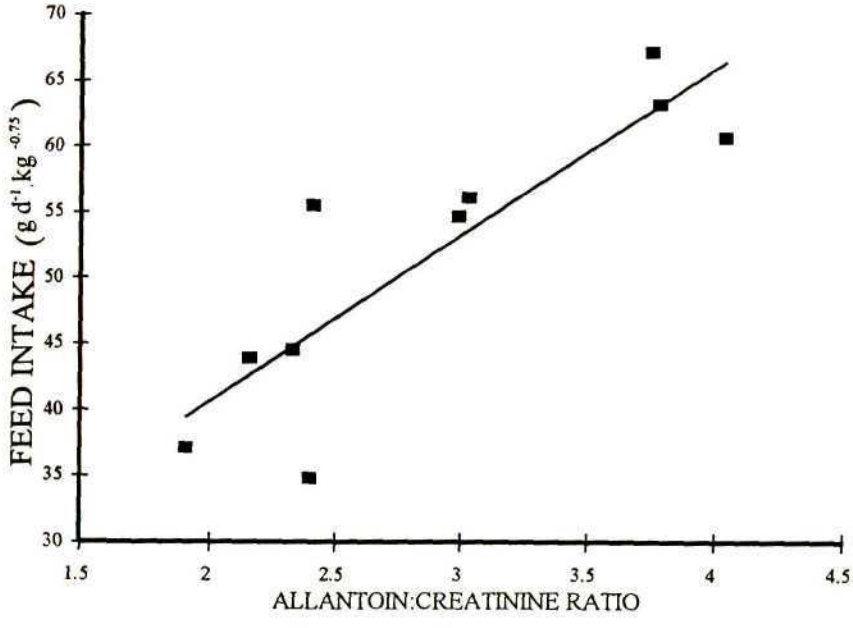

Figure 2.02 Average intake (expressed relative to metabolic mass) plotted against the average allantoin : creatinine ratio of spot urine samples collected from the ten goats during the first evaluation of the purine derivative technique. Solid line depicting the predicted best-fit regression equation.

2.1.3.3 Second run to evaluate the purine derivative technique

Initially when intake (I_mmass) was regressed against the A/C ratio (Appendix 2.03) There was no significant correlation (P>0.05). Two outliers were evident when the data were depicted graphically (Figure 2.03). On investigating the data set, it was found that these were goats 8 and 5 and these were in fact the heaviest two goats in the run, and were also both on the lowest feeding level (the average intakes for goats 8 and 5 were 470 and 476 g d^{-1} respectively). Both these animals were not meeting their maintenance and growth requirements and were thus drawing on body reserves. It is possible that a large proportion of the purine derivatives in the urine were of endogenous origin. This would account for why the allantoin : creatinine ratios were higher than expected. This would only have been the case had the increase in allantoin been proportionally greater than the increase in creatinine excretion associated with a heavier animal. This is likely to be the case since the variation in live mass between the goats was not large. Thus, although intake is expressed relative to metabolic mass to allow for comparison between animals varying in mass, it may not have accounted for any physiological effects associated with mass differences.

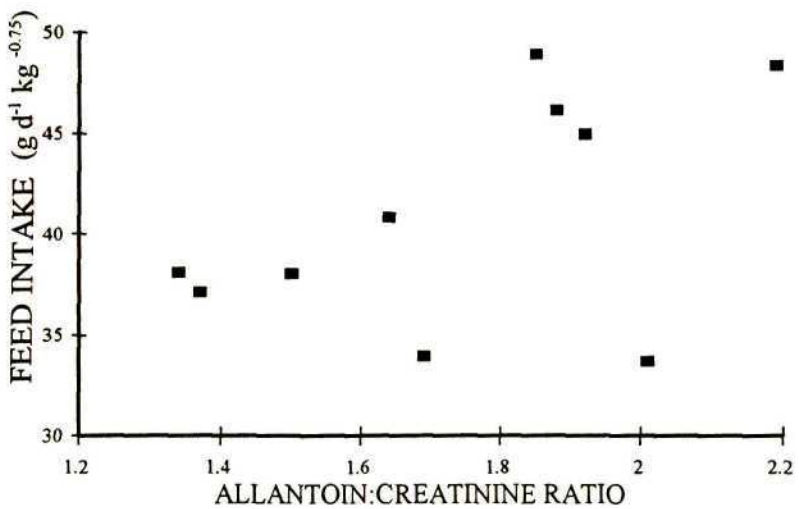

Figure 2.03 Average intake (expressed relative to metabolic mass) plotted against average urinary allantoin : creatinine ratio obtained from spot urine samples collected from all ten goats during the second evaluation of the purine derivative technique.

When goats 8 and 5 were dropped from the data set (Figure 2.04) and the linear regression refitted (Appendix 2.04), the fit improved considerably ($P \leq 0.001$).

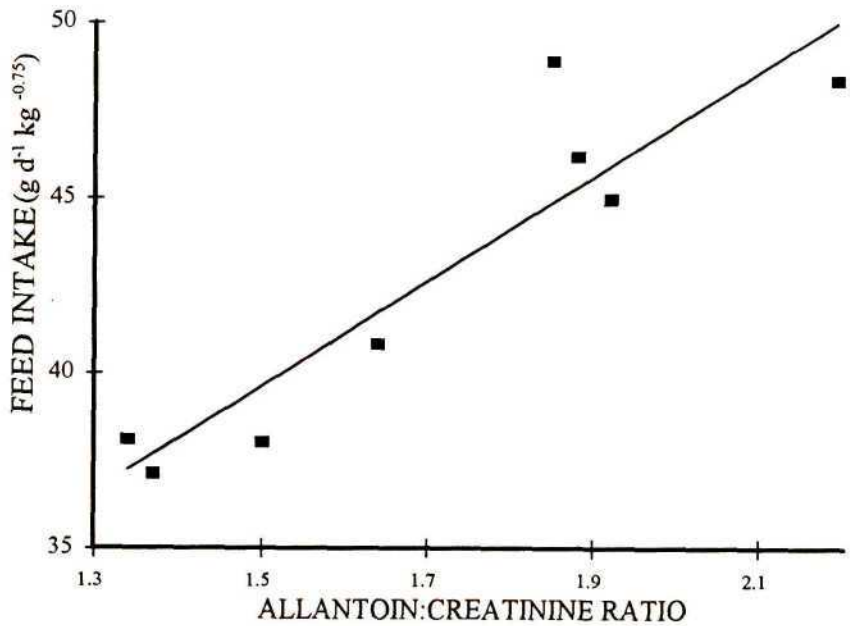

Figure 2.04 Average intake (expressed relative to metabolic mass) plotted against average urinary allantoin : creatinine ratio for the second evaluation of the purine derivative technique when goats 8 and 5 were excluded from the data set. The solid line depicts the predicted best-fit equation.

The following equation holds for the second run:

I_mmass = 14.991 * A/C ratio + 17.166 ($P \leq 0.001$, $r = 0.922$, $R^2_A = 0.824$, SE = 2.038, $n = 8$)

It would appear that there is a positive correlation between intake and the A/C ratio, however it is not sufficiently reliable to be used for predicting intake. The most suitable technique for estimating intake would be one where accuracy is not affected by the level of intake or the composition of the diet.

2.1.3.4 Third evaluation of the purine derivative technique

When I_mmass was plotted against average A/C ratio (Figure 2.06), a general trend showing a positive correlation was apparent. The Linear regression of intake relative to metabolic mass (I_mmass) against the allantoin : creatinine ratio was investigated (Appendix 2.05). Initially the trend was non-significant ($P > 0.05$) however when goat 7, which was seen to have a large standardised residual, was dropped from the regression (Figure 2.07) on the basis of its biological dissimilarity to the other goats, and the analysis was rerun (Appendix 2.06), the trend became significant ($P \leq 0.05$).

The main reason for discarding the data from goat 7 was that it was the only animal in the run that did not lose weight during the experimental period, in fact it gained marginally. It was only offered 800 g d^{-1}, but it actually achieved the second highest average intake. The fact that it was also one of the lighter goats in the run resulted in its having an abnormally large I_mmass value. That is, for its size it had a relatively high level of intake which is why it did not lose weight as did the other goats in the run.

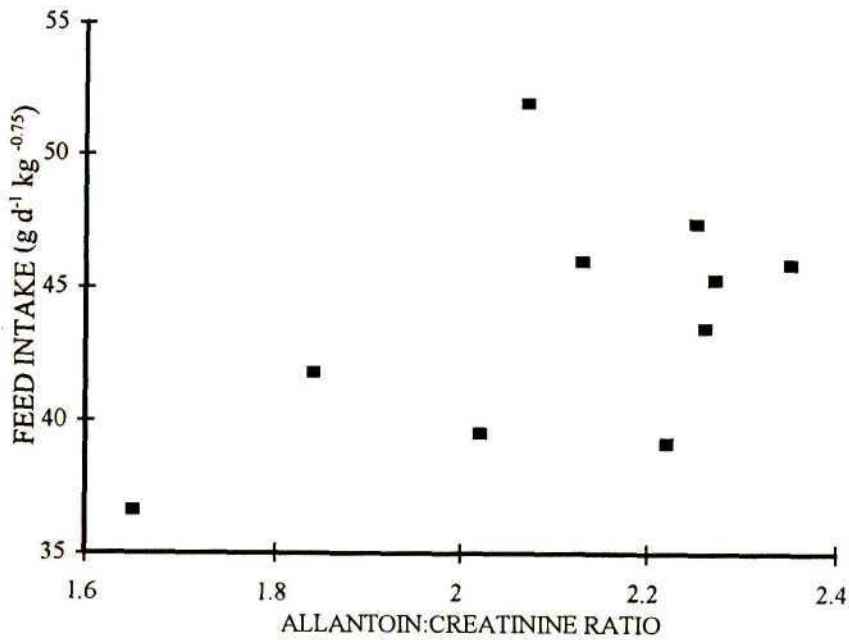

Figure 2.06 Average intake (expressed relative to metabolic mass) plotted against average urinary allantoin : creatinine ratio obtained from spot samples collected from ten goats during the third evaluation of the purine derivative technique for estimating feed intake.

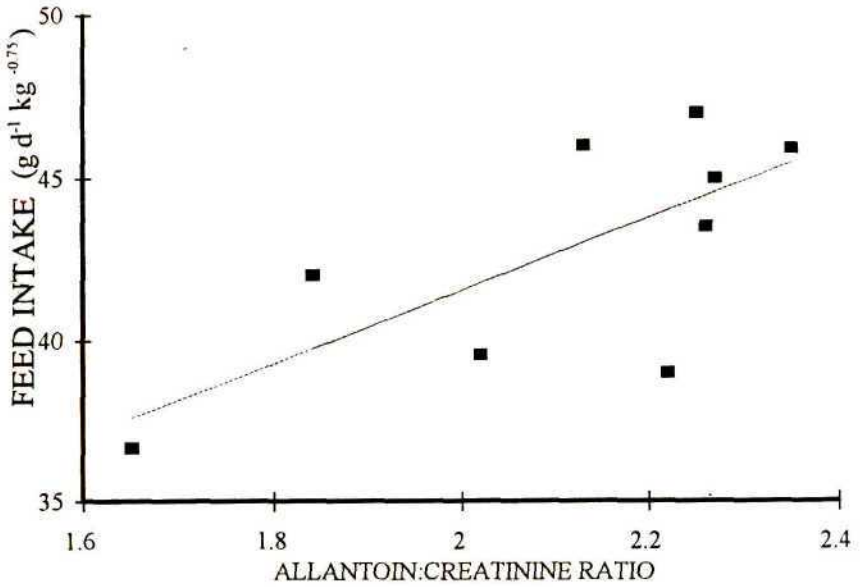

Figure 2.07 Average intake (expressed relative to metabolic mass) plotted against the average allantoin : creatinine ratio for the third run when goat 7 was excluded from the data set. The solid line depicts the predicted best-fit equation.

The following equation describes the relation between intake and A/C ratio for the third run: I_mmass = 11.221*A/C ratio + 19.098 ($P \leq 0.05$, $r = 0.708$; $R^2_A = 0.430$; $SE = 2.775$; $n = 9$). There is quite a wide scatter of points around the predictive line. The fact that this equation only accounts for 43.0% of the variation suggests that it is not a suitable predictive equation for use in determining intake.

2.1.4 Variation in allantoin : creatinine ratio over days

In an exercise to investigate the variation in A/C ratio between days for each of the goats, the A/C ratios obtained from the ten goats for the four collection days were plotted (Figure 2.08). It was found that although the values for any particular goat differed between days, the goats generally maintained the same respective positions relative to each other on any particular collection day. When the data for these goats (Appendix 2.07) were examined, it was found that goats 6 and 10 which showed relatively small A/C ratios consistently, did in fact have relatively low average daily intake values and goat 6 had a low I_mmass value however goat 10 had a fairly average I_mmass value. Goats 4 and 8 both produced relatively large A/C ratios fairly consistently, however although goat 4 had a high average intake (g d^{-1}), goat 8 did not. Even when expressed as

I_mmass, goat 8 did not show the positive correlation expected between intake and allantoin excretion. Thus although the results of this analysis show that the excretion of allantoin is not random, it does not appear to be a direct function of the level of intake. The fact that goats 6 and 10 were on the upper level offered and achieved such low intake levels, indicates that there was much opportunity for selectivity.

The variation in A/C ratios between days makes it difficult to compare ratios obtained from different goats on different days. It may have been the sample handling and analytical procedures which caused the variation between days. Should this be the case then it is necessary to find a procedure which gives more reliable results.

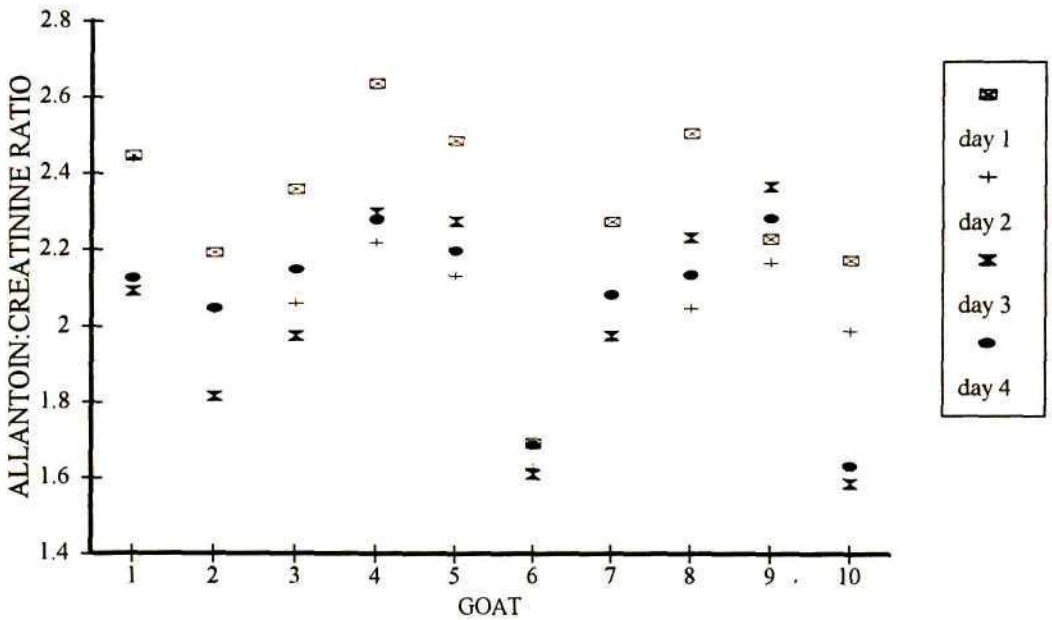

Figure 2.08 Variation in the allantoin : creatinine ratio of spot urine samples for the ten goats on the four collection days of the third evaluation of the purine derivative technique.

2.1.5 General discussion of the purine derivative technique evaluation

The goats on the upper three of the five feeding levels offered, fed selectively, and generally selected a diet that exceeded the 25% leucaena offered. Thus the diets of the goats varied with respect to proportions of hay and leucaena. This may have introduced an error since large differences in the efficiency of microbial synthesis can occur for fresh herbage, hays and silages (Minson 1990) and thus a simple relation between PD excretion and digestible organic matter intake cannot be assumed (Mayes *et al.* 1995).

Another difficulty encountered in all the runs was the management of the goats in the pens. Every effort was made to prevent the goats from stealing feed from those adjacent to them, but on occasion they did manage to. There were also occasions when they managed to escape from the pens and this lead to a need to restrain them within the pens. It is likely that these factors have contributed to the relatively poor correlations found here.

The range of A/C ratios covered in each of the runs varied, and this could have been related to the average live mass of the goats. Although there appears to be a trend of this nature, it is not well defined (Table 2.01). The slopes of the predicted best-fit lines were found not to differ significantly (Appendix 2.08), thus it would appear that a single predictive equation could have been used for estimating intake had the individual runs produced less scattered results (higher R^2_A values).

Table 2.01 Summary of data pertaining to the prerun and the following three runs

Run	average mass (kg)	Minimum A/C ratio	Maximum A/C ratio	Minimum I_mmass	Maximum I_mmass
Prerun	21.10	3.02	5.57	40	71
Run 1	24.90	1.91	4.04	35	67
Run 2	32.49	1.34	2.19	34	49
Run 3	34.52	1.65	2.35	37	52

2.2 Purine derivative excretion investigated in metabolic crates

2.2.1 Introduction

Since a spot-sampling technique was adopted in the pen trial to evaluate the purine derivative technique for estimating feed intake, it became necessary to validate the assumption that the time of collection would not affect the allantoin : creatinine ratio. Allantoin concentration *per se* would be affected because the volume of urine produced will be affected by time of day, but the expression of allantoin relative to creatinine is said to effectively reduce the influence of time of collection (Chen *et al.* 1992a)

2.2.2 Objectives

1. To verify that there is negligible variation in the allantoin : creatinine ratio of samples collected at different times of day
2. To verify that a spot-sampling technique can be reliably implemented.
3. To validate the hypothesis that level of intake is positively correlated with the A/C ratio.

2.2.3 Methods and materials

As only six metabolic crates were available for use, six young male goats with average live weight 30.40±1.01 kg were randomly allocated to two feeding levels: 600 g d^{-1} (treatment 1) and 800 g d^{-1} (treatment 2), both on an air-dried basis. In order to prevent selective feeding, both levels were lower than voluntary intake. This procedure aimed at minimising differences in digestibility between goats. The diet was similar to that used in the pen trials, namely a 25% leucaena : 75% eragrostis hay mix. The daily allowance was fed in two approximately equal meals at 07h30 and 15h30. The goats had unrestricted access to water throughout the experiment. A 14 day adaptation period preceded the collection period. During this time the goats were housed in the crates and received their allotted feed allowance. The collection equipment was fitted so that they became accustomed to it prior to the collection period.

For three days of the collection period (the spot-sampling period) there were four collection times per day at 07h30, 10h30, 13h30 and 16h30. The tyre apparatus was used and urine was collected directly into buckets containing 0.2N hydrochloric acid to prevent deterioration of the urine through bacterial action. The urine removed at each collection time, constituted any which had been produced since the previous collection. Thus the collection at 07h30 contained any urine produced during the night. Urine was diluted 1:5 with water and stored at < 7°C for analysis. Laboratory analyses were conducted in the evening on the day of collection. Orts, which consisted predominantly of herbage dropped whilst the goats were feeding, was collected daily. Percentage dry matter for both the components of the ration and the orts was determined so that actual intake could be established.

The collection apparatus was not satisfactory for obtaining a total daily urine collection even in the metabolic crates, so for three days of the collection period the collection apparatus was removed from the goats and tray funnels were placed underneath the crates. The 24 hourly collections were subsampled and analysed for creatinine and allantoin concentrations, on the day of collection.

*See appendices 5 and 6 for data from the work done in metabolic crates

2.2.4 Results and discussion

2.2.4.1 Period of twenty-four hourly collections

For each of the three 24-hourly collections, a subsample was analysed for allantoin and creatinine. The A/C ratios of the goats on treatment 1 were generally lower than those on treatment 2 for any one day (Figure 2.09). It is apparent that the A/C ratios show similar patterns between goats for any one day, however, the 'between day' variation indicates that it would be impossible to compare ratios obtained from samples collected on different days. For example, samples collected from goat 3 had the lowest A/C ratio for any one day, but are not lowest when the data from all 3 days are considered.

However, when a linear regression of average A/C ratio against I_mmass was fitted to the data set, the fit was found to be significant only at the 10% level and the R^2_A of 0.408 indicates that there is some tendency toward a linear regression, but the equation only accounts for 40% of the variation of the data (Appendix 2.09). When the treatment effect (amount of feed offered) was investigated (Appendix 2.10) the average A/C ratios for the group on treatment 1 were found to vary significantly from those of the group on treatment 2 ($P \leq 0.01$).

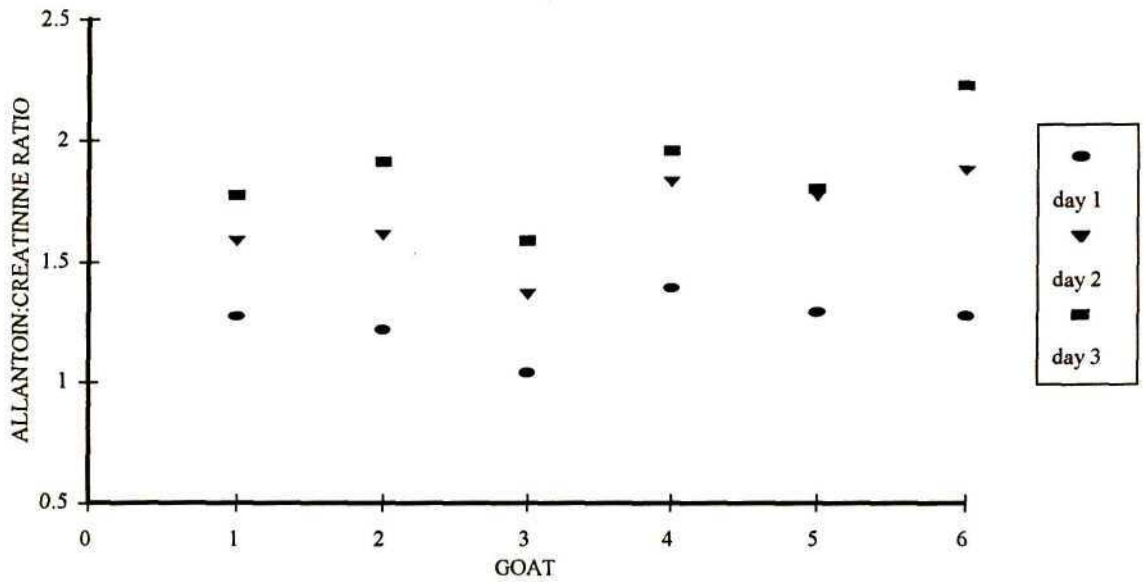

Figure 2.09 Variation in urinary allantoin : creatinine ratio between days for the six goats in the crate trial where goats 1, 2 and 3 were offered 600 g d^{-1} and goats 4,5 and 6 were offered 800 g d^{-1}. Note the variation between days for any one goat.

2.2.4.2 Spot sampling period

The three day spot sampling period yielded 73 samples between the six goats. Due to the unbalanced nature of the data, a regression analysis was conducted to determine whether the time of collection had a significant effect on the A/C ratio(Appendix 2.11). Since the effect of time was found to be non-significant ($P>0.05$), it would suggest that a spot-sampling technique, with

samples collected at any time of day, is valid. It would not have been the case had collection time been seen to have a significant effect on the A/C ratio. The A/C ratios predicted for the two treatments from the regression model are contained in Appendix 2.12.

The mean A/C ratios (calculated over all 6 goats) obtained at each collection time are illustrated in Figure 2.10. A slight trend is apparent, but it can be seen that the means do not differ significantly because the 95% confidence limits overlap.

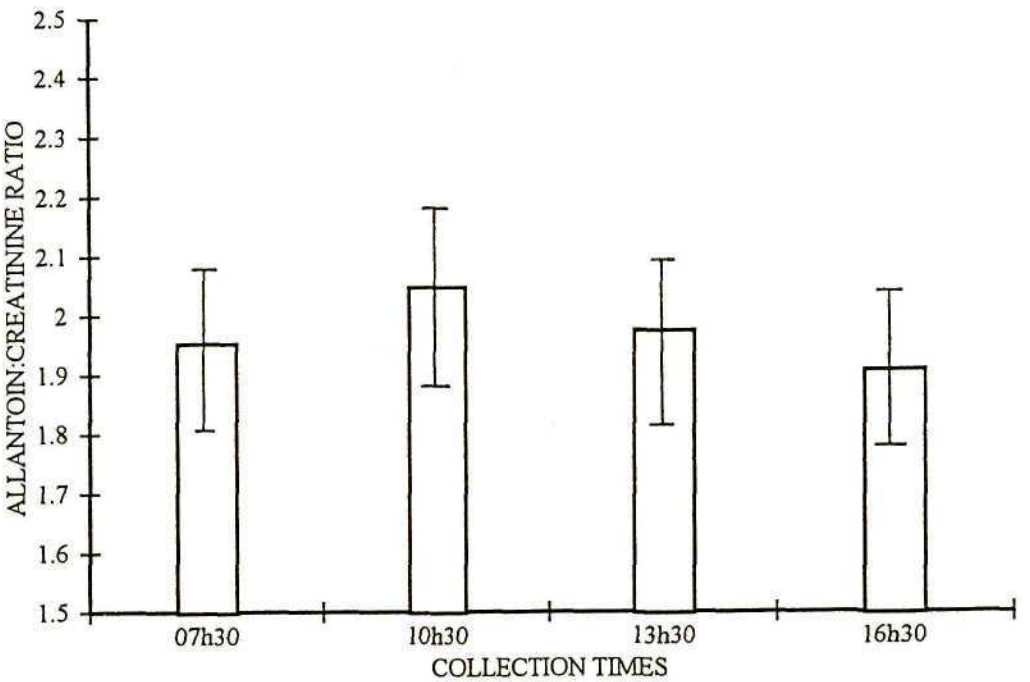

Figure 2.10 Average allantoin : creatinine ratio and 95% confidence limits for spot urine samples collected over different time periods during the day.

The goats in the crates produced approximately half the volume of urine produced by those in the pens. This could have been due to environmental conditions since the crate run was conducted in July when temperatures were cool while the pen trial was conducted from December to March. Unlike the pens which were fairly open, the crates were housed within a closed shed which remained cool throughout the trial and was likely to have reduced the consumption of water, but this was not measured. There was much precipitation of crystals in the urine both prior to

collection and after dilution. This may have been due to the urine being highly concentrated. There was also some deposition of crystals occurring on urination. It would suggest that the animals were stressed in the crates, resulting in their drinking less and their kidneys may have been affected although the trial was not run for an extended period. The crystals were identified under the microscope as uric acid crystals. Before the samples were diluted, these were precipitating out in the urine. They were reddish brown particles, the colour of which is due to the inclusion of urinary pigments. They are found most frequently in the deposit of concentrated acid urines which have been cooled to room temperature and are not characteristic of any pathological condition (Varley 1969)

2.2.5 Conclusions drawn from the work done in metabolic crates

Firstly the A/C ratio was found not to be significantly affected by the period over which the sample was collected. Thus the work done in the metabolic crates showed that a spot-sampling procedure can be implemented. When total daily urine collections were made, the ratio of the concentrations of allantoin to creatinine in a subsample was found to be significantly affected by treatment. In this case treatment being the offered daily feed allowance (600 vs 800 g d^{-1}). It was easier to work with the goats in the metabolic crates and the results were possibly more reliable than those obtained in the pens, however, they may not reflect field conditions.

2.3 Conclusions from the evaluation of the purine derivative technique

The objective of the pen trial was to develop regression equations between feed intake and the ratio of allantoin to creatinine in spot samples which would allow for the prediction of intake in the field. The results of the three runs demonstrate that there is a relation between intake expressed per unit metabolic mass (I_mmass) and the A/C ratio, however, the results of runs 2 and 3 would indicate that the correlation is not strong enough to be used for predictive purposes since without dropping outliers from the data set, the regressions were non-significant ($P>0.05$). In run 2 the outliers were heavy animals on very low levels of feed while in run 3 the outlier was a light animal consuming a relatively large amount of feed. Both these extreme sets of conditions are likely to have resulted in these animals being physiologically different to the rest of the goats

in the run which would have affected the relative urinary concentrations of allantoin and creatinine.

The main objective of the trial conducted in the metabolic crates was to verify that there is no significant variation in the A/C ratio of samples collected at different times of the day. This was achieved and in turn verified the assumption that the use of a spot-sampling technique is justifiable (second objective). The third objective of the metabolic crate work was to demonstrate the positive correlation between feed intake and the A/C ratio. Although a linear regression of intake (I_mmass) against A/C ratio was non-significant, a significant treatment effect was found ($P \leq 0.01$). Treatment being amount offered (g fodder d^{-1}, on air-dried basis).

If the index used for predicting intake had been [A/C ratio * LW * C_{LW}] (modification of equation 3.1; Lindberg 1985) and intake had been kept on a g d^{-1} basis, the technique might have been more applicable to animals differing in mass as occurred in runs 1 and 3 however it would appear that neither correction for differences in mass would take into account any physiological interactions between level of intake and mass. In the field there may be situations when animals are on restricted levels of intake such as just prior to removal from a paddock under a rotational grazing system. It would be a disadvantage to use a technique for predicting intake that was unsatisfactory under these extreme conditions.

Chapter 3

Chromic oxide as an external marker for estimating intake

3.1 Evaluation of the marker technique for estimating feed intake

3.1.1 Introduction

The use of chromic oxide as an external marker for predicting faecal output and feed intake indirectly was evaluated. This was achieved by determining the nature of the relations between measured faecal output and measured feed intake and between measured faecal output and faecal chromium concentration. A positive and negative correlation for the two relations respectively would be expected.

3.1.2 Objective

To validate the use of the marker, chromic oxide contained in gelatin capsules, for determining faecal output and feed intake indirectly.

3.1.3 Method and materials

During the three runs evaluating the purine derivative method, the chromic oxide technique was also evaluated. Gelatin capsules (specified to each contain 10 g Cr_2O_3; Manufacturer: RP Scherer, Berks, England) were used since from the review of the literature it appeared that they would give satisfactory results. Redmon *et al.* (1995) measuring forage intake by beef steers dosed the animals twice daily with 4g of chromic oxide administered orally in a gelatin capsule and sampled faeces twice daily for 4 days following a 6-day equilibration period. Samples were then bulked within animals across the collection period.

In the current study, a 6-day equilibration period was followed by a 5-6 day collection period. This allowed for Cr_2O_3 excretion to become fairly constant by the time samples were collected.

The gelatin capsules, each containing 10g Cr_2O_3 (manufacturer specified) were dosed manually twice daily at meal times (07h30 and 15h30) for the duration of the equilibration period and collection period. Animals were watched closely for possible regurgitation of the capsules.

The attempt to validate the use of the marker method constituted two steps:

Step 1

According to equation 1.1 (**I = F (1-D)**), one would expect a positive linear relation between faecal output and feed intake if digestibility of the diet were constant.

During the trial, while the animals were housed in the pens, total faecal collections were made using faecal bags. Average faecal output (g d^{-1}) for each goat was plotted against its average intake expressed relative to metabolic mass (I_mmass) in order to maintain consistency with the purine derivative technique but when the relation was investigated, the correlation was found to be poor. It improved considerably when faecal output (g d^{-1}) was regressed against absolute intake (g d^{-1}).

Step 2

According to equation 1.2 (**$F = (M_d * R)/M_f$**) there should be a negative linear relation between faecal chromium concentration (M_f) and the amount of faeces produced (F) since the recovery in faeces is complete and the degree of dilution of the marker will depend on the amount of faeces voided.

The concentration of chromic oxide in faeces was determined using atomic absorption spectroscopy (Appendix 1.03). Average faecal chromium concentration (mg kg^{-1}) was plotted against average faecal output (g d^{-1}) for each goat. The significance of the linear regression of average faecal chromium concentration against average faecal output was then established. Intake figures were calculated on a dry matter basis with a knowledge of amount offered, amount refused (orts) and the percentage dry matter for each.

3.1.3.1 Sampling procedure for the first evaluation

The first evaluation of the Cr_2O_3 marker technique coincided with the first evaluation of the PD method. Faecal bags were used to obtain 24 hour collections for each goat. These collections were subsampled in order to determine percentage dry matter so that faecal output (g d^{-1}) could be calculated on a dry matter basis for each goat. In order to simulate twice daily grab sampling without requiring the removal of the faecal collection apparatus, bags were emptied twice daily and the first deposition of faeces into the empty bag was then collected. The two samples for each goat for each day were then combined wet, oven-dried at 60°C for 48 hours, milled and stored until analysis. The mass of the sample was then added to the daily output value.

3.1.3.2 Sampling procedure for the second evaluation

As for run 1 a subsample of the total daily collection was used to determine faecal percentage dry matter and simulated grab samples were collected twice daily and bulked for each goat for each day, dried, milled and analysed for chromium content.

3.1.3.3 Sampling procedure for the third evaluation

As for the first two runs, the faecal bags were emptied twice daily in order to obtain total daily collections, however the sampling procedure differed. In this case the subsample of the total daily collection that was used for determining percentage dry matter was also analysed for chromium concentration. This sample gives mean daily faecal Cr concentration which would be expected to be more closely correlated with faecal output than the grab samples would be.

3.1.4 Results and Discussion

3.1.4.1 Step 1

3.1.4.1.1 First evaluation

A definite positive trend was found to exist between average faecal output (g d^{-1}) and average feed intake (g d^{-1}) for the first run (Figure 3.01). When a linear regression was fitted (Appendix 3.01), the results were found to be highly significant ($P \leq 0.001$) but with R^2_A of only 0.714, the fit was poorer than expected.

The poor fit was the result of one outlying datum point. This was found to be goat 6 on examination of the data. There is no biological reason for this having occurred, so it could not be excluded from the data set, but on examining the data set it was found that the daily intake values for this goat were very inconsistent. This may be why the expected relation between average intake and average faecal output did not hold.

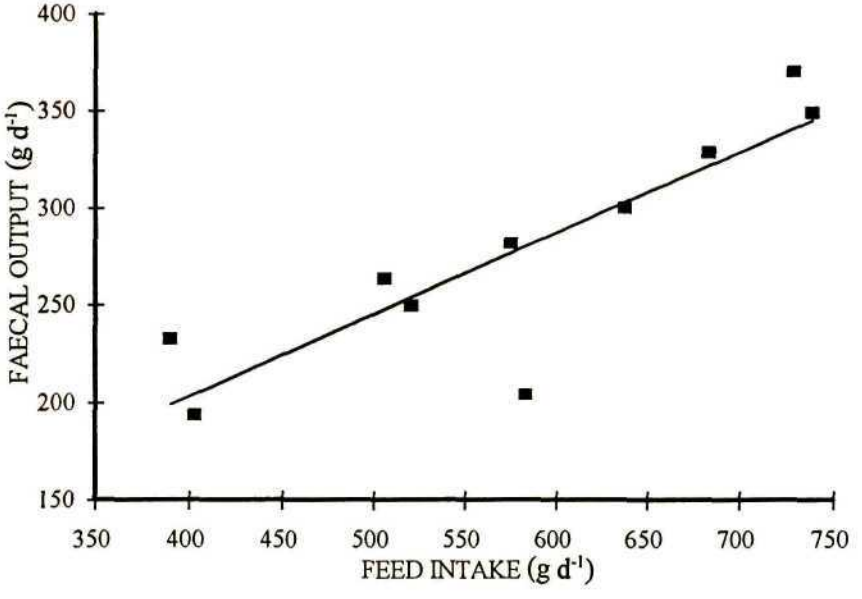

Figure 3.01 Average faecal output (g d^{-1}) plotted against average intake (g d^{-1}) for the first evaluation of the Cr_2O_3 marker method.

The equation describing the relation between faecal output and absolute feed intake for the first run is: Faecal output = 0.417 * feed intake + 37.075
($P \leq 0.001$, $r = 0.864$, R^2_A 0.714, SE = 31.93, n = 10)

3.1.4.1.2 Second evaluation

When average faecal output (g d^{-1}) was plotted against average feed intake (g d^{-1}) for each goat, a definite trend was apparent (Figure 3.02). The correlation was found to be significant when the linear regression of faecal output against feed intake was investigated ($P \leq 0.01$). The results of the analysis are summarised in Appendix 3.02. The equation describing the relation between faecal output and feed intake for the second run is as follows:
Faecal output = 0.484 * feed intake - 0.359 ($P \leq 0.01$, r 0.783, R^2_A 0.565, SE = 32.551, n = 10)

Since this equation only accounts for 56.5% of the variation in the data, the assumption that intake will be linearly correlated with faecal output will not hold here. Should this be a result of differences in the digestibility of the diet, rather than due to poor collection techniques, then the application of a single digestibility figure to all these animals has the possibility of introducing error into the estimation of feed intake by the marker method.

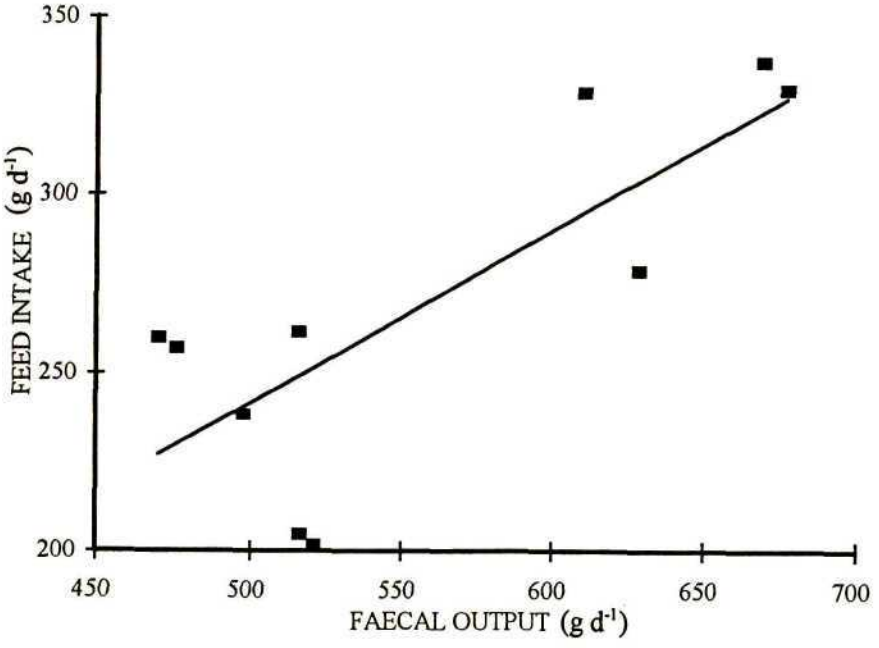

Figure 3.02 Average faecal output (g d^{-1}) plotted against average feed intake (g d^{-1}) for the ten goats during the second run evaluating the marker method.

3.1.4.1.3 Third evaluation

When the relation between average faecal output and average intake (both measured in g d^{-1}) was investigated (Figure 3.03), an obvious outlier was apparent. When the data were examined, this was found to be goat 2. Closer inspection of the data for this goat showed that it had increased its intake quite substantially over the last two days of the collection period. Due to the lag effect of intake on faecal output, I dropped the last two intake values from the calculated mean since they would not have been related to the faecal output measured (Figure 3.04).

Initially when average faecal output was regressed against average feed intake (Appendix 3.03), R^2_A was found to be 0.748. When the analysis was rerun with the intake figure for goat 2 adjusted (Appendix 3.04), the R^2_A increased to 0.862. It was found that the coefficient of intake (the slope of the line) increased with the manipulation of the data and became less similar to that obtained for runs 1 and 2.

The equation describing the relation for run 3 is:

Faecal output = 0.508 * feed intake - 17.648

($P \leq 0.001$, r = 0.937, R^2_A 0.863, SE = 13.431, n = 10)

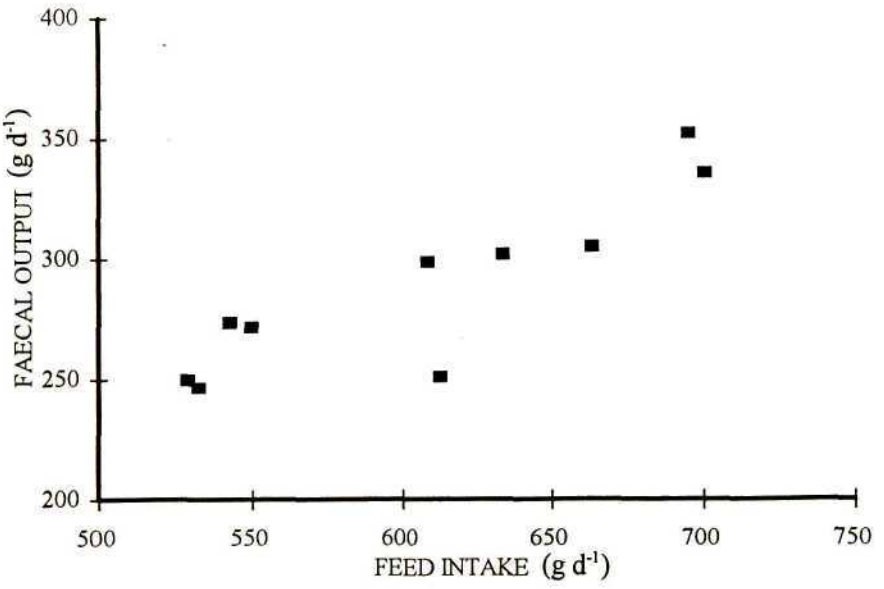

Figure 3.03 Average faecal output (g d^{-1}) plotted against average feed intake (g d^{-1}) for the original data (ten goats) for the third evaluation the marker method.

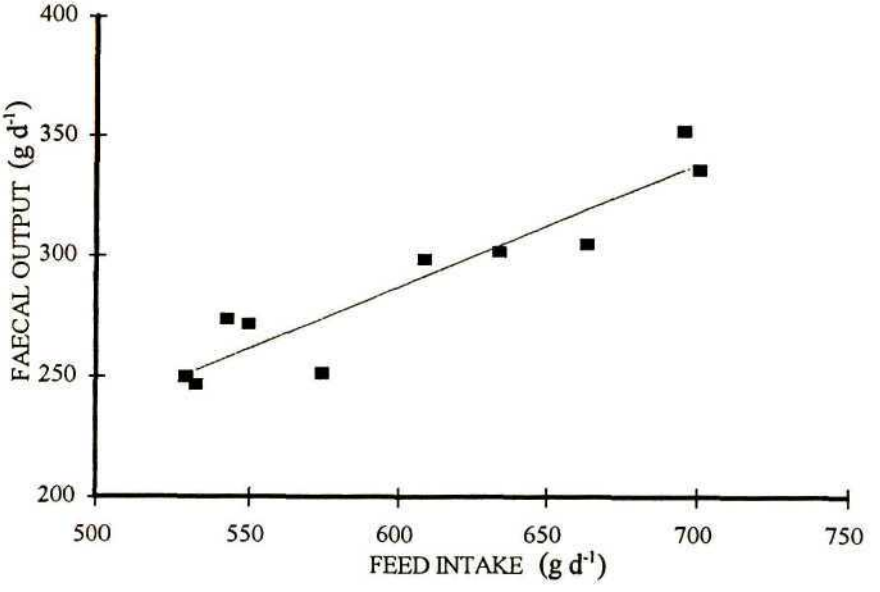

Figure 3.04 Average faecal output (g d^{-1}) plotted against average feed intake (g d^{-1}) for the third evaluation of the external marker technique, when the average intake value for goat 2 was adjusted.

3.1.4.2 Step 2

3.1.4.2.1 First evaluation

When average faecal chromium (Cr) concentration (mg kg^{-1}) was plotted against average faecal output (g d^{-1}), a definite trend was apparent (Figure 3.05). As was expected, faecal Cr concentration appeared to be negatively correlated with faecal output. This was further investigated in the statistical analysis, the results of which are summarised in Appendix 3.05.

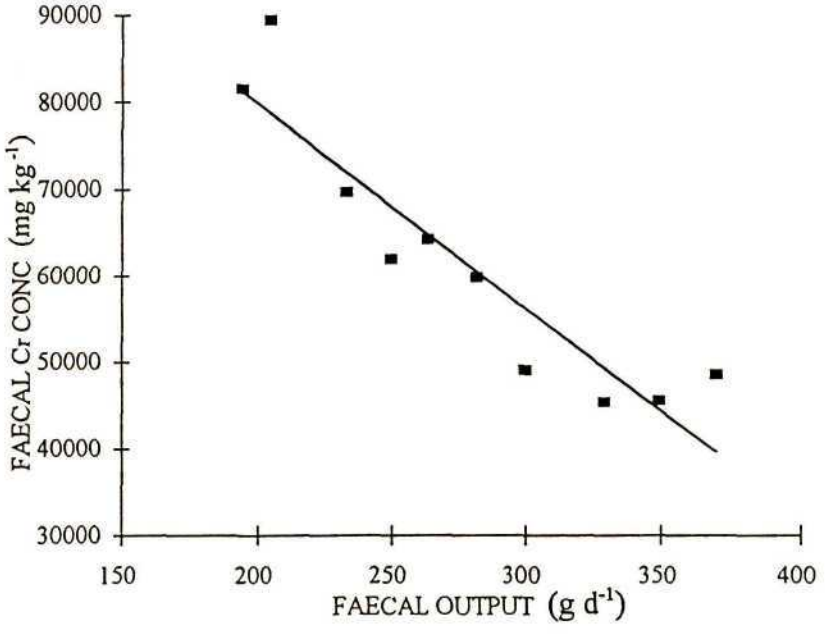

Figure 3.05 Average faecal chromium concentration (mg kg^{-1}) plotted against average faecal output (g d^{-1}) for the first evaluation of the conventional marker method.

The equation describing the relation between faecal chromium concentration and faecal output for the first run evaluating the marker method is:

Faecal chromium concentration = -236.298 * faecal output + 1.27E+05

($P \leq 0.001$, $r = 0.924$, $R^2_A = 0.836$, SE = 6176.617, $n = 10$)

Not only is the relation highly significant, but the R^2_A (0.836) indicates that the line accounts for a large amount of the variation in these data.

3.1.4.2.2 Second evaluation

Figure 3.06 illustrates the relation between average faecal chromium concentration and average faecal output. The trend is well defined and (Appendix 3.06) the fit of the negative linear regression was found to be highly significant ($P \leq 0.001$).

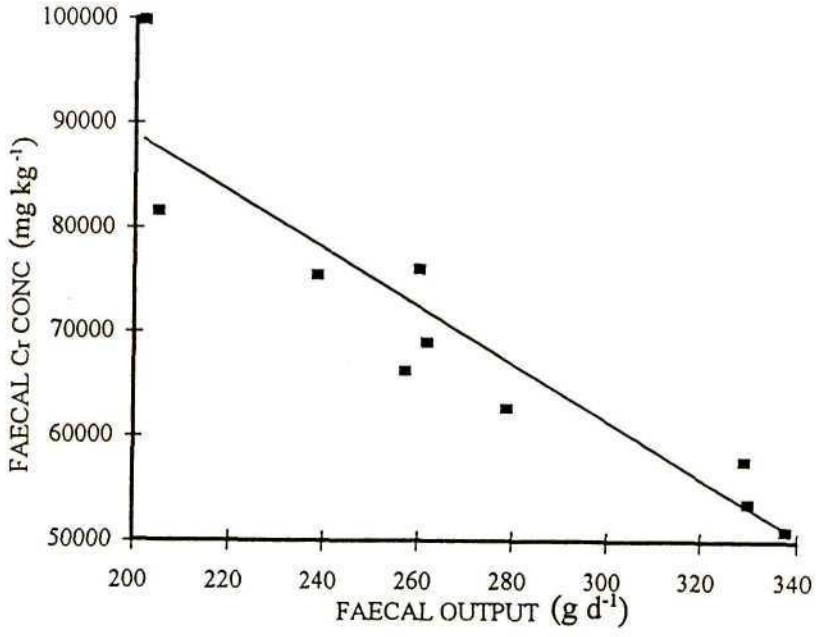

Figure 3.06 Average faecal chromium concentration (mg kg^{-1}) plotted against average faecal output (g d^{-1}) for the ten goats when the marker method was evaluated a second time.

Thus the equation describing the relation for the second run is:

Faecal chromium concentration = - 275.019 * faecal output + 1.44E+05

($P \leq 0.001$, $r = 0.925$, R^2_A 0.837, SE= 5933.900, $n = 10$)

The high correlation obtained for step 2 leads me to believe that the poor result obtained for step 1 of this run, is not due to an inaccurate measurement of faecal output. The fact that the collection of orts was carried out by two people who may not have been equally meticulous may have lead to an error in the measurement of feed intake. Alternatively, it may again have been due to discrepancies in the digestibility of the diets selected by the ten goats.

3.1.4.2.3 Third evaluation

As for runs 1 and 2, average faecal chromium concentration (mg kg^{-1}) was plotted against average faecal output (g d^{-1}) and seeing that a negative correlation was apparent as expected (Figure 3.07), this was then defined statistically (Appendix 3.07). As was to be expected from the sampling procedure, the correlation between faecal output and faecal Cr concentration was greater for the third evaluation than for the first and second as a result of the improved sampling procedure.

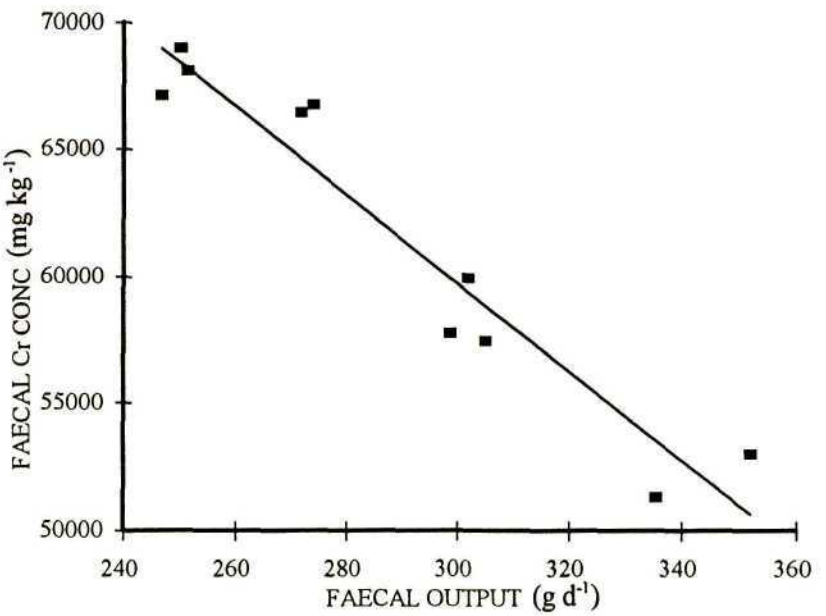

Figure 3.07 Average faecal chromium concentration (mg kg^{-1}) plotted against average faecal output (g d^{-1}) obtained for the ten goats during the third evaluation of the external marker method.

The following equation holds for run 3.

Faecal chromium concentration = -174.273 * faecal output + 1.120E+05

($P < 0.001$, $r = 0.960$, R^2_A 0.912, SE = 1959.580, n = 10).

3.1.4.2.4 General discussion of the three runs

Step 1

Although in the real situation, intake would be calculated from faecal output together with a digestibility figure, faecal output is in fact a function of the amount of feed consumed. Thus, since faecal output is the dependent variable, it must be the Y variable, while feed intake (the independent variable is the X variable. The predicted equations describing the relation between faecal output and feed intake are not significantly different (Figure 3.08). The 95% confidence limits (Appendix 3.08) all overlap so a single equation could be developed for the pooled data (Figure 3.09). The following equation describing the relation between faecal output (g d^{-1}) and feed intake (g d^{-1}) was determined from the statistical analysis (Appendix 3.09) of the pooled data:

Faecal output = feed intake * 0.448 + 19.341 ($P \leq 0.001$, r 0.853, R^2_A 0.718, SE 25.664, n = 30)

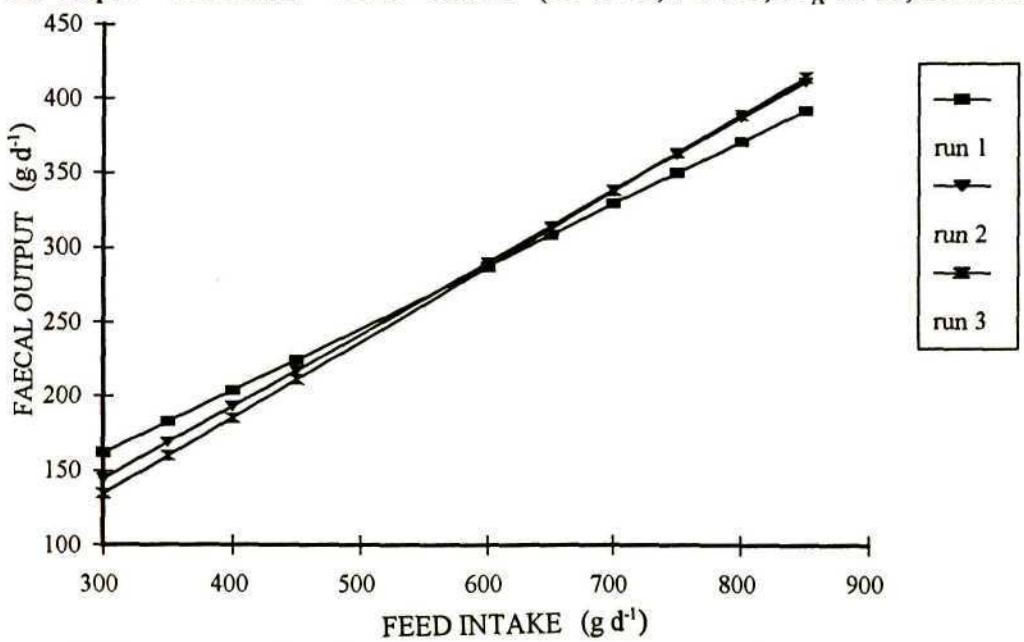

Figure 3.08 Predicted lines describing relation between faecal output (g d^{-1}) and feed intake (g d^{-1}) for the three runs evaluating the external marker method of determining feed intake.

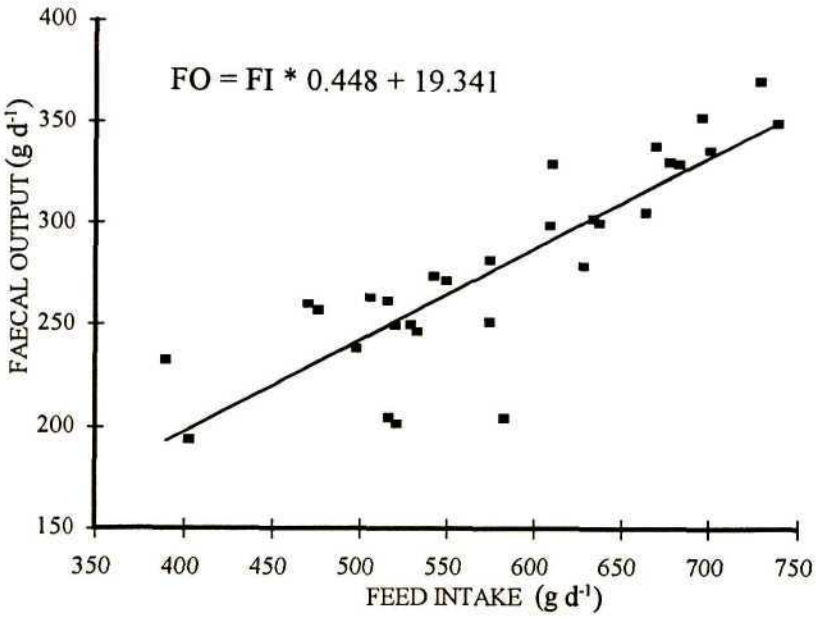

Figure 3.09 Pooled data from the three runs evaluating the external marker method. The solid line depicts the predicted best-fit relation between faecal output (FO) (g d^{-1}) and feed intake (FI) (g d^{-1}).

Step 2

Similarly the slopes of the predicted lines describing the relation between faecal chromium concentration and faecal output do not differ significantly (Appendix 3.10; Figure 3.10). Again the data from the three runs were pooled (Appendix 3.11; Figure 3.11)

The equation obtained from the statistical analysis of the pooled data is:
Faecal Cr conc = faecal output * -241.5469 + 1.315E+05
($P \leq 0.001$, r 0.904, R^2_A 0.811, SE 5603.788, n = 30).

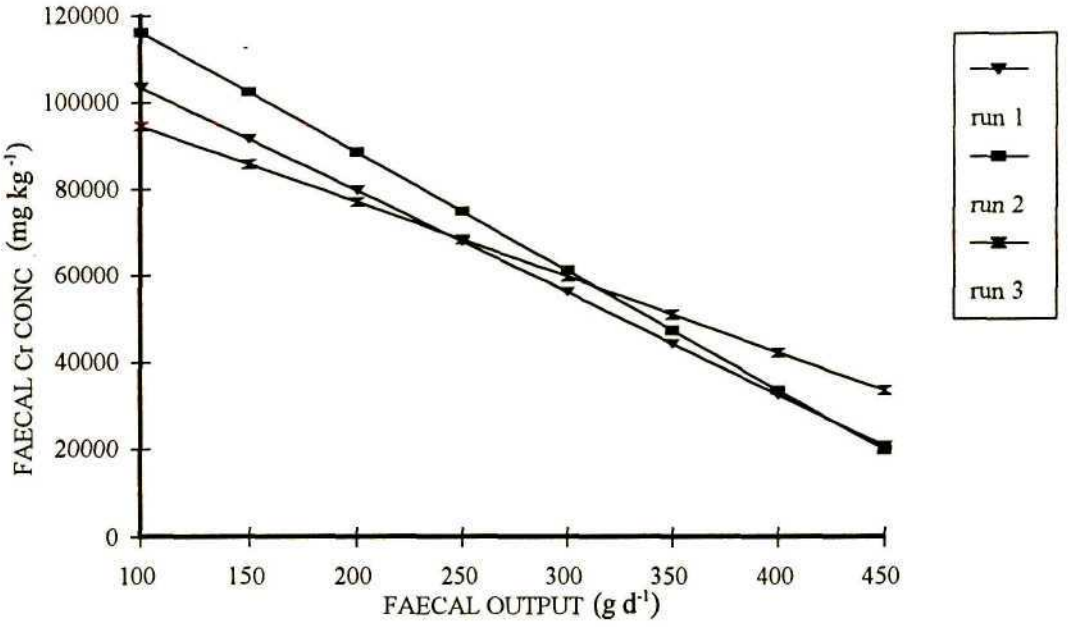

Figure 4.12 Predicted lines describing relation between faecal chromium concentration (mg kg^{-1}) and faecal output (g d^{-1}) for runs 1, 2 and 3.

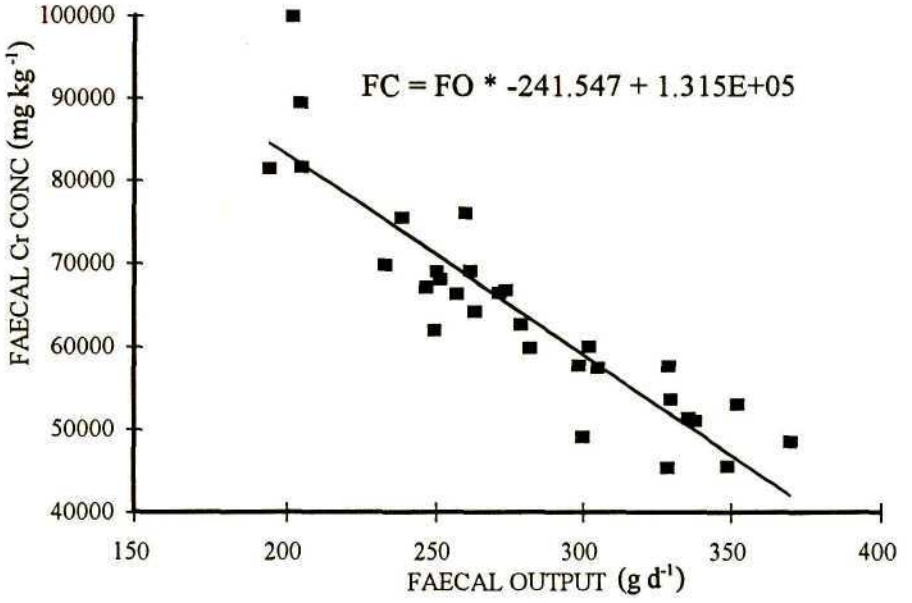

Figure 4.13 Pooled data for the three runs evaluating the marker method. The solid line depicts the predicted best-fit line describing the relation between faecal chromium concentration (FC) (mg kg^{-1}) and faecal output (FO) (g d^{-1}).

3.1.5 Conclusions drawn from the evaluation of the marker method

It would appear that the use of chromic oxide as a marker to estimate the output of faeces and indirectly the intake of feed, would be satisfactory for goats if a reliable method of determining 0digestibility were employed.

The fact that the regressions obtained for each of the runs for each of the steps did not differ significantly suggests that the mass of the goat within the range covered during these three runs, does not affect the slope of the regressions. It is however, not possible to extrapolate to masses outside of this range.

With a knowledge of how faecal output is related to intake, and, how faecal chromium concentration is related to faecal output under these dosing conditions, it is actually possible to predict intake from faecal chromium concentration.

The relations are defined by the following equations:
Faecal output = feed intake * 0.448 + 19.341
Faecal Cr conc = faecal output * -241.547 + 1.315E+05

3.2 Digestibility trial

3.2.1 Introduction

If the conventional marker method of using an estimation of faecal output and an estimate of indigestibility is to be adopted in order to calculate feed intake, then digestibility of the diet must be determined.

It was possible to determine the *in vivo* digestibility of the 25% leucaena : 75% hay diet in the

metabolic crates. Furthermore, an *in vitro* method was also used to determine digestibility. In this case the digestibilities of a range of percentage leucaena mixes were determined. This gives some indication of how the digestibility of the diets selected may have varied from the offered diet.

3.2.2 Method and materials

At the time when the urinary excretion of purine derivatives was investigated in the metabolic crates, the digestibility of a 25% leucaena : 75% hay diet was also determined. During the preliminary period consistent levels of intake were established. This was followed by the collection period during which both faecal output and feed intake were measured accurately and averaged for each goat. Digestibility was then determined according to the equation:

$$Digestibility = \frac{intake - faecal\ output}{intake} * 100 \qquad \text{----------(4.1)}$$

In Vitro digestibility values of a range of leucaena : hay mixes (20% leucaena : 80% hay to 60% leucaena : 40% hay) were determined in the laboratories of the Range and Forage Institute, ARC, Roodeplaat. This was the range selected by the goats during the pen trial.

3.2.3 Results and discussion

The digestibility values obtained for the 25% leucaena: 75% hay diet in the metabolic crates ranged between 45.89 and 51.11 % (Table 3.01). Quite a variation in digestibility values is apparent even though the goats all consumed a largely similar diet, there being very little selectivity possible, due to the restricted feeding levels offered. From the data in Table 3.01, digestibility appears to be related to the level of intake. Both of the levels offered were below voluntary intake and the lower level (600 g d^{-1} on an air dried basis) was possibly below the requirements of the goats for maintenance and growth. This may have caused the differences in digestibility values obtained for the six goats.

When the digestibilities of a range of leucaena : hay mixtures was determined by *in vitro* methods, the range of digestibility figures was found to be much narrower than the range of mixtures investigated (Table 3.02). The results of the 24% and 26% leucaena components (52.51 and 46.32% respectively) compared favourably with the results of the digestibility trial. It would appear that the percentage leucaena in the leucaena : hay mix did not affect the digestibility of the diet in any consistent manner. The 54% leucaena mix did have a substantially greater digestibility value than the rest of the samples. Inconsistencies in the quality of the hay across the samples may have impacted on the results obtained, although attempts were made to minimise this.

The range of percentage leucaena mixes that were analysed for *in vitro* digestibility extended from 20 % leucaena to 60% leucaena because this was the range of selectivity shown by the goats, although they were all offered a 25% leucaena diet. It might have been useful to have analysed a 0% leucaena and a 100% leucaena sample, however at the time these were not included.

3.2.4 Conclusions

In a grazing trial, especially where animals have access to veld, they will display a degree of selectivity in their grazing habits. It would be necessary to apply an average digestibility figure (obtained say from a number of samples collected from oesophageally fistulated animals) to all the animals in the group. This is likely to be a source of error in estimating intake for individual animals in the group.

Table 3.01 Average intake (g d^{-1}) and faecal output figures (g d^{1}) and calculated digestibility figures for the 25% leucaena : 75% hay mix obtained from the digestibility trial conducted in metabolic crates

Goat	Average intake (g d^{-1})	Average faecal output (g d^{-1})	Digestibility (%)
1	518.43	280.52	45.89
2	552.68	282.40	48.90
3	549.68	287.00	47.79
4	660.08	327.47	50.39
5	702.93	344.90	50.93
6	687.95	336.35	51.11

Table 3.02 *In vitro* organic matter digestibility (%IVOMD) figures for a range of leucaena : hay mixes

Percentage leucaena	% IVOMD
20	46.26
24	52.31
26	46.32
30	44.92
34	43.87
40	46.95
44	46.40
50	47.06
54	64.41
60	53.44

CHAPTER 4

Final conclusions

That which is called the purine derivative technique in the current study, is actually a modification of the technique, since allantoin rather than total PD is used as the index for predicting feed intake. Further modification of the technique used by Lindberg (1985) involved the use of a spot sampling technique (suggested by both Osuji *et al.* 1993 and Mayes *et al.* 1995) since in the field it would be difficult to obtain total daily urinary collections.

The technique which I adopted could only be used for goats of similar live mass since expressing allantoin relative to creatinine in order to standardise it, required that total daily creatinine output was similar for all goats. Since it has been found to be a function of live mass, it is generally expressed as mg creatinine. kg LW^{-1}. The variation in live mass was greater for runs 1 and 3 than it was for the second run. It would have been optimal to have minimised this variation.

During the evaluation of the PD technique, intake was expressed relative to metabolic mass (I_mmass) and was regressed against the ratio of allantoin : creatinine (A/C) in spot urine samples.

The following equations were obtained from the statistical analysis:

(1) I_mmass = 12.637 * A/C ratio + 15.383 ($P \leq 0.001$, R^2_A 0.715, SE 5.910, n = 10)

(2) I_mmass = 14.991 * A/C ratio + 17.166 ($P \leq 0.001$, R^2_A 0.824, SE 2.038, n = 8)

(3) I_mmass = 11.221 * A/C ratio + 19.098 ($P \leq 0.05$, R^2_A 0.430, SE 2.775, n = 9)

The outliers dropped in run 2 were dropped on the basis of their biological dissimilarity from the other goats in the run since they were heavy animals on low levels of feed intake which may well have been below their maintenance requirements. The goat dropped in run 3 was dropped because it was the only animal which did not lose weight during the course of the run, in fact it gained marginally.

Without dropping these outliers both runs 2 and 3 did not produce significant relations. A technique for predicting intake which does not hold under slightly unusual conditions is unsatisfactory for research work (Lambourne 1957) especially under free-range conditions where many more variables are at play .

In assessing the conventional marker method using chromic oxide contained in gelatin capsules, two relations were investigated and the following results were obtained:

When faecal output (Faecout, g d^{-1}) was regressed against feed intake (FI, g d^{-1}):

(1) Faecout = 0.417 * FI + 37.075 ($P \leq 0.001$, R^2_A 0.714, SE 31.93, n = 10)

(2) Faecout = 0.484 * FI - 0.359 ($P \leq 0.01$, R^2_A 0.565, SE 32.55, n = 10)

(3) Faecout = 0.508 * FI - 17.648 ($P \leq 0.001$, R^2_A 0.863, SE 13.43, n = 10)

When faecal chromium concentration (Faec Cr , mg kg^{-1}) was regressed against faecal output (Faecout, g d^{-1}):

(1) Faec Cr = -236.298 * Faecout + 1.27E+05 ($P \leq 0.001$, R^2_A 0.836, SE 6176.62, n = 10)

(2) Faec Cr = -275.019 * Faecout + 1.44E+05 ($P \leq 0.0001$, R^2_A 0.837, SE 5933.90, n = 10)

(3) Faec Cr = -174.273 * Faecout + 1.12E+05 ($P \leq 0.001$, R^2_A 0.912, SE 1959.58, n = 10)

Since all the regressions for this part of the trial were significant and most were highly correlated (except for run 2: Faecout against FI), it would appear that this method is more reliable than the purine derivative technique. The data for the three runs were pooled and single equations describing the relations between faecal output and feed intake and between faecal chromium concentration and faecal output were established:

Faecal output = feed intake * 0.448 + 19.341 ($P \leq 0.001$, r 0.853, R^2_A 0.718, SE 25.664, n = 30)

Faecal Cr conc = faecal output * -241.5469 + 1.315E+05 ($P \leq 0.001$, r 0.904, R^2_A 0.811, SE 5603.788, n = 30).

This would allow for the prediction of feed intake of grazing goats on a similar diet, under the same dosing and sampling regimes from a knowledge of faecal chromium concentration. It should however be noted that these relations cannot be expected to hold under conditions differing from

those of the current study, in any way.

The difficulty associated with obtaining a reliable and accurate measure of dietary digestibility remains. Thus the conventional marker method of determining feed intake from an estimation of faecal output and digestibility of diet selected by the animal will still have its limitations. Where conditions allow, the double alkane technique would probably be superior for estimating intake as it does not require a digestibility value.

The purine derivative technique deserves further investigation as it shows much potential as a method of predicting feed intake under free-range conditions. Lack of funds and inadequate equipment may have been largely responsible for the relatively poor results obtained in the current study. Various other factors may also have contributed. Amongst these are possible differences in the quality of the grass hay and leucaena offered to the goats, although every effort was made to minimise this type of variation. The selectivity of the goats also contributed to differences in the quality of the feed consumed by the goats.

Further research might possibly involve the establishment of regressions between intake and the ratio of allantoin to creatinine in urine samples collected from goats in metabolic crates. These could then be tested in free-movement pens. If they were found to hold under these conditions, then it could possibly be assumed that they would also hold under free-ranging conditions.

APPENDICES

Appendix 1

Chemical analysis procedures

Appendix 1.01

The analysis of urine for allantoin

The method used was a convenient rugged technique described by Borchers 1977, which depends on the possibility of forming the 2.4-dinitrophenylhydrazine of glyoxylic acid which is conveniently converted to a sensitive chromophore after making alkaline.

Procedure:

1 A 2.5 ml aliquot of diluted urine (1:250 or 1:500) is heated with 0.5 ml of 0.6M sodium hydroxide in a boiling-water bath for 10 - 15 minutes;

2 To this is added 1 ml of 0.1% 2,4-dinitrophenylhydrazine in 2M hydrochloric acid, and heating is continued for 2-4 minutes. This step hydrolyses the allantoic acid and forms the hydrazone of the resulting glyoxylic acid;

3 The tubes are then cooled to room temperature in cold water and then made alkaline with 5 ml 2.5M sodium hydroxide;

4 After standing at room temperature for 9 - 11 minutes, absorbance is read on the spectrophotometer at 520 nm;

5 The blank is provided by omitting the heating and adding the acid hydrazine solution first before the alkali.

Appendix 1.02

The analysis of urine for creatinine

The analysis used was one described by Varley (1969) as the alkaline picrate method of Bonsnes & Tausskey (1945). This analysis relies on the production of a red colour with an alkaline picrate solution - the Jaffe reaction. It is the presence of chromogens which give the red colour, but in urine a maximum of 5% of the total chromogens are non-creatinine substances.

Procedure:

1 Urine is diluted 1: 100;

2 To 3 ml of the diluted urine is added 1 ml 0.04M picric acid followed by 1 ml 0.75N sodium hydroxide;

3 A stock solution of creatinine containing 1 mg creatinine ml^{-1} is made by dissolving 1 g of pure dry creatinine in 0.1N hydrochloric acid and making up to 1 l with the acid. The standard solution is prepared by diluting 1 ml of the stock solution to 100 ml with water. This contains 0.01 mg creatinine ml^{-1}.

4 3 ml of a prepared standard solution is similarly treated, as well as a blank;

5 After a 15 minute stand, the samples are read in the spectrophotometer at transmission of 500 millimicrons.

Calculation:

Since the standard is prepared to contain 0.03 mg creatinine, and 3 ml of diluted urine corresponds to 0.03 ml of the original urine.

Grams creatinine per litre of urine

$$= \frac{reading\ of\ unknown}{reading\ of\ standard} * 0.03 * \frac{1000}{0.03} * \frac{1}{1000} \qquad = \frac{reading\ of\ unknown}{reading\ of\ standard}$$

or

$$\frac{u}{s} * 100 = mg\ 100\ ml^{-1}$$

where u is the value obtained for the unknown and s is the value obtained for the standard

Appendix 1.03

The analysis of faeces for chromium content

The method was developed by Costigan & Ellis (1990) and was actually developed for use with controlled release devices where the concentration of chromium would have been much lower, which required slight modification of the method in the current study. The procedure relies upon a sulphuric/phosphoric acid digestion and bromate oxidation step to solubilise the chromic oxide, and uses atomic absorption spectroscopy with nitrous oxide flame to measure the chromium concentration in solution

Procedure:

1 Faeces are dried and milled and placed in a pre-ashed and tared tube;

2 The required amount of faeces are dried for 24 hours at 105 °C and ashed at 600 °C for at least 4 hours;

3 The sample is then reweighed, antibumping granules and 0.6 ml acid digestion mix (250 ml conc sulphuric acid (98%), 250 ml orthophosphoric acid (85%), 50 ml 10% aqueous Mn $SO_4.4H_2O$, 500 ml distilled water) are added;

4 The tube is then heated to boiling (140 °C) in a heating block for approximately 90 minutes. The hot plate is then turned off and the tubes removed and cooled to below 100°C;

5 0.3 ml of 4.5% potassium bromate is added and the tubes are then heated to the final temperature (220°C) over 90 minutes in the heating block;

6 The tubes are removed, cooled, and 9.72ml distilled water is added to each by dispenser;

7 Tubes are then shaken, allowed to settle for a minimum of 2 hours centrifuged and aspirated into the atomic absorption spectrophotometer (AAS) where they are read. The ASS is calibrated prior to reading of samples. A chromium-free blank, and a range of standards are prepared for this purpose.

Appendix 2

Appendices for chapter 2

Appendix 2.01

Analysis of variance for the linear regression of average intake (expressed relative to metabolic mass) against the average allantoin :creatinine ratio of spot urine samples from ten goats for the prerun

	df	Sum of Squares	Mean Square	F	P
Regression	1	10.764	10.764	0.109	0.750
Residual	8	790.450	98.806		
Total	9	801.214			

Appendix 2.02

Analysis of variance for the linear regression of intake (expressed relative to metabolic mass) against the average allantoin : creatinine ratio in spot urine samples collected from the ten goats during the first evaluation of the purine derivative technique

	df	Sum of Squares	Mean Square	F	P
Regression	1	824.080	824.080	23.592	0.001
Residual	8	279.446	34.931		
Total	9	1103.526			

Appendix 2.03

Analysis of variance for the linear regression of intake (expressed relative to metabolic mass) against average urinary allantoin : creatinine ratio obtained for spot samples collected from the ten goats during the second evaluation of the purine derivative technique

	df	Sum o f Squares	Mean Square	F	P
Regression	1	75.889	75.889	2.774	0.134
Residual	8	218.844	27.356		
Total	9	294.733			

(I_mmass = 10.364 * A/C ratio + 22.998)

Appendix 2.04

Analysis of variance of linear regression of intake (expressed relative to metabolic mass) against average urinary allantoin : creatinine ratio obtained from spot samples collected during the second evaluation of the purine derivative technique (goats 8 and 5 excluded from the data set)

	df	Sum of Squares	Mean Square	F	P
Regression	1	140.355	140.356	33.808	0.001
Residual	6	24.909	4.152		
Total	7	165.265			

Appendix 2.05

Analysis of variance for linear regression of intake (expressed relative to metabolic mass) against the ratio of allantoin to creatinine in spot urine samples obtained from the ten goats during the third evaluation of the purine derivative technique

	df	Sum of Squares	Mean Square	F	P
Regression	1	49.763	49.763	2.907	0.127
Residual	8	136.961	17.120		
Total	9	186.724			

Appendix 2.06

Analysis of variance of linear regression of intake (expressed relative to metabolic mass) against the allantoin : creatinine ratio in spot urine samples for run 3 when goat 7 was removed from the data set

	df	Sum of Squares	Mean Square	F	P
Regression	1	54.191	54.191	7.038	0.033
Residual	7	53.895	7.699		
Total	8	108.085			

Appendix 2.07

Data for the set of goats used in the third run comparing the purine derivative technique and the conventional marker method

Goat	Offered (g d^{-1})	Live Mass (kg)	Feed intake (g d^{-1})	Leucaena component of diet (%)	I_mmass
1	1100	35.80	663.4	39	45
2	900	38.62	574.0	34	40
3	800	37.71	700.4	28	46
4	700	31.37	608.6	27	46
5	600	29.43	549.6	26	43
6	900	36.34	542.4	44	37
7	800	31.77	695.2	29	52
8	600	32.40	532.4	28	39
9	700	31.68	633.8	27	47
10	1100	29.46	528.8	52	42

Appendix 2.08

Estimated slopes and their 95 % confidence limits for the linear regressions of intake (expressed relative to metabolic mass) against allantoin : creatinine ratio for the three runs evaluating the purine derivative technique

Run	Average slope	Lower confidence limit	Upper confidence limit
1	12.637	6.637	18.637
2	14.991	8.683	21.301
3	11.221	1.220	21.222

Appendix 2.09

Analysis of variance of linear regression of average intake (expressed relative to metabolic mass) against the daily allantoin : creatinine ratio for the six goats for the 3 collection days

	df	Sum of Squares	Mean Square	F	P
Regression	1	99.24	99.24	4.44	0.103
Residual	4	89.31	22.33	($r = 0.408$, SE = 4.725, $R^2_A = 0.408$)	
Total	5	188.55			

Appendix 2.10

Analysis of variance summary determining significance of the treatment effect (amount offered) on allantoin : creatinine ratio of 24 hour urine collection subsamples

Source of variation	df	Sum of Squares	Mean Square	F	P
day stratum	2	1.216	0.608	34.78	
day.*Units* stratum					
offered	1	0.233	0.233	13.36	0.003
Residual	14	0.245	0.017		
Total	17	1.69			

Appendix 2.11

Summary of the analysis (adjusted for covariate I_mmass) determining the effect of collection time (Time) on the allantoin : creatinine ratio during the spot-sampling trial

Change	df	Sum of Squares	Mean Square	F	P
Treat	1	0.79139	0.79139	26.57	<.001
animal	4	0.32314	0.08078	2.71	0.038
Time	3	0.18183	0.06061	2.03	0.118
Treat.Time	3	0.09005	0.03002	1.01	0.396
Residual	61	1.81715	0.02979		
Total	72	6.92081	0.09612		

Appendix 2.12

Allantoin : creatinine ratios and their standard errors for each treatment at each collection time predicted from the regression model (Appendix 2.09)

time	Treatment 1	se	Treatment 2	se
07h30	1.8203	0.0642	2.0760	0.0649
10h30	1.8059	0.0738	2.2313	0.0652
13h30	1.7481	0.0779	2.1600	0.0635
16h30	1.7335	0.0689	2.0371	0.0581

Appendix 3

Appendices for chapter 3

Appendix 3.01

Summary of the analysis of variance for the linear regression of average faecal output (g d^{-1}) against average feed intake (g d^{-1}) for ten goats during the first evaluation of the conventional marker method

	df	Sum of Squares	Mean Square	F	P
Regression	1	23907.8	23907.8	23.454	0.001
Residual	8	8154.9	1019.4		
Total	9	32062.7			

Appendix 3.02

Analysis of variance for linear regression of average faecal output (g d^{-1}) against average feed intake (g d^{-1}) for the second evaluation of the marker method

	df	Sum of Squares	Mean Square	F	P
Regression	1	13432.963	13432.963	12.678	0.007
Residual	8	8476.711	1059.589		
Total	9	21909.675			

Appendix 3.03

Regression analysis of faecal output (g d^{-1}) against absolute feed intake (g d^{-1}) for the ten goats during the third run (original data)

	df	Sum of Squares	Mean Square	F	P
Regression	1	9229.594	9229.594	27.752	0.001
Residual	8	2660.637	332.580		
Total	9	11890.231			

Appendix 3.04

Summary of variance of regression analysis of average faecal output (g d^{-1}) against average feed intake (g d^{-1}) for the third evaluation of the conventional marker technique, where the data has been manipulated

	df	Sum of Squares	Mean Square	F	P
Regression	1	10447.083	10447.083	57.913	≤0.001
Residual	8	1443.148	180.394		
Total	9	11890.231			

Appendix 3.05

Analysis of variance for linear regression of average faecal chromium concentration (mg kg^{-1}) against average faecal output (g d^{-1}) for the first evaluation of the conventional marker method

	df	Sum of Squares	Mean Square	F	P
Regression	1	1.790E+09	1.790E+09	46.927	<0.001
Residual	8	3.052E+08	3.815E+07		
Total	9	2.095E+09			

Appendix 3.06

Analysis of variance summary of the regression of average faecal chromium concentration (mg kg^{-1}) against average faecal output (g d^{-1}) for the second run evaluating the conventional marker method

	df	Sum of Squares	Mean Square	F	P
Regression	1	1.657E+09	1.657E+09	47.063	0.0001
Residual	8	2.817E+08	3.521E+07		
Total	9	1.939E+09			

Appendix 3.07

Analysis of variance of regression analysis of average faecal chromium concentration (parts per million) against average faecal output (g d^{-1}) for run 3

	df	Sum of Squares	Mean Square	F	P
Regression	1	3.611E+08	3.611E+08	94.042	<0.001
Residual	8	3.071E+07	3.840E+06		
Total	9	3.918E+08			

Appendix 3.08

Confidence limits for slopes of regressions of faecal output (g d^{-1}) against feed intake (g d^{-1})

Run	lower 95% confidence limit	upper 95% confidence limit
1	0.218	0.616
2	0.171	0.798
3	0.271	0.694

Apendix 3.09

Analysis of Variance of faecal output (g d^{-1}) against feed intake (g d^{-1}) for the pooled data from the three runs evaluating the conventional marker method

	df	Sum of Squares	Mean Square	F	P
Regression	1	49197.148	49197.148	74.694	≤0.001
Residual	28	18442.213	658.65046		
Total	29	67639.361			

Appendix 3.10
Confidence limits for slopes of regressions of faecal chromium concentration (mg kg^{-1}) against faecal output (g d^{-1})

Run	lower 95% confidence limit	upper 95% confidence limit
1	-315.843	-156.753
2	-367.464	-182.575
3	-215.714	-132.832

Appendix 3.11
Analysis of Variance for linear regression of faecal chromium concentration (mg kg^{-1}) against faecal output (g d^{-1}) for the pooled data from all three runs evaluating the conventional external marker method

	df	Sum of Squares	Mean Square	F	P
Regression	1	3.947E+09	3.947e+09	125.694	≤0.001
Residual	28	8.793E+08	3.140E+07		
Total	29	4.826e+09			

Appendix 4

Summary of data for the prerun and following three runs evaluating both the conventional external marker technique and the purine derivative technique

Animal	Mass	Feed offered	Feed intake	I_mmass	Average creatinine concentration	Average A/C ratio	Average faecal output	Average chromium concentration
	(Kg)	(g d^{-1}; air dried)	(g d^{-1}; dry matter basis)	(feed intake/ metabolic mass)	(mg.100 ml^{-1})		(g d^{-1}; dry matter basis)	(mg kg^{-1})
PRERUN								
1	24.50	1100	758	69	59.10	3.49		
2	23.50	950	764	72	66.25	4.00		
3	20.00	800	614	65	65.54	4.08		
4	19.50	650	549	59	62.71	3.02		
5	20.50	500	407	42	21.44	4.16		
6	21.50	950	574	57	105.09	3.30		
7	19.50	800	608	65	40.23	5.03		
8	18.00	500	437	50	52.04	3.11		
9	21.50	650	487	49	15.85	5.57		
10	22.50	1100	592	57	70.82	4.19		
RUN 1								
1	22.00	1100	673	66	48.87	3.75	341	45394
2	30.50	950	713	55	76.25	3.03	383	48527
3	23.00	800	639	61	56.75	4.04	309	49093
4	26.00	650	511	44	116.25	2.16	278	64254
5	25.00	500	386	35	52.93	2.40	242	69747
6	23.00	950	523	50	122.33	2.41	252	89456
7	23.00	800	585	56	81.85	2.99	294	59840
8	24.00	500	404	37	134.10	1.91	210	81475
9	26.50	650	512	44	79.01	2.33	257	61955
10	26.50	1100	718	61	65.50	3.78	326	45539
RUN 2								
1	33.22	1100	769	48	67.70	0.87	338	51037
2	33.24	900	756	49	147.86	0.74	330	53636
3	32.34	800	680	45	101.62	0.76	329	57695
4	31.84	700	559	37	221.73	0.56	239	75465
5	33.78	600	528	34	153.27	0.63	257	66325
6	29.46	900	574	41	121.71	0.65	205	81641
7	32.48	800	698	46	96.71	0.74	279	62730
8	33.58	600	522	34	70.83	0.81	260	76048
9	32.32	700	578	38	160.81	0.60	262	69076
10	32.68	1100	578	38	233.91	0.54	202	99917

RUN 3								
1	36	1100	663	45	157.98	2.27	305	57419
2	39	900	574	40	150.91	2.02	251	68108
3	38	800	700	46	102.01	2.13	336	51329
4	31	700	609	46	125.41	2.35	299	57765
5	29	600	550	43	124.02	2.26	272	66456
6	36	900	542	37	185.18	1.65	274	66742
7	32	800	695	52	145.53	2.07	352	53015
8	32	600	532	39	137.83	2.22	247	67120
9	32	700	634	47	133.49	2.25	302	59954
10	29	1100	529	42	149.67	1.84	250	69015

Appendix 5

Data for the goats from the 24-hour collections in the metabolic crates

Animal	Feed offered	average intake	average mass	Faecal output	I_mmass	Average creatinine concentration	Average A/C ratio
	($g\ d^{-1}$; air dried basis)	($g\ d^{-1}$; dry matter basis)	(kg)	($g\ d^{-1}$; dry matter basis)	(Intake. metabolic $mass^{-1}$)	(mg $100\ ml^{-1}$)	
1	600	518	29.94	280.52	41	182.16	1.54
2	600	553	29.39	282.40	44	226.06	1.57
3	600	550	30.95	287.00	42	234.39	1.33
4	800	660	32.16	327.47	49	235.95	1.72
5	800	703	30.11	344.90	55	172.97	1.62
6	800	688	29.82	336.35	54	225.59	1.79

Appendix 6

Data from the work done in metabolic crates determing the effect of time of collection on the allantoin : creatinine ratio

Animal	Day	Collection time	Creatinine concentration (mg. 100 ml^{-1})	A/C ratio	Animal	Day	Collection time	Creatinine concentration (mg. 100 ml^{-1})	A/C ratio
1	a	07h30	97.50	1.89	4	a	07h30	179.45	2.22
1	a	10h30	158.69	1.90	4	a	10h30	49.80	2.41
1	a	16h30	172.17	1.90	4	a	13h30	92.81	2.45
1	b	07h30	196.09	1.97	4	a	16h30	136.95	2.13
1	b	10h30	127.99	1.89	4	b	07h30	195.17	2.15
1	b	16h30	128.92	1.68	4	b	10h30	129.45	2.27
1	c	07h30	188.00	1.62	4	b	16h30	192.78	2.22
1	c	13h30	51.49	2.46	4	c	07h30	245.22	2.16
2	a	07h30	175.88	1.70	4	c	10h30	134.05	2.11
2	a	10h30	156.22	2.00	4	c	13h30	141.50	1.96
2	a	16h30	137.51	1.79	4	c	16h30	149.59	1.97
2	b	07h30	155.53	2.26	4	d	07h30	218.89	2.18
2	b	10h30	113.91	1.63	4	d	10h30	108.57	2.15
2	b	13h30	103.82	1.58	4	d	16h30	161.36	2.16
2	b	16h30	155.07	1.46	5	a	07h30	185.99	2.65
2	c	07h30	252.52	1.50	5	a	10h30	102.99	2.52
2	c	13h30	133.41	1.58	5	a	16h30	135.69	2.31
2	c	16h30	172.19	1.46	5	b	07h30	123.11	1.87
2	d	07h30	232.47	1.73	5	b	10h30	55.08	2.38
2	d	13h30	140.53	1.74	5	b	13h30	114.02	2.26
3	a	07h30	232.31	1.54	5	b	16h30	148.95	2.13
3	a	10h30	144.74	1.66	5	c	07h30	225.86	2.03
3	a	13h30	155.58	1.52	5	c	13h30	148.43	2.15
3	a	16h30	138.52	1.51	5	c	16h30	135.19	2.10
3	b	07h30	224.71	1.63	5	d	07h30	255.16	1.91
3	b	10h30	147.64	1.54	5	d	13h30	170.44	2.16
3	b	16h30	179.77	1.58	5	d	16h30	144.02	2.03
3	c	07h30	275.09	1.67	6	a	07h30	169.39	2.35
3	c	13h30	198.25	1.52	6	a	10h30	80.86	2.58
3	c	16h30	140.09	1.90	6	a	13h30	133.93	2.42
3	d	10h30	147.77	1.42	6	a	16h30	96.58	1.88
3	d	13h30	158.77	1.67	6	b	10h30	157.06	2.13
3	d	16h30	149.46	1.46	6	b	13h30	153.21	2.25
					6	b	16h30	135.16	1.95
					6	c	13h30	163.86	1.93
					6	c	16h30	157.74	1.70
					6	d	07h30	238.59	1.85
					6	d	10h30	81.39	1.95
					6	d	13h30	77.51	2.10
					6	d	16h30	82.95	2.11

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