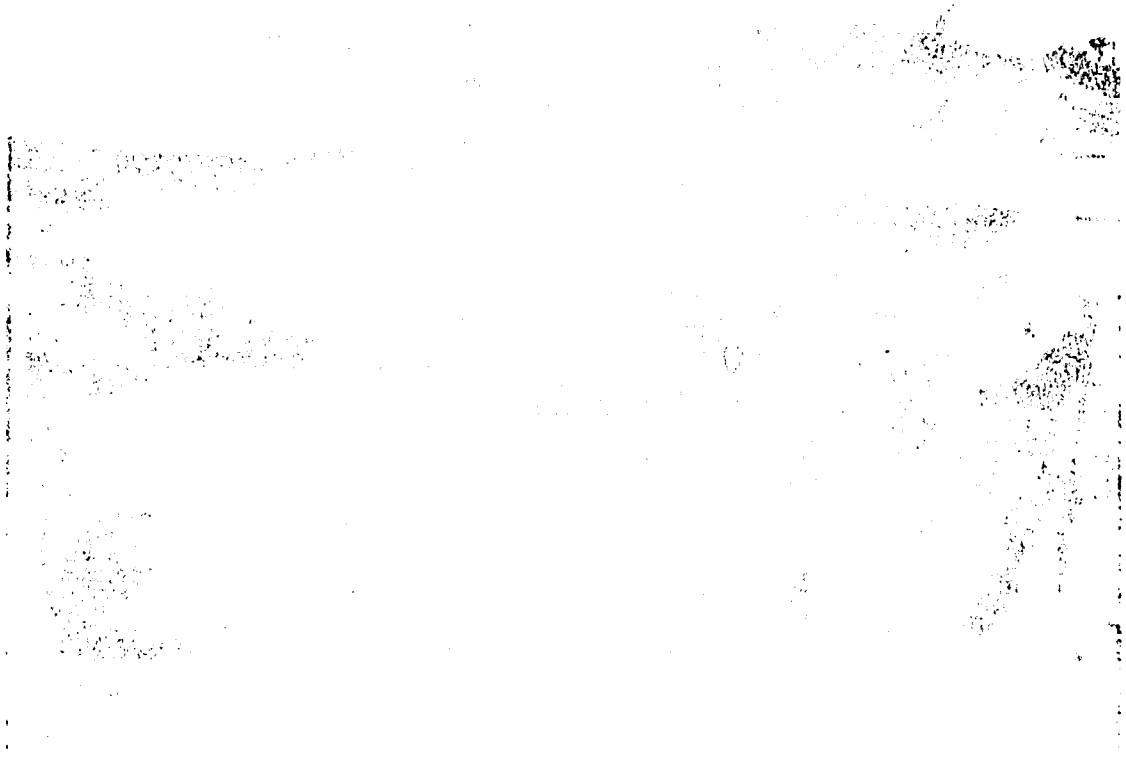
ILCA Manual 5

Feed evaluation



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Feed evaluation

P.O. Osuji, I.V. Nsahlai and H. Khalili

International Livestock Centre for Africa
Addis Ababa, Ethiopia
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Preface

This manual has been produced in order to standardise some analytical procedures at ILCA and among those NARS scientists who may be interested in the aspects of feed evaluation which the manual attempts to address. Furthermore, it is expected that the methods presented will, through use, stimulate discussion that may lead to their improvement or modification.

We would like to acknowledge the contributions of other ILCA nutritionists who reviewed this manual at various stages. For reviewing the manual, from a NARS perspective, we are grateful to Weizero Zinash Seleshi of the Institute of Agricultural Research (IAR), Holetta, Ethiopia.

Ms Anne Nyamu, of ILCA Publications Unit, did the final editing and pre-publication arrangements. For this we thank her. Finally we would like to acknowledge the contribution of Weizerit Yemesrach Mamo for patiently typing the various drafts of the manual during its preparation and Ato Negusu Ababu for preparing the illustrations.

P.O. Osuji

ILCA Debre Zeit Research Station

Introduction

Background

Poor nutrition is one of the major constraints to livestock productivity in sub-Saharan Africa (SSA). This is because animals thrive predominantly on high-fibre feeds (straws, stovers and native pasture hay) which are deficient in nutrients (nitrogen, sulphur, minerals, phosphorus etc) essential for microbial fermentation. Consequently, the digestibility and intake of digestible nutrients are unavoidably low. These deficiencies can partly be mitigated by supplementing roughage diets with feeds containing the deficient nutrients. Feeding practices developed in temperate countries are often inappropriate when applied to ruminant production systems in the tropics because temperate animals are fed straw as bulk in high density diets.

Roughage diets and supplements may differ vastly in quality and therefore in the quantity eaten by the animal. Previously digestibility and chemical composition were used to describe the nutritive value of fibrous feeds. This proved inadequate because these attributes give little indication of the quantity of such feed an animal will eat and the quality of nutrients derived through digestion. An understanding of the factors which affect rumen degradability of low-quality basal feeds and microbial protein production will assist scientists in designing diets that will be utilised more efficiently. In addition to determining responses (performance) from feeds, there is a need to establish causal relationships.

Feed-evaluation strategy

ILCA's feed evaluation programme is aimed at developing feed evaluation techniques that can be used in support of national agricultural research systems (NARS) in Africa. Table 1 gives a comparison of different feed-evaluation techniques. The methods described in this manual are aimed at standardising methods used

Table 1. *Comparison of four feed-evaluation techniques to predict digestibility in terms of the possibility of being adopted successfully in SSA countries.*

	Two-stage *in vitro*	Gas production	Nylon bag	Fungal enzymes
1. Requirements:				
a. Incubator	Yes	Yes	No	Yes
b. Electricity	Yes	Yes	No	Yes
c. Chemicals for buffer	Yes	Yes	No	Yes
d. CO_2 tank	Yes	Yes	No	No
e. Fistulated animals	Yes	Yes	Yes	No
f. Relative labour needs	Low	Low	Low	Low
2. Technical features:				
a. Relative precision	High	Low	Low	High
b. Ease of standardisation	Easy	Easy?	Diff*	Diff*
c. Estimate rate of digestion	Yes	Yes	Yes	No
d. Estimate extent of digestion	Yes	Yes	Yes	Yes
e. Relative number of samples/batches	High	High	Low	High
3. Relative cost of analysis:				
a. Instrument	High	High	Low	High
b. Chemicals	High	High	Zero	High
c. Labour, laboratory technician	Low	Low	Low	Low
d. Feed, labour for fistulated animals	High	High	High	Zero
e. Other materials (glassware etc)	High	High	Low	High
4. Relative overall possibilities of adoption in SSA	Low	Low	High	?

? : Not well defined.
* : Difficult.

by both ILCA and NARS scientists so as to make cross-laboratory and cross-site comparisons of results easier.

ILCA has a comparative advantage in the evaluation and utilisation of crop residues for the following reasons:

1. Low-quality crop residues and fibrous feeds are the primary components of animal diets in SSA whereas these feeds are used as bulk in developed countries.
2. The predominance of indigenous animals offers the opportunity to study the interaction between animals, feeds and the environment.
3. Smallholders in sub-Saharan Africa depend mainly on the fermentation of fibrous feeds to provide the protein and energy needs of their livestock because of the limited supply and high cost of conventional protein and energy supplements.

To ultimately have an impact on animal production in sub-Saharan Africa, ILCA has to work with and through NARS where the majority of animal production resources are found. To do this effectively, a major objective of the feed evaluation work at ILCA is to develop and provide standardised tools for ILCA and the NARS to use in animal production. These tools have to be easy to use, within reach of most NARS and independent of foreign funds.

A lot of effort has been put into the evaluation of feeds in SSA using diverse procedures. This manual gives details of selected techniques and procedures that could be used by both ILCA and NARS scientists. Their use will help generate information that, when put together, could be used to develop feeding systems for improved and sustainable ruminant production. It is expected that these procedures will be added to as new methods evolve or are modified in the light of experience within ILCA and the NARS.

1. Determination of voluntary intake, digestion and retention coefficients

1.1 Introduction

Voluntary intake is determined by offering animals a known quantity of feed and determining the amount remaining at the end of the feeding period. Digestion and retention coefficients are determined by collecting all the excreta (mainly urine and faeces) and analysing feed and excreta samples. The amounts of some of the nutrients absorbed and retained in the body or stored can also be determined by analysing urine and products such as milk.

Preparing for an experiment:

- Choose an appropriate experimental design.
- Set aside enough of the experimental feed for at least 42 days' feeding particularly for voluntary intake estimations.
- One week before the experiment, confine the animals in a barn, preferably in pens with a slatted floor. Deworm the animals and start feeding them the experimental diet. Ensure that water and mineral blocks are available *ad libitum*.
- Fit the animals with faecal collection bags (Figure 1) if they are not kept in stalls that permit faecal collection (Figure 2). Manual collection can also be done.

1.2 Direct method

The experiment takes 23 days if feed is in short supply, otherwise 28 days are preferable. Offer the animals 50 g DM/kg LW (dry matter/kg live weight) of feed daily (for roughage, e.g. stovers) or a minimum of 20–25% uneaten feed. Uneaten feed should not be refed. Follow this procedure throughout the experiment.

The first 14 (or 21) days of the experiment are a preliminary or adaptation period. Days 15 through 21, or 21 through 28 (7 days), form the intake measurement period.

1.2.1 Feed-intake measurement and collection of faeces and urine

(a) Weigh the animals on the first day of the experiment and place them in individual metabolism cages. Attach the faeces bags and provide feeds according to the experiment design. The type of metabolism cage will determine whether bags are needed or not. If faeces bags are available, however, they should be preferred because their use will reduce the chances of contamination of urine by faeces.

(b) On each of the intake measurement period days collect a sample (5–10%) of the feed offered and save it in a large bin with an airtight lid, in a plastic bucket, or in strong plastic bag. Freeze samples of fresh feed (e.g. silage and green forage).

(c) Clean the feeders thoroughly before feeding on day 15. Each day, collect all (or a fixed proportion) of the feed refused by each animal and save it in a paper sack. The uneaten feed collected on days 16 through 22 corresponds to the feed offered on days 15 through 21 (7 days). Use one sack for each animal for the 7 days. Freeze fresh material samples.

(d) Empty faeces bags daily throughout the experiment. This should be done immediately after removing and weighing uneaten feed and before feeding. On day 16, the faeces bags are emptied completely.

Note: Attaching bags from day 1 is to allow the animals sufficient time to adapt to the idea of carrying faecal bags. Collection starts from day 17 so bags should be emptied completely on day 16.

(e) On days 17 through 23 (7 days) collect all the faeces voided during the previous 24 hours in a bucket. Weigh, mix and take a sample for dry matter (for each day). Place a sample (5–10% aliquot) in a plastic bag and save it frozen or dried pending chemical analysis.

Figure 1. *Plan for sheep metabolism crates (ILCA Debre Zeit Research Station).*

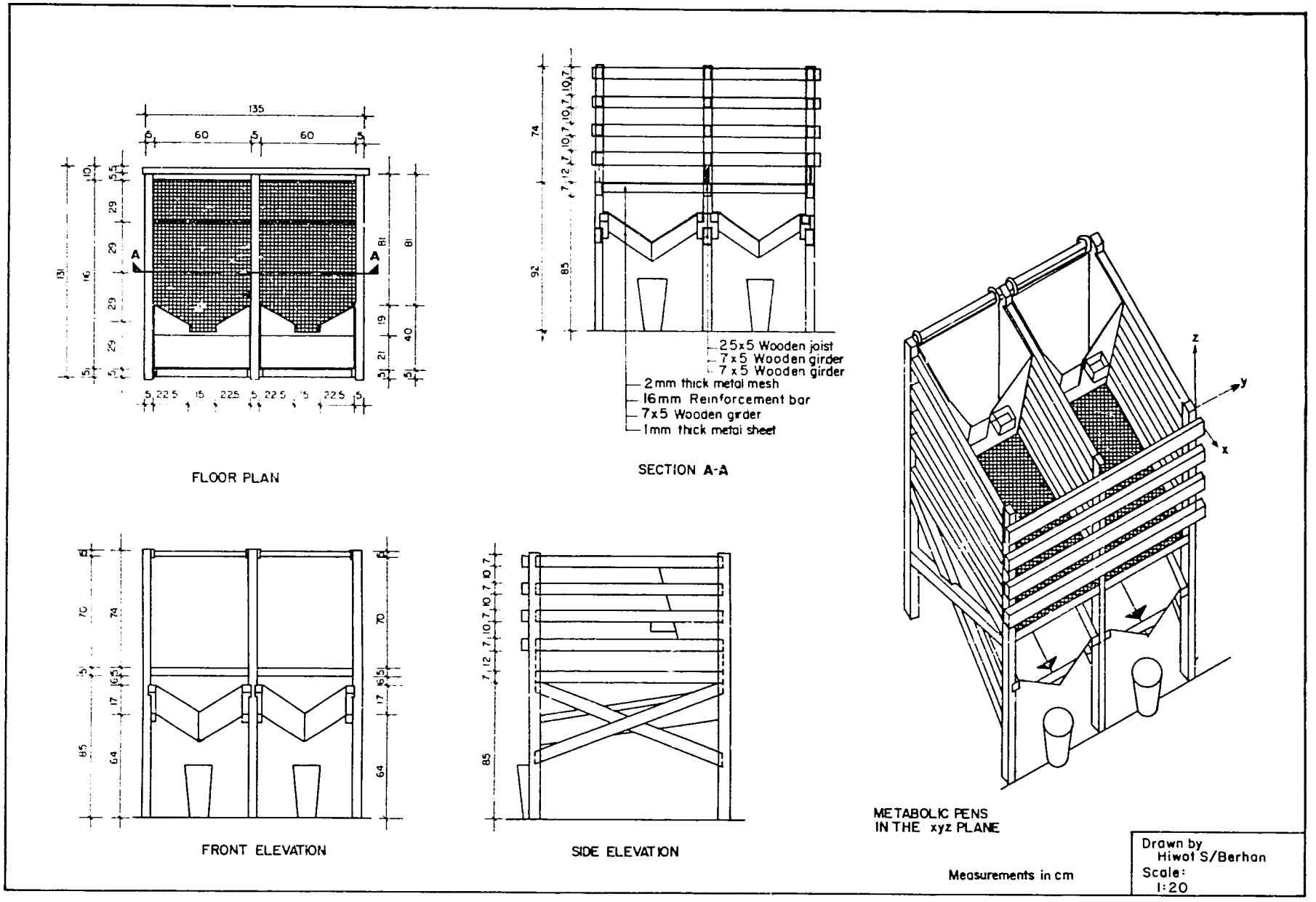
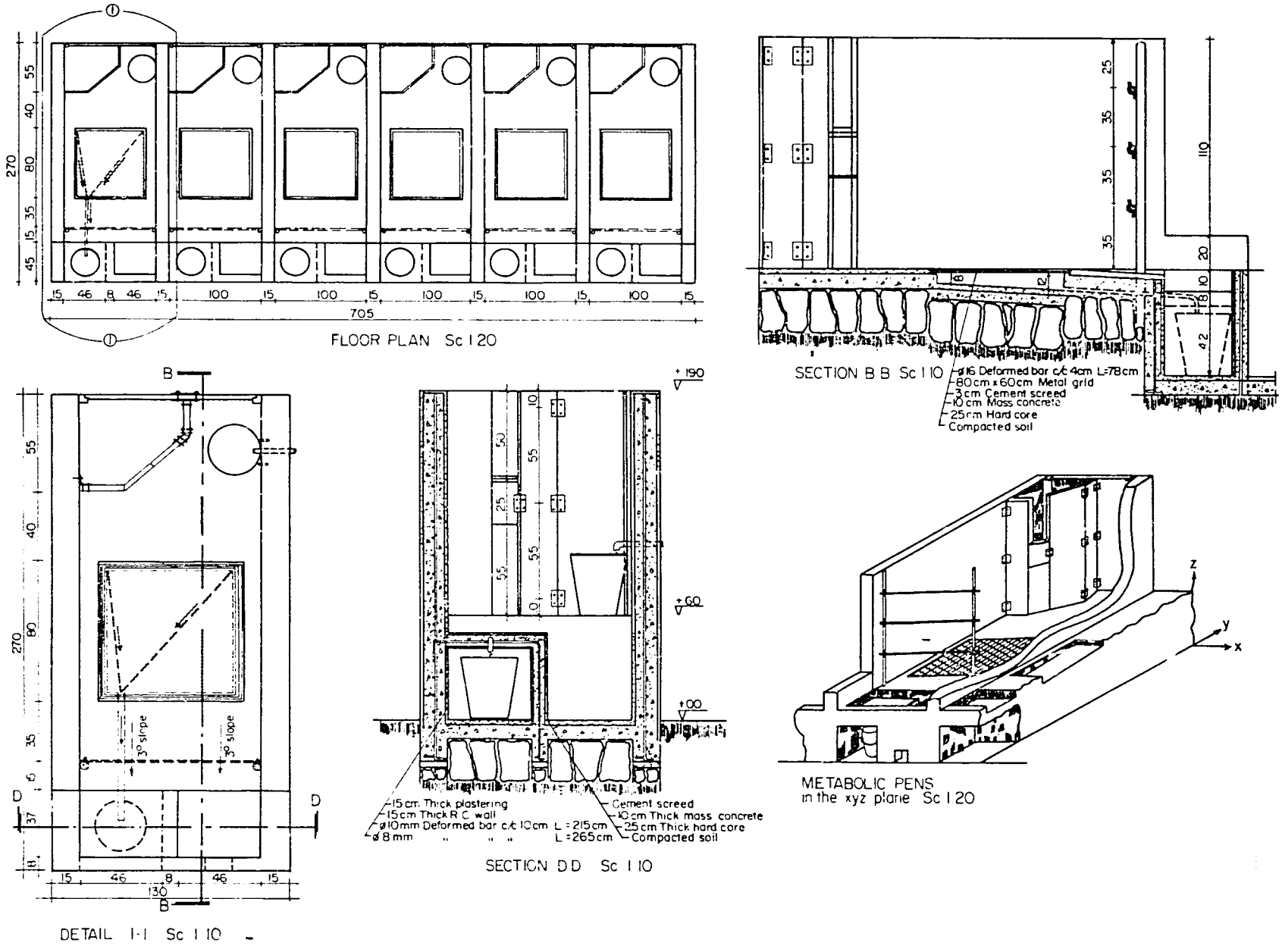


Figure 2. *Plan for cattle metabolism crates (ILCA Debre Zeit Research Station).*



(f) Collect and sample urine in the same way. Add acid (e.g. 0.2N HCl, 0.1N H_2SO_4) to ensure that pH is less than 3 to avoid loss of nitrogen (N).

(g) Weigh the animal after faecal collection and before feeding on day 23.

The experimental procedure can be summarised as follows:

Day	Action			
1	Weigh animal, start feeding experimental diets, determine voluntary intake			
2 to 14	Adaptation period			
15	Collect feed sample			
16	Collect feed sample, collect uneaten feed			
17	Collect feed sample, collect uneaten feed, collect faeces and urine			
18	"	"	"	
19	"	"	"	
20	"	"	"	
21	"	"	"	
22	"	"	"	
23		"	"	weigh animal

Note: When voluntary intake is being estimated the measurement time is better extended to 28 days.

1.2.2 Preparing samples for chemical analysis

Feed samples

Mix the daily feed samples and grind through a 2 mm screen. Mix and place 2 kg of the ground sample in a plastic bag and seal the bag to prevent changes in moisture content. Take an 800 g sample and seal it in a bottle for long-term storage as a precaution against loss or damage of sample while forwarding to the laboratory for analysis. Grind about 200 g through a 1 mm screen and store in an airtight container such as a 250 ml glass or plastic bottle.

Uneaten feed

Reweigh, subsample and immediately grind the uneaten feed from each animal. Place 300 g in a plastic bag and seal it to prevent changes in moisture content. Regrind this sample through the hammer mill (1 mm screen), as above, and store in an airtight container.

Faeces

Dry the faeces samples from each animal under forced air at 60°C. Equilibrate the sample with atmospheric moisture for at least 48 hours, weigh it and immediately grind through a 2 mm screen. Regrind a 300 g representative subsample through a 1 mm screen and store it in an airtight container.

Dry-matter analysis

This should be conducted as soon as possible on the prepared samples. Weigh the sample, dry at 100°C for 24 hours and weigh again. The weight difference represents the moisture content.

Take samples of the composited daily urine collected for each animal for analysis of nitrogen, minerals, urea, purine derivatives, creatinine etc; the kind of analysis is determined by the hypothesis being tested.

1.2.3 Calculations

(a) Voluntary dry-matter intake and apparent dry-matter digestibility of the feed are calculated for each animal as follows:

Average daily dry-matter intake = A – B

$$\text{Apparent dry-matter digestibility} = \left(\frac{A - B - C}{A - B}\right) \times 100$$

where:

A = average dry matter offered daily

B = average dry matter refused daily

C = average dry matter voided in faeces daily.

A, B and C are calculated as follows:

$$A = \frac{\text{Average weight of feed offered daily (kg)}^{1} \times \text{dry matter content of feed (\%)}}{100}$$

$$B = \frac{\text{Average weight of uneaten feed daily (kg)}^{2} \times \text{dry matter content of refusals (\%)}}{100}$$

$$C = \frac{(\text{\% dry matter of partial dry faeces}) \times 7 \times (\text{weight of faeces aliquot}^{3})}{100 \times 7}$$

1. Average weight of forage offered according to daily barn records for days 15 through 21.
2. Weight of uneaten feed before grinding through hammer mill, divided by the number of days in collection period.
3. Weight of faeces before grinding through the 2 mm screen of the hammer mill (this represents 20% of the total faeces voided), divided by the number of days in collection period.

(b) Digestion coefficients for all other nutrients are calculated using the dry-matter values A, B and C above and the percentage of nutrients in feed, refusals and faeces expressed on a dry-matter basis.

2. Special methods for measuring digestibility

2.1 Indicator method

2.1.1 Introduction

Apparent digestibility of a diet can be estimated using a natural constituent of the feed as an indicator. Acid insoluble ash (AIA) can be used in this way (van Keulen and Young, 1977). The ratio between the concentration of AIA in the feed and the concentration of AIA in the faeces gives an estimate of digestibility.

$$\text{Digestibility of DM} = 100 - \left[\frac{100\ (\text{AIA concentration in diet})}{(\text{AIA concentration in faeces})}\right]$$

2.1.2 Sampling

Collect samples of every feed used in the experiment and analyse for AIA.

During the last six days of each collection period or experiment collect faecal grab samples at five representative times a day. The precise timing is determined by the individual trials. Pool samples for each animal and period and freeze them. The samples are subsequently thawed, dried at 60°C and stored at room temperature for later analyses. This procedure stops the faeces growing mould.

Digestibility of dietary constituents can be calculated after chemical analyses. For example, the digestibility of neutral detergent fibre (NDF) is calculated as:

$$\text{Digestibility of DM} = 100 - \left[\frac{100\ (\text{AIA concentration in diet} \times \text{NDF concentration in faeces})}{(\text{AIA concentration in faeces} \times \text{NDF concentration in diet})}\right]$$

2.2 The nylon-bag technique

2.2.1 Introduction

The nylon-bag technique provides a means of ranking feeds according to the rate and extent of degradation of dry matter, organic matter, nitrogen or other nutritional parameters. It involves incubating samples of feeds in the rumens of fistulated animals for periods of from 6 to 120 hours and subsequent determination of the disappearance of the different feed components. The nylon-bag technique is appropriate for use by NARS because it does not involve large amounts of imported inputs.

The nylon-bag technique uses bags (6.5 x 14 cm) made of nylon mesh (30–50 µm). A sample of known weight is tightly sealed in the nylon bags and placed in the rumen of a fistulated animal. After the required period of time, the sample is removed, washed, dried and weighed. Degradability (or disappearance) of the substrate is determined by the weight loss during the incubation periods. The dried residue may also be used for chemical analyses.

The pore size of the bags must allow entry of rumen microbes and escape of accumulated gases, and keep solid particle losses to a minimum. The efflux of feed particles from the bags without breakdown by rumen microbes is corrected for by using zero-hour bags. These bags are filled with the substrate but are not incubated in the rumen; they are washed and dried in the same way as the incubated bags. Furthermore, the zero-hour bags are used to correct for passage of material from pressure applied to the nylon bags during washing.

The major factors which affect nylon-bag degradation include how the bags are placed in the rumen, particle size of sample *vis-à-vis* the pore size of the nylon bag, loss of feed particles through the bag cloth (a function of fineness of grinding, cloth pore size and feed material), method of washing, the length of time that the samples are incubated in the rumen and the rumen environment in which degradability is determined.

If the intention is to rank feeds with regards to their nutritive value without imposing any treatment on the fistulated animals, then the diet of the animals must be adequate in rumen-degradable nitrogen (N).

When N disappearance of low N feed is of interest then the nylon-bag technique is not precise enough as microbial contamination will bias the results. In such cases there is need to allow for correction, for example by analysing for microbial N.

The nylon-bag technique is a very simple and useful biological tool for *in vivo (in sacco)* animal-nutrition studies. If determination of the dry-matter disappearance is the only analysis of importance, then the main analytical equipment needed would be accurate balances and drying ovens. In general, the following materials are required to determine the degradability of feeds using the nylon-bag method:

- Nylon bag
- Nylon string/cord
- Analytical balance
- Drying oven or drying device
- Washing machine or running tap water
- Desiccator.

The detailed procedure of the nylon-bag method can be modified depending on the availability of the required materials. However, the basic procedures should remain the same. The size of bag relative to sample size is very important and is governed by the amount of residues required for analysis. The incubated material must be able to move freely within the bags to avoid formation of micro-environments in the bags which would result in poor replication.

2.2.2 The nylon-bag procedure

At ILCA's Debre Zeit Research Station, the following procedure is used:

- Grind the feeds through a 2 mm screen (mesh).
- Dry the ground samples in an oven at 100–105°C overnight to determine the dry matter (DM).
- Number 6.5 x 14 cm nylon bags with a pore size of 41 µm, oven dry at 60–65°C for 30 minutes and measure their empty weights immediately, or after allowing to cool to room temperature in a desiccator. The ratio of width to length of the bags should be between 1:1 (i.e. square) and 1:2.5.
- Place about 2.0 g DM of sample in each nylon bag. Sample size should be related to the total surface area of the bag; although there is no consensus 15 mg of sample material per square centimetre of bag (bag area includes all the external surfaces) has been suggested.
- Tie the bags tightly using nylon string which is resistant to rumen micro-organisms.
- Anchor the bags with about 50 cm of nylon chord (about 25 cm are required for sheep/goats) to the cannula top. Place the bags deep into the rumen of a fistulated animal.

Incubation

Incubate samples for 0, 6, 12, 24, 48, 72, 96 and 120 hours for forages and up to 48–72 hours for concentrates. Place the 120-hour samples into the rumen on the morning of day 1 of incubation in each fistulated animal. On the next morning (day 2), insert the 96-hour samples at the same hour as the day 1 samples. Continue the activity in the same manner until all samples are in the rumen.

Three or four rumen-fistulated animals are needed to determine animal variation but the number may vary according to specific experimental designs. The samples, which are mostly prepared in triplicate for every feed and each incubation hour, are incubated in each of the experimental animals in the same way. The number of replicates and sample size depend on the amount of residue required for further analysis. For cattle, 40 to 60 bags can be incubated at the same time while for sheep/goats 8 to 10 bags can be used for each animal. All the bags are taken out at the same time. This method is referred to as sequential addition. The alternative method is to incubate all the bags on day 1 at the same time and withdraw them at different

times (sequential withdrawal). The advantage of sequential addition over sequential withdrawal is that there is less disturbance of the rumen environment. In addition, sequential removal is more prone to error.

Washing and drying

Immediately wash the bags (including the zero hour samples) with cold water for about 30 minutes in a washing machine or under running tap water while rubbing gently between thumb and fingers until the water runs clear. Dry the washed bags in an oven at 60–65°C for about 48 hours. Either allow them to cool down in a desiccator or weigh immediately. Determine the dry matter of the residue samples using the same method as with feed samples. Finally, calculate the disappearance using the formula:

$$\text{Disappearance} = \frac{(SWa - BW) \times DMa - (SWb - BW) \times DMb}{(SWa - BW) \times DMa}$$

where:

SWa = Weight of the original sample + nylon bag

BW = Weight of empty nylon bag

SWb = Weight of the sample + nylon bag after incubation

DMa = Dry matter of feed sample

DMb = Dry matter of residue sample.

2.2.3 Handling nylon-bag data

The nylon-bag technique generates considerable data because of the number of hours, replicates and animals involved. It is therefore desirable to process such data electronically to minimise human error. To process:

- the data are plotted against time
- outliers are edited out
- the model of DM disappearance proposed by Ørskov and McDonald (1979) or by McDonald (1981) is fitted to summarise the data and derive degradation parameters.

$Y = a + b\,(1-e^{-ct})$ ..... Ørskov and McDonald (1979)

$Y = a + b\,(1-e^{-c(t-tl)})$ ..... McDonald (1981) model with lag

where:

Y = degradability at time (t)

a = intercept

b = potentially degradable fraction

c = rate of degradation of b

tl = lag time.

Computer analysis

Any computer program that can fit non-linear models to data can be used to estimate degradability, e.g. SAS, SPSS, NAWAY, NEWAY. NAWAY and NEWAY were developed by the Rowett Research Institute. Copies can be acquired either from the Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB2 9SB, UK, or from ILCA's Debre Zeit Research Station. At Debre Zeit a simpler model is frequently used to handle a large volume of data. The data are entered as given in Appendix 1.

Manual calculation

Unlike computer programs that give precise and non-subjective estimates of the degradation constants, manual calculations only give rough approximations.

The following data will illustrate how manual calculation could be done:

Time	Dry-matter disappearance (DMD, %)
0	15.8
6	27.7
12	30.8
24	43.1
48	52.7
72	54.4
96	55.9

The data are plotted and extrapolated, if necessary, until the asymptote (Figure 3) to estimate degradation characteristics as defined in the equation $Y = a+b(1-e^{-ct})$ (Ørskov and McDonald, 1979)

where:

Y = degradability at time (t)

a = intercept

b = potentially degradable fraction

c = rate of degradation of b.

The asymptote represents (a+b) or the potential degradability and in this example it is 56%. The intercept of the curve is represented by (a) and gives the DMD value at time 0 hour.[1] The intercept in this example is approximately 19%. The (b) value can be calculated as the difference between the asymptotic DMD and the intercept (a). In this example b = 56 – 19 = 37%.

To calculate the rate of degradation, the above equation needs to be transformed:

$$Y = a + b\,(1-e^{-ct}) = (a + b) - be^{-ct}$$

$$\frac{Y - (a + b)}{-b} = e^{-ct} = Y^1$$

Take the natural logarithmic derivative of both sides:

$$\text{Ln}\, Y^1 = -ct$$

$$\text{Hence } c = \frac{\text{Ln}\, Y^1}{-t}$$

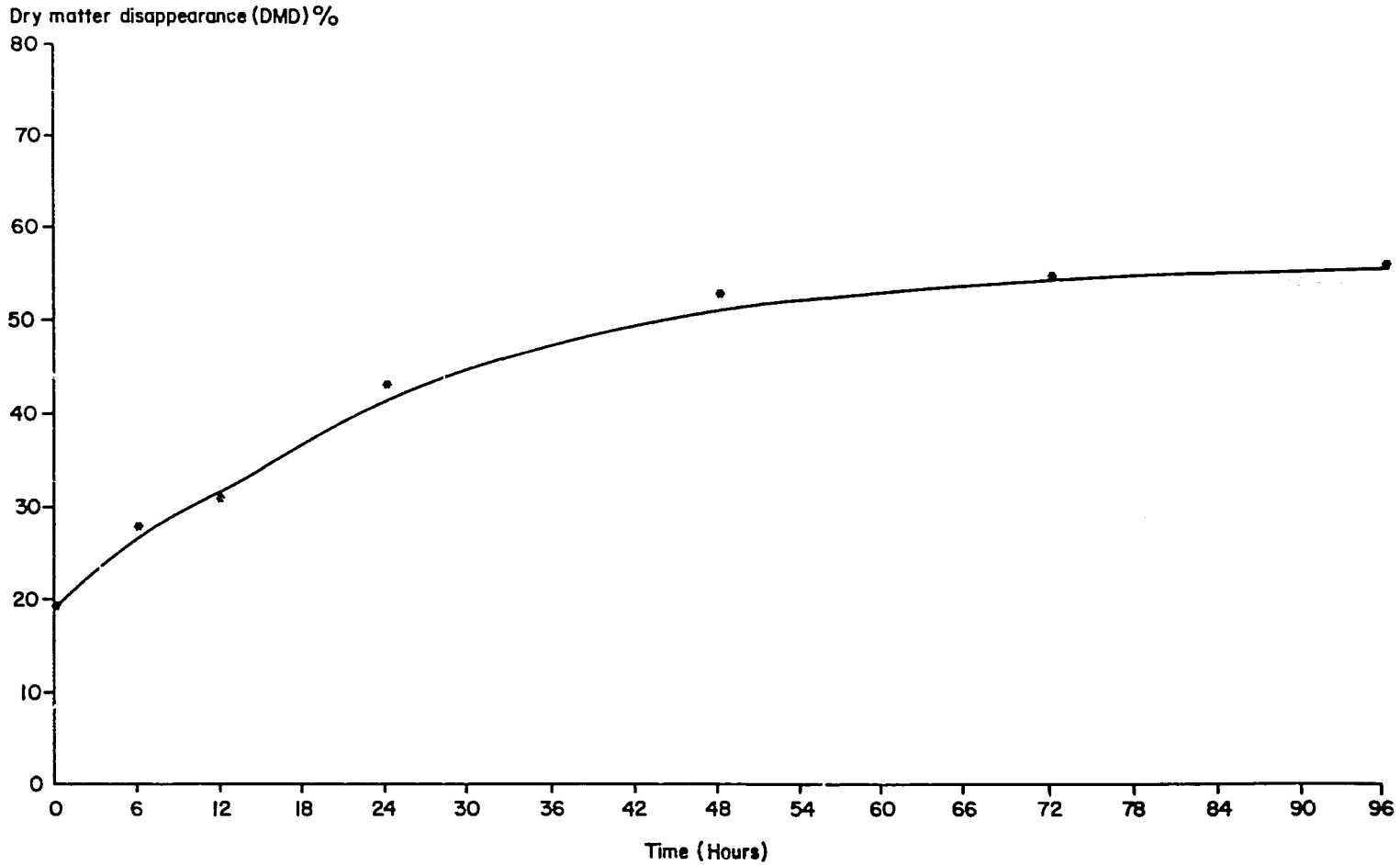
To get good estimates of (c), select Y (i.e. DMD% at time t) when the curve is changing most rapidly. From the above graph 30 h of incubation is about the time when DMD is most sensitive to changes in time and corresponds to a DMD of 45.5%.

Hence,

$$Y^1 = \frac{45.5 - (19 + 37)}{-37} = 0.28378$$

1. Note that the washing value (%) is plotted on the curve to serve as a guide to the intercept and frequently is not used in the computer program to fit the curve. It can therefore be excluded when extrapolating to the intercept.

Figure 3. *Disappearance of dry matter with time.*



$$c = \frac{Ln\ (0.28378)}{30} = 0.0420$$

It is important to determine how much these approximations deviate from values obtained using computer non-linear regression models (Table 2).

Table 2. *Comparison of manual and computer-generated degradation constants.*

Parameter	SAS estimates	Manual estimates	Manual/SAS ratio
a	17.7	19	1.07
b	39.2	37	0.94
c	0.0416	0.0420	1.01
(a+b)	56.9	56	0.98

Although manual approximations are unlikely to yield degradation constants of comparable accuracy to computer estimates, manual calculations are quite adequate for exploring the relative differences between feeds or diets.

2.3 The Menke *in vitro* gas-production technique

2.3.1 Introduction

The Menke *in vitro* gas-production technique (Menke et al, 1979) is commonly used to determine the amount of gas produced over a 24-hour incubation period. The amount of gas released when a feed is incubated *in vitro* with rumen fluid is closely related to the digestibility of the feed.

At ILCA's Debre Zeit Research Station, this method has been modified. The gas produced is read either at a fixed incubation time, 24 hours, or at a series of incubation times (sequential incubation), mainly 6, 12, 24, 48, 72 and 96 hours. Sometimes, 3 and 120 hours of incubation are included if the asymptote of the gas production curve is to be clearly defined. Both fresh and dry samples can be assayed and the gas produced from 200 mg DM of both samples is determined for comparison. Grass hay from Sululta is used as a standard sample. Three syringes containing the Sululta grass hay and three other syringes each containing 70% hay and 30% starch are incubated with every set of samples.

The data from the incubation of these standard samples are collected for six months. The average gas production from the standard samples for all incubation periods are determined separately and used as correction factors.

2.3.2 Gas-production procedure

The following apparatus is needed:

- 200 mm long glass syringes (piston pipettes) calibrated to 100 ml, with capillary attachment
- Silicon rubber tube about 45 cm long
- Plastic clip
- Analytical balance
- Water bath with grid for supporting syringes

- Suction bottle/Erlenmeyer flask (2 litres)
- Carbon dioxide cylinder with regulator
- Glass syringe rack for storing the syringes
- Buckner funnel and flask
- Plastic buckets (4 litres) and cheese cloth
- 10 ml automatic syringe (an aliquot dispenser)
- Magnetic stirrer
- Thermometer.

Preparation and weighing the feed sample

Before weighing, grind the dry material through a 1 mm screen. Avoid very fine grinding because of observed differences in digestibility (*in vivo*) and gas production (*in vitro*) between coarse and finely ground roughage. For fresh samples, use a cutting mill, a slow rotating meat cutter or a pair of scissors to chop the roughage.

Weigh about 200 mg DM of the sample on a weighing boat (Figure 4).[2] Push the piston (greased with vaseline to ensure easy movement and precise fitting) down the cylinder. Close the silicon rubber tube attached to the capillary attachment (needle) of the syringe with a plastic clip. Fermentation is carried out in this glass syringe.

Rumen fluid

Not more than 15 minutes before the trial starts, collect rumen fluid (about 1 litre) in equal proportions from two rumen-fistulated donor cows/small ruminants under the same feeding regime (at Debre Zeit, grass hay given *ad libitum* and a total of 2.4 kg cottonseed cake given in two meals daily). Filter the sample through two layers of cheese cloth into a warm flask (kept in a bucket of water at 37–38°C) and flush with carbon dioxide (CO_2). Take the rumen fluid before the morning feed or before feeding the diet supplement.

Solutions

Prepare five different solutions as media and mix with rumen liquor. The composition of the solutions are as follows:

Solution A (Micro mineral)

13.2 g calcium chloride ($CaCl_2.2H_2O$)

10.0 g manganese chloride ($MnCl_2.4H_2O$)

1.0 g cobalt chloride ($CoCl_2.6H_2O$)

8.0 g iron chloride ($FeCl_3.6H_2O$)

made up to 100 ml with distilled water.

Solution B (Buffer solution)

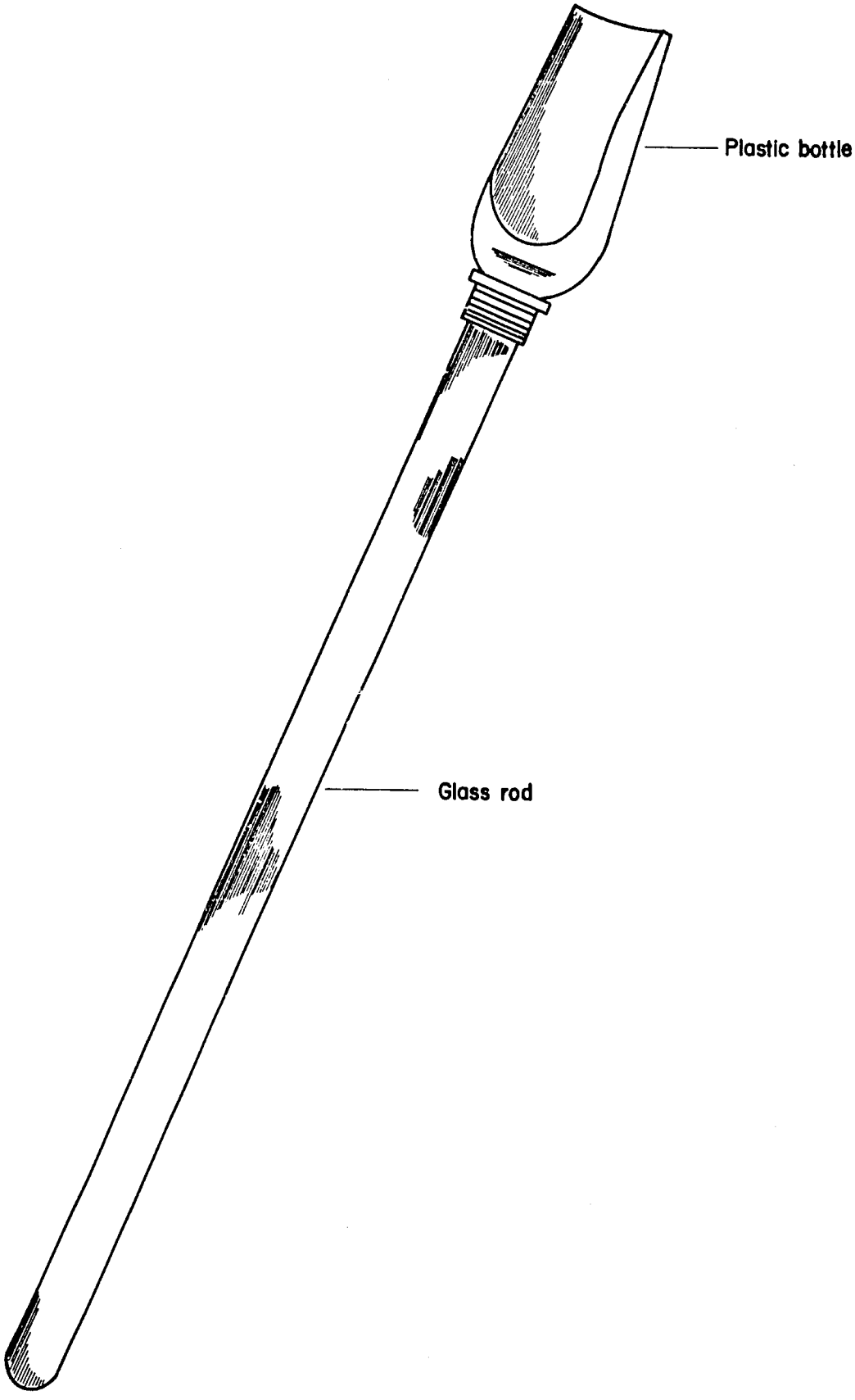
39.0 g sodium hydrogen carbonate ($NaHCO_3$) or

35.0 g $NaHCO_3$ + 4.0 g ammonium hydrogen carbonate ($(NH_4)HCO_3$)

made up to 1 litre with distilled water.

2. The boat can be locally prepared. Cut a very small plastic bottle and fit the neck with a glass rod. This helps deposit the sample at the bottom of the glass syringe (piston pipette) without it sticking to the sides of the glass.

Figure 4. *Weighing boat.*



Solution C (Macro mineral)

5.7 g disodium hydrogen phosphate (Na_2HPO_4)

6.2 g potassium dihydrogen phosphate (KH_2PO_4)

0.6 g magnesium sulphate ($Mg\ SO_4.7H_2O$)

made up to 1 litre with distilled water.

Resazurin solution

100 mg resazurin made up to 100 ml with distilled water.

Reducing solution

4 ml sodium hydroxide (1N NaOH)

625 mg sodium sulphide ($Na_2S.9H_2O$)

added to 95 ml distilled water.

The reducing solution must be freshly prepared each time shortly before the rumen fluid is taken from the animal. The other solutions can be made up and stored.

Preparation of media

Pour 400 ml distilled water, 0.1 ml solution A, 200 ml solution B, 200 ml solution C and 1 ml resazurin into a Buckner flask. You will observe a bluish colour. Add 40 ml reducing solution while mixing with a magnetic stirrer. Flush the mixture with CO_2 gas while the reducing solution is being added. The colour will change from bluish through a reddish colour (oxidised) to colourless (reduced).

Add the rumen fluid. The ratio of rumen fluid to buffer medium is 1:2 (v/v).

Preparing syringes for incubation

Place the glass syringes containing the substrates in a water bath at 38–39°C an hour before incubation starts. During incubation, remove the glass syringe from the water bath and firmly fix the rubber tube on to the needle of the automatic syringe.

Pipette 30 ml of the rumen fluid/medium mixture with an automatic syringe into each of the pre-warmed glass syringes. Bring any air bubbles trapped in the syringe to the surface by gentle shaking and remove them through the capillary attachment by careful upward orientation and pushing the piston. Close the clip on the tube, read the initial volume and record it as V_0. Place the syringe back in the syringe rack for incubation in the water bath at 38–39°C.

Twenty-four hour incubation

Incubate the feeds in triplicate in at least two different sessions (with different rumen fluids), yielding six parallel measurements. Include four glass syringes containing rumen fluid/media mixture without substrate (blank), three glass syringes containing Sululta hay (200 mg DM), i.e. the standard, and three syringes containing 140 mg DM Sululta hay and 60 mg starch in every set to control differences in composition and activity of the rumen fluid (control incubations). The readings from the blank, grass hay and grass hay + starch are GP_0, GPH and GPHS, respectively. The exact reading where the end mark on the piston lies is regarded as the initial volume (V_0).

Read the position of the piston 6 h after incubation begins and record it as intermediate volume ($V_{int.}$). Move the piston gently beforehand to make sure that it is not sticking. If gas production exceeds 60 ml, open the clip and move the piston back to the 30 ml mark, while keeping it vertical, thus allowing most of the gas which has formed to escape. Record the exact reading before the piston is moved back to 30 ml as V_1 for the next incubation hour. Continue the incubation and take the final reading after 24 hours (V_{final}).

Sequential incubation (3, 6, 12, 24, 48, 72, 96 and 120 h)

To determine the volume of gas produced at 3, 6, 12, 24, 48, 72, 96 and 120 hours, a slightly modified procedure is followed regarding the number of parallel measurements. Except for the blank which is incubated in triplicate, the substrates and both standards are all incubated in duplicate for every incubation period (time).

In the 3- and 6-hour incubations, the gas produced is not expected to exceed 60 ml and thus there is no V_1. For the rest of the incubation periods conducted in series, i.e. 12, 24, 48, 72, 96 and 120 hours, consider the 12-hour reading as the first calibrated volume ($1V_1$). During calibration reset the piston to the 30 ml position for all of the syringes except for the blanks. Since there is no calibration before the 12-hour reading, the net gas production at 3, 6 and 12 h incubation periods is simply the final reading of gas produced minus the sum of V_0 and blanks at these hours.

For all other readings taken at and after 24 hours of incubation, calibrate only when the gas produced exceeds 60 ml. Release the gas produced and set the piston back to 30 ml (second calibration). The second calibrated volume ($2V_1$) is the sum of the $1V_1$ and the most recent reading taken before the second calibration. Use the $2V_1$, like the $1V_1$, only for the calculation of the net gas production for the succeeding incubation hour. Likewise, if there is a need to release the gas for the third time, the $3V_1$ is the sum of the $2V_1$ and the most recent reading before the gas is released. The same method of calculation applies in this case.

Calculations

Use the volumes of gas recorded at different times to estimate the *in vitro* gas production during incubation of the feeds. Gas production (GP) is defined as the total increase in volume minus the blank (GP_0). Subtract the mean blank value (GP_0) from the recorded gas production of all samples and standards to give the net gas production. Relate the gas volume from which the blank value has been deducted to the weight of exactly 200 mg DM of the sample taken.

After collecting ample data for the standards, calculate the standard value for, in the case of Debre Zeit, the Sululta grass hay and the Sululta hay + starch. Estimate the mean gas production (in ml/200 mg DM) with each of the standard feeds at each incubation period and calculate the correction factor for the corresponding periods. Divide the standard value for the Sululta grass hay by the measured net value of the same standard hay for the particular incubation session to give the correction factor (FH). The correction factor for hay + starch standard (FHS) is derived similarly. Use the mean of these two factors FH and FHS for correction of the sample measurements.

It is necessary to check from the standards included in every set how far the recorded values deviate from the standard values. The difference between FH and FHS is expected to be insignificant. The theoretically accepted values for FH and FHS lie between 0.9 and 1.1. If the factors do not fall within this range, the test must be repeated.

The general formula for calculating the corrected gas production is:

$$GP\ (ml/200\ mg\ DM) = \frac{(XV_1 - 30X + V_{final} - V_0 - GP_0) \times 200\ ((FH + FHS)/2)}{\text{weight in mg DM}}$$

where:

X = the number of times that the gas is released from the syringe and the volume is set back to 30 ml

V_0 = the initial volume of gas recorded before incubation starts

V_1 = the volume of gas recorded before the gas is released from the syringe and the volume is set back to 30 ml

V_{final} = the final volume of gas recorded at the end of incubation time

GP_0 = the mean blank value

FH = the correction factor for the standard grass hay

FHS = the correction factor for the grass hay/starch standard

DM = dry matter.

Data from gas production may be processed like data obtained with the nylon-bag technique. More often the following model is fitted to the data:

$$Y = b(1-e^{-ct})$$

where:

Y = the volume of gas produced with time (t)

c = the gas production rate

b = the potential extent of gas production.

The intercept is not included in the model with the understanding that no gas is produced from unfermented feed.

3. Kinetics of digestion and of passage

3.1 Flow rates

The extent of digestion of a feed depends on its rate of digestion and on the time the feed spends in the digestion pool. The animal's requirements are met from the digested component of intake. This section describes methods for calculating flow rate constants.

Flow rate is the rate (mass/time) at which digesta leaves a compartment. Fractional outflow rate is the proportion of a component of feed or of a marker which leaves the compartment per unit time. Flow rate or fractional outflow rate are estimated to determine the mean duration feed remains in the gastrointestinal (GI) tract, usually called the mean retention time (MRT). The time available for digestion in each pool ($t_{1/2}$) is also estimated since it is reported to have a strong positive correlation with organic matter (OM) digestibility (Grovum and Williams, 1977). Markers or rumen evacuation can be used to estimate both rumen volume and passage rates.

3.2 Rumen-evacuation technique

3.2.1 Introduction

The complete removal and mixing of reticulorumen contents appears to be a valid technique in digestion studies. Towne et al (1986) found no differences in rumen microbial activity between evacuated and non-evacuated animals.

Rumen-evacuation-derived rates of digestion of fibre (kd) are highly correlated with *in vivo* digestibility of fibre (Tamminga et al, 1989; Huhtanen and Khalili, 1991; Khalili, 1993), lending validity to this technique in estimating the rate of fibre digestion.

Estimating the rate of fibre digestion in the rumen using the nylon-bag technique gave results lower than the results based on rumen evacuation data (Tamminga et al, 1989). These results supported the suggestion by Aitchison et al (1986) that the rate of digestion may be underestimated by using nylon bags. The porosity and closed surface area of the bags and the lack of rumination may provide some explanation for the different results. According to Tamminga et al (1989), including rumen evacuation data in a rumen digestion model resulted in a level of rumen digestion which was much closer to data observed *in vivo* than those based on nylon-bag incubations.

The passage rate of neutral-detergent fibre (NDF) was much lower (Huhtanen and Khalili, 1991) than that based on chromium (Cr)-mordanted straw particles (Huhtanen, 1988) in cattle fed similar diets. Similarly, Aitchison et al (1986), Robinson et al (1987) and Tamminga et al (1989) observed a greater rate of passage (kp) for Cr-mordant than the value based on rumen evacuation. The reason for these different results is that mordant particles are not digested. Recent data (Tamminga et al, 1989) show that Cr-mordanted particles give a fairly accurate estimate of the passage of indigestible cell-wall materials.

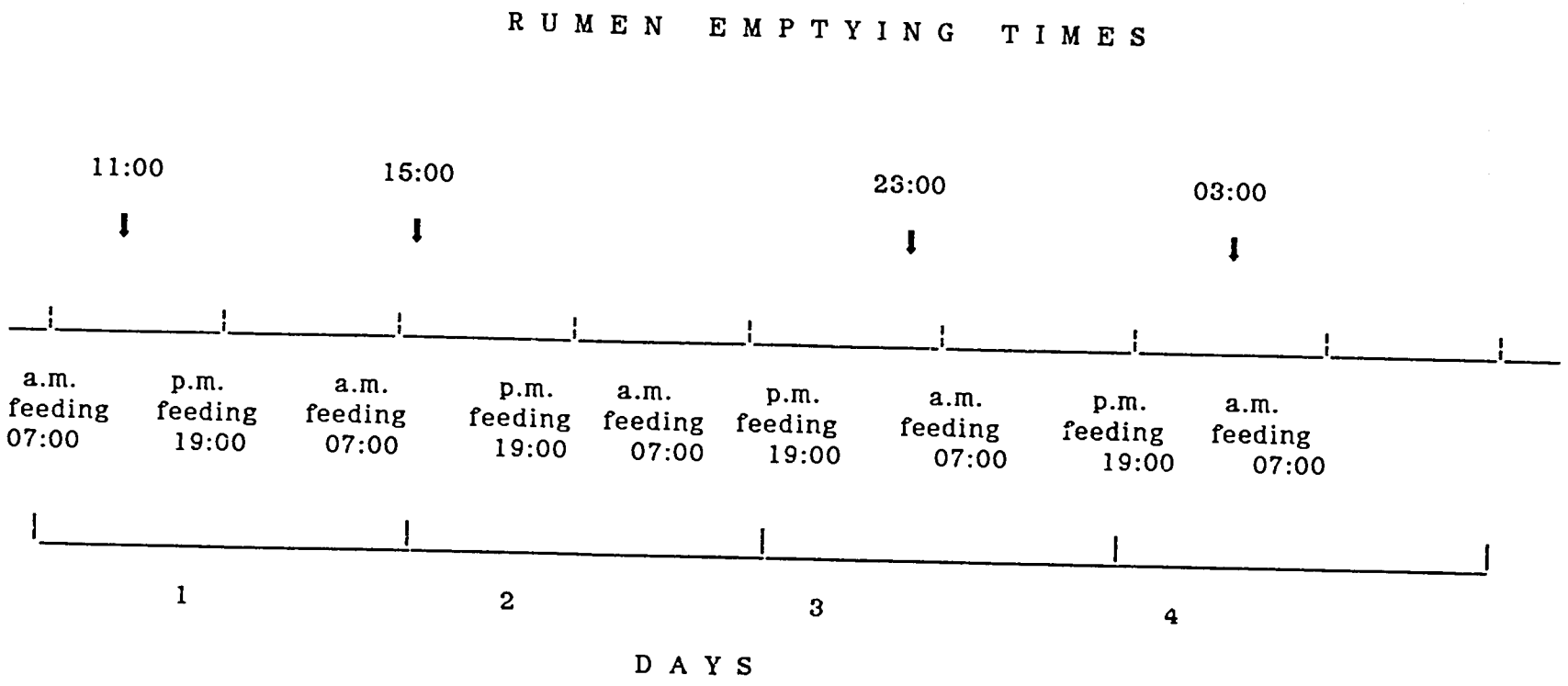
3.2.2 Implementing the rumen-evacuation technique

The total weights of rumen contents can be estimated by manually emptying the rumen of each animal at different times. There should be a minimum of 24 hours between consecutive emptyings (Figure 5) (see Towne et al, 1986; Robinson et al, 1987; Tamminga et al, 1989).

Procedure for estimating rumen volume by evacuation:

- Remove the cover of the rumen cannula and empty all rumen contents by hand into a barrel (size depending on total weights of rumen contents). If possible, keep the barrel in a container with warm water.
- Weigh all the material, mix thoroughly and take a sample (2.0–2.5 kg).
- Return the remaining material to the rumen as soon as possible.

Figure 5. *Schematic example for emptying the rumen.*



- The entire procedure should not exceed 10 minutes per animal and the rumen should be "empty" for only 2–3 minutes.
- Dry rumen content samples at 100°C for 24 h to determine the DM content of rumen digesta.
- Dry samples of rumen content for chemical analysis at 60°C for 48 h.

The rumen pool size of several constituents can be calculated from the average (if emptied more than once, which is preferable) rumen DM pool size and chemical analysis of a pooled sample that represents the average of the consecutive evacuations made.

It is also possible, if need be, to divide NDF into rumen-indigestible NDF (INDF) and degradable, rumen-digestible NDF (DNDF), fractions (Tamminga et al, 1989; Huhtanen and Khalili, 1991). This technique determines if there is selective retention of different fibre fractions in the rumen.

- Incubate rumen-content samples in nylon bags for 336 h in the rumens of two or three animals fed the basal diet. Analyse the residues for DM, NDF etc according to the experimental plan.
- Nylon-bag residues are rumen-indigestible DM (IDM), INDF etc and the degradable fractions are rumen-digestible DM etc.

The kinetics of rumen NDF intake, passage and digestion can be calculated using the model suggested by Robinson et al (1987) assuming steady state conditions in the rumen:

- Rate of intake (k_i per hour) = 1/24 x (intake, kg/day)/(rumen pool size, kg)
- Rate of passage (k_p per hour) = 1/24 x (faecal flow, kg/day)/(rumen pool size, kg)
- Rate of digestion (k_d per hour) = $k_i - k_p$

where:

intake = kg NDF (dry-matter basis) per day

pool size = kg NDF (dry-matter basis) in the rumen

faecal flow = kg NDF (dry-matter basis) excreted per day.

This model is used bearing in mind that the assumption concerning the steady state conditions may not be totally true. However, the relative differences between diets should be valid. In addition, faecal flow is often used to calculate these values because of the difficulties associated with duodenal cannulation.

3.3 The use of markers to estimate passage rates

3.3.1 Marker preparation

Solid-phase marker: Chromium-mordanting of fibrous materials

Carry out chromium-mordanting of straw following the procedure described by Uden et al (1980).

Weigh the material to be labelled in quantities of 250 g DM. Transfer the material into a solution of potassium dichromate or an equivalent amount of sodium dichromate in a 5-litre beaker (plastic, enamel or galvanised buckets could also be used) at a concentration of 30–33% (w/w) of chromium to mordanting sample. While ensuring complete immersion in the solution, seal the beaker with several layers of heat-resistant polythene film or aluminum foil and bake in an oven at 100°C for at least 24 h.

Wash the baked material thoroughly with tap water until the water is only faintly coloured. Transfer the mordanting material (straw) into a solution of ascorbic acid at a concentration of 1/2 (w/w) of ascorbic acid to straw and leave for one hour. Terminate the procedure by thoroughly washing several times in tap water. Dry at 65°C for 24–48 hrs.

Grind the mordanted straw through a 5 mm screen of a hammer mill or chop it to approximately similar length and seal in a plastic bag. Thoroughly hand-mix the straw and store it dry pending administration.

Preparation of chromium mordant straw (plant fibre)

Step 1. Soak a known quantity of straw (plant fibre) in water overnight and then wash thoroughly in tap water.

Step 2. Add sodium dichromate ($Na_2Cr_2O_7$) or potassium dichromate ($K_2Cr_2O_7$).
Dissolve sodium or potassium dichromate equivalent to 33% of the straw weight to get a Cr concentration equivalent to 12–14% of the straw weight.

Step 3. Place the straw in the dichromate solution and add enough water to completely cover the straw. Place a weight (e.g. block, stone etc) on top and cover properly with aluminum foil and tie with string.

Step 4. Bake the straw in dichromate solution in an oven at 100°C for at least 24 hours.

Step 5. Safely dispose of the liquid (it is highly poisonous). Thoroughly wash the material with tap water until the water is only faintly coloured.

Step 6. Suspend the mordant (material being mordanted) in tap water and mix ascorbic acid, equivalent to half of the original straw weight, with water to ensure that the material is completely covered. Leave for at least one hour, stirring occasionally. The pH should be acidic.

Step 7. Wash the plant fibre several times in tap water until it is free of soluble green matter and dry in an oven at 65°C for 24 hours.

Step 8. Grind the dried mordant using a screen size of at least 5 mm or chop to similar lengths.

Liquid-phase marker: Cobalt ethylenediaminetetraacetic acid (Co-EDTA)

To prepare Co-EDTA, dissolve 297.2 g Na-EDTA, 190.4 g $CoCl_2.6H_2O$ and 32.0 g NaOH in 1600 ml distilled water in a 5-litre beaker while gently heating (Uden et al, 1980). You may need to add more NaOH pellets to ensure that all the reagents dissolve. Allow the solution to cool to room temperature and add 160 ml hydrogen peroxide. Leave the mixture to stand at room temperature for 4 h, then add 2400 ml of 95% (v/v) ethanol. Store the mixture in a refrigerator overnight. Filter the resulting crystals, repeatedly wash them with 80% (v/v) ethanol and dry overnight at 100°C.

3.3.2 Marker administration and sampling procedures

Introduce solid-phase marker(s) into the rumen 30–60 minutes before feeding time. Use a plastic funnel to introduce marker material into the dorsal rumen via the rumen fistula. Wash the particles left in the funnel into the rumen with water. For non-fistulated animals, mix the mordant with a small portion of the supplement and offer as feed to ensure complete consumption during feeding time. Collect faeces samples 12, 24, 27, 30, 33, 36, 48, 54, 60, 72, 96, 120 and 144 hours after administration.

Pour the liquid-phase marker (Co-EDTA) solution into the rumen via the rumen fistula before feeding. Repeatedly wash the flat-bottomed flask with 0.1 litre of water. Sample rumen liquor 0.5, 1, 2, 4, 6, 9, 12 and 24 hours after administration of Co-EDTA.

Oven-dry the faeces at 100°C for at least 24 hours, grind through a 2 mm screen and store pending analysis. Centrifuge the rumen liquor and determine the cobalt concentration in the supernatant using an atomic absorption spectrophotometer.

3.3.3 Mathematical procedures

Estimation of k1, k2 and TT using the graphical method (Grovum and Williams, 1973)

Although only one model to estimate passage kinetics will be used, it is worth mentioning that many other models have been developed such as that of Ellis et al (1979) where k1 and k2 represent events occurring in the rumen; the model of Ponds et al (1984, cited in France et al, 1988); and the multicompartmental model of Dhanoa et al (1985) developed because Grovum and Phillips (1973) observed a poor fit between the observed concentration of marker and the predicted values for the two-pool model. The latter model considers digesta flow as a multicompartmental exponential process and was found to be superior to the other models in that it fitted all the (82) data sets successfully (Dhanoa et al, 1985). According to France et al (1988) it appears that there is little choice between the models when estimating the faeces production rate.

The mathematical procedures described below are from the Grovum and Williams (1973) version of the Blaxter et al (1956) model that can be represented thus:

$$Y = 0 \text{ when } t < TT$$

$$Y = Ae^{-k1(t-TT)} - Ae^{-k2(t-TT)} \text{ when } t \geq TT$$

where:

Y and A = adjusted marker concentrations in the faecal DM

k1 and k2 = rate constants

TT = the calculated time for first appearance of marker in the faeces

t = the sampling time (h) after a single injection.

Graphically, the natural logarithm of the marker concentration in faeces DM is plotted against time (Figure 6) and regression analysis performed on the linear portion of the descending slope.

The regression coefficient and the Y-intercept correspond to the slowest rate constant (k1) and A1, respectively (Figure 6). Fitted values are estimated for all collection times that corresponded to the ascending phase and the peak portions of the curve. The anti-logarithm of the fitted values minus the actual concentrations measured at these times gives the residuals.

Regression analysis involving the natural logarithm of the residual concentrations and the collection time would give the Y-intercept (A2) and the second slowest rate constant, k2. The two lines intersect at the point (TT,A). Hence TT can be calculated thus;

$$TT = (A2 - A1) / (k2 - k1)$$

Note that A1 and A2 above are natural logarithmic derivatives. The parameters estimated here can be used as the initial estimates in the computer fitting of the model of Dhanoa et al (1985).

Estimation of $t_{1/2}$ and mean retention time (MRT)

The time available for digestion in each pool denoted as $t_{1/2}$ is estimated as follows:

$$t_{1/2} = 0.693/k \text{ (Grovum and Phillips, 1973)}$$

where:

k = either k1 or k2.

The MRT is estimated as follows:

$$MRT = 1/k1 + 1/k2 + TT \text{ (Grovum and Phillips, 1973)}$$

where:

TT = the transit time.

Flow rate and rumen DM pool size

To estimate the flow rate and rumen DM pool size, continuous infusion of the markers should be used. However, with a knowledge of the faeces production rate (F) and the assumption that the proportion of digestible DM and OM apparently digested in the rumen (MADR and OMADR) are equal, the flow rate (FR) of DM out of the rumen could be estimated thus:

$$FR = F + (I - OMADR)\ DMI$$

where:

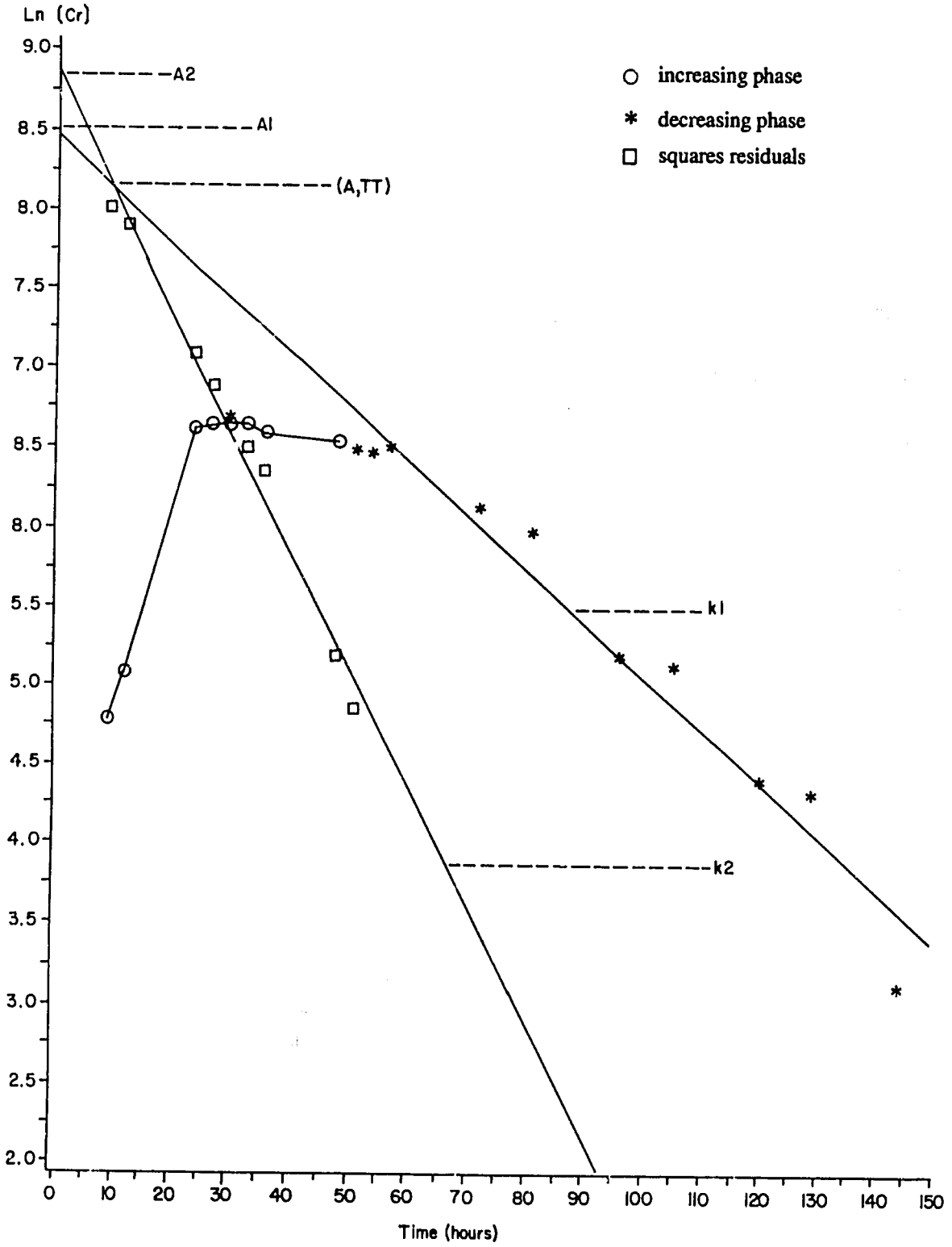
DMI = the digestible DM intake

OMADR = 0.65 (ARC, 1980; 1984) or more appropriately 0.75 for straw based diets (Osuji et al, 1993b).

The critical issue here is having the appropriate value of OMADR that corresponds to the dietary treatments imposed. The rumen volume, V, can be assessed according to the following relationship:

$$FR = V.k1 \text{ (Faichney, 1980)}$$

Figure 6. *The relationship between the natural logarithm of chromium concentration in faeces and time after dosing.*



Estimation of passage rate of liquid marker

After a single injection of liquid marker into the rumen the concentration (Y) decreases very rapidly with time (t) following an exponential pattern:

$$Y = Ae^{-kt}$$

where:

A = the intercept.

Regressing the natural logarithm of marker concentration against time will give an intercept, Ln(A), and the regression coefficient that corresponds to k. The rumen liquid volume (V_L) can then be calculated thus:

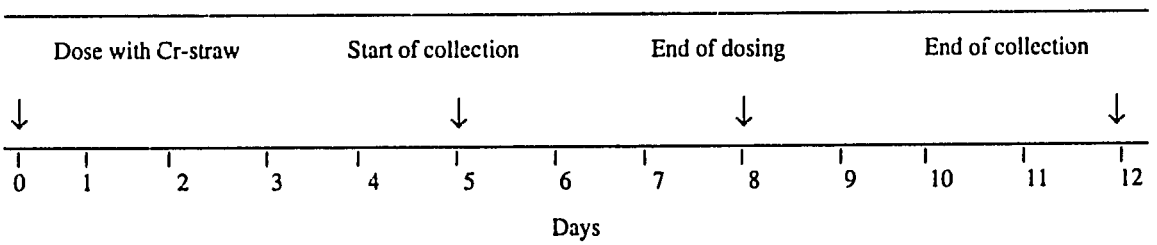
$$V_{(L)} = Dose/e^{Ln(A)}$$

Note: If the marker concentrations are expressed in µg/g, then the dose should be converted to µg too.

3.4 Continuous dosing with chromium-mordanted straw

Continuous dosing with mordant is used to estimate rumen volume. At the beginning, give a primer dose (which is about four times the regular dosage, i.e. 60 g Cr-straw with cattle and 20 g with sheep). Thereafter give the regular dose at similar times for 8 days. Collect representative rumen samples at 0800 and 1600 every day for 7 days counting from day 5 (Figure 7) by about which time the marker concentration in the rumen would have stabilised.

Figure 7. *Schematic example for dosing and sample collection in rumen-volume estimation.*



Plot the logarithm of the concentration of chromium in rumen digesta against time to verify that the concentration of Cr has stabilised. The mean concentration within this zone represents the equilibrium concentration (E_0). The logarithm of Cr concentration at the descending phase of the excretion curve is regressed against time and the regression coefficient corresponds to fractional outflow rate from the rumen (k1). The infusion rate divided by E_0 will give the rumen volume. The product of the rumen volume and k1 give the flow rate.

4. Estimation of microbial protein supply using total urine excretion of purine derivatives

4.1 Introduction

Several methods have been proposed for the estimation of microbial protein synthesis by rumen organisms. Most of them require elaborate cannulation of the animal and expensive instruments in the chemical procedures for the analysis of the diverse microbial markers.

Some researchers have recently recognised the need for simpler and non-invasive procedures for the determination of the daily synthesis of microbial protein. Such methods involve the use of either total urinary purine derivatives (PD), i.e. the sum of allantoin, hypoxanthine, xanthine and uric acid (Fujihara et al, 1987; X B Chen, Rowett Research Institute, Aberdeen, UK, unpublished data) or of allantoin alone (Dewhurst and Webster, 1988). These methods are currently being developed such that the ratio of the concentration of either PD or allantoin to creatinine in spot urine or plasma samples could be used to diagnose the nutritive state of the animal (Chen et al, 1992; Osuji et al, 1993a). Results obtained with these methods are comparable to those obtained with conventional methods. However, because of assumptions about the proportions of purines salvaged and the endogenous contribution, the urinary purine derivatives are good indicators only of relative dietary differences.

4.2 Sample preparation

1. Using any convenient metabolism facility, collect 24 h urine from the animal for 5–7 days.
2. Ensure the urine pH is less than 3 by adding acid to the containers in which urine is collected as follows:

   sheep/goats – 100 ml of 10% H_2SO_4

   cattle – 1200 ml of 10% H_2SO_4 or an equivalent amount of HCl.
3. Each morning, weigh the quantity of urine voided, add water to make the weight 30–50 kg for cattle and 3–5 kg for sheep/goats. This provides enough dilution to avoid precipitation of uric acid.
4. Take an appropriate subsample of the daily diluted urine, filter and store frozen. Analyse as a single batch for purines.
5. Using a suitable sampling procedure, take a representative sample of the bulked urine for purine analysis. The laboratory requires about 2 ml for each run, therefore a sample of 20–50 ml should be enough.
6. The laboratory should be provided with information on the live weight and DM intake of the animals and urine volume (final volume of the diluted urine).

   The urine collected from the field is diluted in the laboratory as follows:

   Sheep/goats

   Allantoin: 30 times (1 ml of urine + 29 ml distilled water)

   Uric acid: 8 times (1 ml of urine + 7 ml distilled water)

   Cattle

   Allantoin: 50 times (1 ml of urine + 49 ml distilled water)

   Uric acid: 10 times (1 ml of urine + 9 ml distilled water)

These dilution factors are chosen to ensure that the samples analysed fall within the range of the standards.

4.3 Mathematical procedures

How microbial protein supply is calculated from the purine derivative (mmol/day) estimated from laboratory analysis is dealt with here. The relationships between urinary PD output, Y (mmol/day), and microbial PD absorbed after duodenal and intestinal digestion, X (mmol/day), are:

(1) $Y = 0.35\,X + (0.385W^{0.75})$ .....for cattle (Verbic et al, 1990)

(2) $Y = 0.84\,X + (0.150\,W^{0.75}\,e^{-0.25x})$ ....for sheep (Chen et al, 1990)

For cattle

$$X = (Y - 0.385W^{0.75})/0.85.$$

Note: The parameter estimates in the above equations were determined with temperate sheep and cattle. These will be modified if necessary to suit tropical ruminant species.

However, for sheep/goats the situation is slightly different since the endogenous contributions vary as a function of exogenous input. Chen et al (1990) have demonstrated with sheep that if daily urinary excretion is greater than or equal to 0.6 mmol/kg $W^{0.75}$, then the endogenous contribution approaches zero (i.e. negligible). Therefore, correcting for the endogenous contribution is necessary only when PD (mmol/kg $W^{0.75}$) is less than 0.6. In such a situation the authors suggest that X can be estimated using the Newton–Raphson iteration procedure (Chen et al, 1990).

Suppose that the urinary PD excretion (mmol/day) is denoted by d. Then for sheep, if endogenous contribution tends toward zero,

$$X = d/0.84$$

If a function f(X) is set up such that

$$f(X) = 0.84\,X - (0.150\,W^{0.75}\,e^{-0.25X}) - d = 0$$

Taking the first derivative of f(X)

$$f^1(X) = 0.84 - 0.038\,W^{0.75}\,e^{-0.25X}$$

Given the above information, X can be estimated after correcting for the endogenous contribution by fitting the function

$$X_{(n+1)} = X_{(n)} - f(X_n)/f^1(X_n)$$

The fitting process will go through a series of iterations until $X_{(n+1)}$ is constant. The value of $X_{(n+1)}$ for which further iterations cause no change is the best estimate of microbial purine absorbed (X) from the intestines. The value for X can be estimated using a SAS program (Appendix 2) or a calculator. When the value of X is estimated, it is used to calculate the numerical value of the function f(X) and the first derivative of the function (f^1X) (denoted in the program by fun and dfun, respectively). The iterative estimates of X (i.e X1, X2, X3, X4, X5.....X(n+1)) are then calculated accordingly (Appendix 2). The best estimate of X is then used to estimate microbial N supply.

You may therefore need to use X (estimated as above for sheep or for cattle) to estimate microbial protein supply.

$$\text{Microbial N (g/day)} = \frac{X\,(\text{mmol}) \times 70}{0.83 \times 0.116 \times 1000} = 0.727X$$

where:

70 represents mg N per mmol of purine derivative

0.83 represents average digestibility of mixed microbial purines

0.166 represents the proportion of microbial N that is purine N

1000 is to convert the estimate from mg to g/day.

Appendix 1

Suppose the data structure is defined by the following classes:

Animal number ..... (ANO)
Period ..... (PER)
Roughage or feed type ..... (Feed)
Time ..... (time)
DM disappearance ..... (DMD)
N disappearance ..... (ND)

Log on to SAS (SAS Institute Inc., 1989) and type the following instructions on to your program editor window. When the data are plotted, visually inspect them and edit out the outliers, if any, by replacing with a period or point (.).

```
libname perm 'b:';
DATA INCU;
    INPUT ANIMAL PERIOD FEED TIME DMD;
CARDS;
    30   1   1   6   27.7
    30   1   1  12   30.8
    30   1   1  24   43.1
    30   1   1  48   52.7
    30   1   1  72   54.4
    30   1   1  96   55.9
    40   2   1   6   27.2
    40   2   1  12   34.7
    40   2   1  24   43.6
    40   2   1  48   51.6
    40   2   1  72   58.9
    40   2   1  96   60.1

run;
PROC SORT DATA=INCU;
    BY ANIMAL PERIOD FEED;
RUN;
PROC PLOT DATA=INCU;
    BY ANIMAL PERIOD FEED;
    PLOT DMD*TIME;
    RUN;
```

When the data are edited proceed to fit the model onto the 'clean' data as follows:

```
PROC NLIN BEST = 9; BY ANIMAL PERIOD FEED;
```

```
PARMS A = 9 TO 15 BY 1
      B = 39 TO 44 BY 1
      C = 0.03 TO 0.05 BY 0.005;
MODEL DMD=A + B*(1-EXP(-C*TIME));
OUTPUT OUT =PERM.TCH PARMS = A B C;
RUN;
```

This program has been written such that the NLIN procedure will by default use the DUD method. Run the program to calculate feed degradation characteristics. Then use a, b, c values to analyse for the effect of treatment depending on the experimental design and structure.

Note: To fit the model with lagtime insert the parms statement at the position between b and c : ltime = 1 to 10 by 1. Also change the model statement to read: Model dmd = a + b(1–exp(–c*(time–ltime));*

The next set of program lines will help to print just the degradation constants and the effective degradability.

```
DATA PERM.TH1;
    SET PERM.TCH;
    PD = A + B;
    ED = A + B*C/(C + 0.03);
RUN;
PROC MEANS MEAN NOPRINT DATA = PERM.TH1;
    BY ANIMAL PERIOD FEED;
    VAR A B C PD ED,
    OUTPUT OUT=PERM.TH2 MEAN=A B C PD ED;
RUN;
PROC PRINT DATA = PERM.TH2;
RUN;
```

Note that in the printout there are five additional variables:
a, b and c are the degradation constants
pd the potential degradability (a + b)
ed the effective degradability (a + bc/(c+0.03)) calculated assuming a passage rate of 0.03 (Ørskov and McDonald, 1979).

Appendix 2

This SAS procedure can also be evaluated with a suitable hand calculator:

```
data pur ;
    w = 17 ;
    d = 3.4 ;
    X = d/0.84;

    fun = 0.84*X + 0.15*w**0.75*exp(–0.25*X) – d;
    dfun = 0.84 – 0.038*w**0.75*exp(–0.25*X);
    X1 = X – fun/dfun;

    fun = 0.84*X1 + 0.15*w**0.75*exp(–0.25*X1) – d;
    dfun= 0.84 – 0.038*w**0.75*exp(–0.25*X1;
    X2 = X1 – fun/dfun;

    fun = 0.84*X2 + 0.15*w**0.75*exp(–0.25*X2) – d;
    fun= 0.84 – 0.038*w**0.75*exp(–0.25*X2);
    X3 = X2 – fun/dfun;

    fun = 0.84*X3 + 0.15*w**0.75*exp(–0.25*X3) – d;
    dfun= 0.84 – 0.038*w**0.75*exp(–0.25*X3);
    X4 = X3 – fun/dfun;

    fun = 0.84*X4 + 0.15*w**0.75*exp(–0.25*X4) – d;
    dfun= 0.84 – 0.038*w**0.75*exp(–0.25*X4);
    X5 = X4 – fun/dfun;

run;
proc print data = pur;
run;
```

*Note: * stands for multiplication sign, and ** for power and exp for exponent (where exp is the base of natural logarithm); where w = animal weight and d = daily PD production in mmol/day.*

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