THEORETICAL AND APPLIED ASPECTS OF VOLUNTARY FEED INTAKE BY RUMINANTS, WITH SPECIAL REFERENCE TO THE KINETICS OF RUMEN DIGESTION

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ABSTRACT

The aim of these studies was to examine the factors which determine voluntary feed intake and feed quality in ruminants. In the first experiments, the concepts of ruminal digestion kinetics were conceptualised and measured in animals. These concepts were applied practically in feed evaluation studies which followed. *In vivo* studies on alkali treated wheat straw explained why voluntary intake of ruminants increased when roughages are treated with alkali. The effect of washing the treated feed to remove excess sodium was also studied. The explanation was found in terms of ruminal digestion kinetics, showing that the mean rate of digestion was not changed, but chemical treatment improved the potential digestibility, thereby increasing the active pool size in the rumen which resulted in a faster clearance rate from the rumen. A study of the effect of starch fermentation on the kinetics of roughage fermentation in the rumen, revealed that the fermentation of different diets were affected in a different manner. The paramount factor was found to be a reduced rate of forage fermentation in the presence of starch fermentation in the rumen. A study of *Pennisetum clandestinum* revealed the reasons why animal performance on kikuyu pasture is often lower than what would be expected from the digestibility and chemical composition of the material. It was shown that a high soluble nitrogen content of the material was the most likely reason for low voluntary intakes, low ruminal fill and therefore poor animal performance on lush kikuyu pasture. A method was developed by which the concepts of ruminal digestion kinetics (MRT method) are used to determine voluntary feed intake with grazing animals. The method gave a mean intake that was similar to the mean obtained when intake was calculated from faecal collections, but had the advantage of a clearer pattern of intake. The accuracy obtained when using the MRT method to estimate voluntary feed intake was confirmed in a second experiment where actual intakes were known, and predicted intake was very close to actual intake. Indirect methods were developed by which two important determinants of voluntary intakes, i.e. rate and extent of digestion may be estimated. The Tilley & Terry *in vitro* method was adapted to allow the estimation of fermentation rates from rates of gas production. Digestion rates obtained with *in vitro* gas production agreed well with *in sacco* estimates. *In vivo* digestion rates were much

slower than those obtained *in vitro* or *in sacco*. This discrepancy is yet unexplained, and is in contrast with the results of a previous experiment where *in sacco* and *in vivo* results were in good agreement. Increasing the mean particle size of the fermenting forages resulted in a small but statistically significant decrease in fermentation rate. Stirring the fermentation vessels did not have any positive effect on fermentation rate. Total volume of gas produced was not a good indicator of *in vitro* digestibility because gas production measures ruminal digestion, while *in vitro* digestibility includes both a ruminal and an acid pepsin phase. The rate of *in vitro* gas production, as measured by pressure changes in the fermentation vessels, is a practical method that was easily automated by using a data logger. The automated measurement of rate and extent of digestion allows their inclusion into routine analyses for feed evaluation and the results obtained so far indicate that the system is sufficiently accurate to give useful estimates of voluntary feed intake and animal production.

I hereby declare that this entire thesis and the associated research, unless indicated to the contrary in the text, comprises my own, original work.

J. P. Pienaar

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Chapter 1

General Introduction

The control of voluntary intake in ruminants, is multifaceted. The effects of many metabolic and physical limits of the body are reflected in the control of voluntary feed intake (Forbes, 1993). "Often the controls involved are too complex to be stated in qualitative terms, and certainly cannot be fully explored without being expressed quantitatively", Forbes (1993) concluded.

Factors such as dietary effects, metabolic status, growth and fattening, oestrus, pregnancy and lactation, are responsible for changes in the longer term (daily) voluntary intake, whereas time of the day and changes of blood hormone and metabolite concentrations affect short term (hourly) intakes. In the very short term (minute by minute), both metabolic and digestive processes control intake (Forbes, 1993).

Despite the complexities of the controls involved, the expression of feed quality cannot be complete if an estimate of the voluntary intake of that feed is not included (Crampton, Donefer & Lloyd, 1960; Dulphy, Faverdin & Jarrige, 1989). Because of its association with feed quality, many attempts have been made to predict the intake of a feed (ARC, 1980, NRC, 1987), mostly with little success (Beever, 1993). Despite the fact that little success has been obtained so far, and that it is unlikely that a high degree of accurary will ever be obtained due to the complexities of the animal associated factors involved, it should not discourage attempts to include this aspect into feed evaluation. It is important to express feed quality in terms of the voluntary intake on all types of diets, since it determines the potential animal production from that feed (Beever, 1993).

The matter may be viewed from two sides. The first being the complex side of animal factors which makes the accurate prediction of intake for all conditions seem to be unlikely or impossible. This could discourage any attempts to work on the prediction of voluntary feed intake. The other view is to accept the fact that it is going to be difficult to define animal factors accurately, but rather to concentrate on the factors in the feed which influence voluntary intake, since these are so important when assessing feed quality. This might imply that during the validation of the model, some of the predictions may not be axactly what the animal is consuming. However to be of any value, the predictions must at least be reasonably accurate. The most important however, is the accuracy of the relative differences between feeds. These should be better than any presently available method if the method should have any merit for future implementation.

On concentrate diets the potential intake of a diet may exceed the physiological capacity of the animal to metabolise the nutrients. Thus the **concentration** of nutrients in the diet can be used to calculate intake and animal production from that diet. However, an estimate of intake is very important with **roughage** type diets where intake is controlled by the digestive capacity of the animal, the so-called physical control (Conrad *et al.*, 1964). Under this type of control the concentration of nutrients in the diet is not the limiting factor, but the actual "rate at which previously ingested meals are removed from the rumen" is (Beever, 1993).

In the past numerous attempts have been made to relate various factors to voluntary feed intake of ruminants. These include the physical properties of the feed (Moore, Shenk, Norris & Barnes, 1976) as well as its chemical composition (Golding, Moore, Franke & Ruelke, 1976). Ruminal digestion kinetics have also been related to voluntary intake (Gill, Conrad & Hibbs, 1969). Recently, Ørskov, Reid & Kay, (1988) described how intake in cattle can be predicted from the degradation characteristics of roughages. Both these attempts to relate ruminal digestion kinetics to voluntary intake have, however, assumed that linear relationships exist between, for instance, fermentation rate and voluntary intake. From the general intake

equation given under 4.2 below, it can be seen that a linear relationship exists only between ruminal fill and voluntary intake. All the other parameters are inversely related to voluntary intake.

The most important approaches currently in use for predicting voluntary feed intake may be classified under one of the following three categories: 1) static models, 2) dynamic models and 3) estimates obtained from direct (*in vivo*) measurements of intake as used by Institute National de la Recherche Agronomique (INRA) in France.

1.1 Static models

Static models are currently in use in most of the important feeding standards such as the NRC (1988) and ARC (1980) and also in an approach proposed by Mertens (1985), which is widely used in the USA. In all these models intake is expressed as a function of a specific component of the diet, be it the metabolizable energy- or neutral detergent fibre (NDF) content or the concentrate proportion of the diet. Additivity is assumed between the energy values of dietary components. The advantage of such an approach is that it allows the use of linear programming and least cost computer techniques when balancing diets. Diets are merely formulated to a specific composition which should give the desired intake.

Unfortunately the accuracy of all these methods had been rather disappointing. For example, the ARC (1980) predicted intake for sheep and cattle from regression equations using metabolizability and in some cases also the proportion of concentrate in the feed and animal live mass. These predictions of intake should be used cautiously since the results, according to the ARC (1980), "may be inaccurate in some circumstances and exclude many additional factors, some of which are known but could not be included in the equations". The multiple correlation coefficients of the regressions they use to predict intake for sheep and cattle vary between only 0.27 and 0.67.

The approach of Mertens (1985), where maximum NDF intake is expressed as a constant (1.1%) relative to body mass, was criticized by Williams, Oltenacu & Sniffen (1989) for underestimating intake on legume based diets and overestimating intake of grass based diets. At the same NDF content of 36%, cows had a voluntary intake of about 19 kg per day on a grass based diet while a lucerne based diet yielded an intake of about 23.7 kg per day. Williams *et al* (1989) attempted to rectify this, and obtained very good predictions of voluntary intake and even milk production, with their final corrected model. However, local results (Pienaar & Roux, 1989b) with this corrected model were rather disappointing, and showed no advantage compared to the original model of Mertens(1985).

Any advantage of using computers, and more specifically least cost diet formulation, in ruminant nutrition may be offset by the limitations of the traditional static models in terms of their poor accuracy for predicting voluntary intake and animal performance.

1.2 Dynamic models.

Dynamic models are used to describe ruminal flow and digestion kinetics. They can be divided into mathematical models and simulation models. Mathematical models are expressed in algebraic formulae and usually have an explicit mathematical solution. Simulation models are written in a simulation language, are always run on computers and can be solved normally only by iteration.

The first mathematical modelling of the rate at which previous meals are removed from the rumen, by both passage through the rumen and fermentation, was published by Waldo *et al.* (1972). His model, although basically correct, was based on simple first order kinetics and was not meant to be used as such for feed evaluation. The chemical entities used (cellulose

and lignin) were not practical for routine analyses, nor were there methods available by which the rates of fermentation or passage could be estimated on a routine basis. His model was followed by the simulation model of Mertens (1977).

An experiment with sheep, published by Pienaar, Roux, Morgan & Grattarola (1980) and summarised in section 2.1.1 in this thesis, was the first attempt to quantify the parameters needed in a first order model to calculate the rate at which previous meals were removed from the rumen. More recently a dynamic first order mathematical model was published by Allen & Mertens (1988) while Fisher, Burns & Pond (1987) published a simulation model using inputs that differ from the previous simulation models. Pienaar & Roux (1989a) modified their first order mathematical model by using the gamma function to describe both the fermentation and outflow curves of organic matter disappearance from the rumen. This paper is summarised under 2.1.2.

The advantage of dynamic models is that they include more of the relevant variables and usually yield more realistic estimates of voluntary feed intake than static models. Their big disadvantage for acceptance and application in practical animal nutrition, is their complexity. They need more complicated inputs and the simulation models can be run only on a computer. Until now, simulation models have not been used widely in practical ruminant nutrition. They have been useful rather as research tools in studying the processes of flow and fermentation. The model of Fisher *et al.* (1987) uses inputs of particle size, NDF content and *in vitro* dry matter disappearance of masticated dry matter. These can be obtained from relatively simple laboratory analyses.

Since dynamic mathematical models of voluntary intake usually have explicit mathematical solutions they are reasonably simple to use in practice. They also include dynamic parameters of ruminant digestion combined in the correct manner, but usually do not include as many variables and rate constants as do simulation models.

Potential voluntary feed or energy intake intake, predicted from both dynamic mathematical and simulation models, can not be used in linear programming to formulate diets since the potential intake of a component would determine the final composition of the feed. Also if rumen fill is assumed to be a function of energy demand (Fisher, Burns & Pond, 1987 and discussed under section 4.3 of this thesis) intake would not be suitable for least cost formulation since rumen fill would differ for each component of the diet depending on the energy yield from that component. In that case mean retention time (MRT[1])) and energy concentration would be the parameters of choice to be used in linear programming. The diet will then be formulated for a specified energy (ME) concentration and a specified MRT according to the energy needs and rumen fill that is specified for the animal. Calculating the MRT of the diet in this manner results in exactly the same than the Danish fill units (Madsen, Stensig, Weisbjerg & Hvelplund, 1994)

When using least cost procedures the MRT's and ME values of feeds are assumed to be additive. That is not perfectly true, since it is shown under 2.2.2 that the fermentation rate of some roughages is changed when they are fermenting together with starch. The effects of these deviations on the prediction of intake and animal performance become clear in Section 4.4 when intakes are predicted for animals on complete diets (roughage and concentrate mixtures). The problem of non-additivity however applies to all the approaches used so far for formulating ruminant diets on a least cost basis.

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1 For an explanation of MRT see section 3.1

1.3 *In vivo* measurements of voluntary intake.

Apparently the *in vivo* measurement of voluntary intake is the simplest method by which voluntary intake may be predicted. However, as soon as the intake value obtained for a specific feed has to be used for types of cattle and sheep other than the ones used to determine the intakes, the situation becomes complicated. This is clear when the "INRA fill unit" system (Jarrige, Demarquilly, Dulphy, Hoden, Robelin, Beranger, Geay, Journet, Malterre, Micol, & Petit, 1986) is studied. When using the INRA fill unit, the voluntary intake of a group of reference animals fed an unknown grass is compared to intakes obtained on a reference grass. These values are tabulated and used to predict voluntary feed intake in the practical feeding situation for different kinds of animals. Hence, it also serves as an excellent example of the complexities which arise, what ever the method used, when voluntary feed intake has to be estimated for different kinds of animals.

One of the two main problems arises when an attempt is made to scale animals to comparable sizes in terms of their voluntary intake. Sheep and cattle are not comparable on a metabolic weight basis. Therefore, different norms have to be used when intakes are compared on a reference diet. The same is true for animals of the same species but in different physiological stages. Thus, a different norm, and tabled set of values for feeds have to be defined for each type of animal. The possibility that interactions might occur between type of animal and quality of diet cannot be excluded. In a more mechanistic approach such as the one proposed in this thesis corrections such as the one proposed by Fisher *et al* (1987) can be used to predict the performance of different types of animals and using only one table of feed characteristics (See Section 4.3.1).

The work of Ketelaars & Tolkamp (1992) suggested an interesting approach to the regulation of feed intake. If it is verified by other experiments, it may contribute to clarify the problem of the control of feed intake. However, it is difficult to accept their total rejection of some sort of restriction on intake by ruminal fill. The fact that animals tend to eat more of a better quality feed is clearly shown in their own graph (Ketelaars & Tolkamp 1992, Fig 2a). This relationship cannot be ascribed to the protein content of the feeds, since the work of Pionnaar et

al. (1980) has shown the same relationship, but sufficient protein was supplemented on all diets in the form of fishmeal in order to eliminate the effect of a protein deficiency in the animal on voluntary feed intake.

Another problem entails the interactions between the digestion of roughages and concentrates. Since the magnitude of this interaction depends on both the amount of concentrate and the quality of the roughage fed, it causes a significant bias when formulating least cost diets using linear programming techniques. Linear programming assumes additivity when feed components are mixed. In this approach, as with the dynamic models, roughage concentrate mixtures are shown to be non-additive and large interactions are apparent, especially with high quality forages. This precludes the use of this approach when linear programming is used to formulate diets.

A publication entitled "Ruminant Nutrition, Recommended allowances & feed Tables" by the INRA group (Jarrige,1989) was published at the end of 1989. It contains comprehensive lists of the different forages and their "fill values" for different types of animals, enabling the prediction of voluntary feed intake and diet formulation with feeds which are listed.

1.4 The scope and hypothesis of this thesis.

The work reported in this thesis commenced in 1976. Much of it has already been published and the references of those are given as foot notes below the appropriate section headings. Only short summaries of some of the earlier publications are included, while more lengthy summaries of other publications are included. Some of the more recently published - and unpublished work are presented unabridged.

The thesis describes the development in the understanding of ruminal digestion kinetics with the objective to describe feed quality and voluntary feed intake in ruminants. After a general introduction to certain aspects of voluntary feed intake and feed quality, it shows how the principles of ruminal digestion kinetics were developed. The application of a first order model, originally used to predict intake as well as the model based on the gamma distribution function, which lends more flexibility to the description of the curves, are briefly discussed. The application of flow and fermentation dynamics to practical nutrition, such as the chemical treatment of roughages and the effect of starch fermentation in the rumen on voluntary intake and the kinetics of forage digestion, are also shown briefly. The application of ruminal digestion kinetics to elicit the factors which limit animal production on kikuyu pasture, is presented unabridged in two papers. The ability of some indirect methods which are normally used to measure intakes in grazing animals are compared under constant intakes, and also when abrupt changes in intake occur.

In order to use the principles of ruminal digestion kinetics for routine feed evaluation, the variables which determine voluntary feed intake *in vivo* have to be estimated by methods which can be executed relatively easily, within a reasonable period of time and at a cost that would be acceptable to the potential user. In Chapter 3 the possible application of the *in sacco* and manometric *in vitro* techniques is presented and discussed as possible indirect methods of estimating some of the variables that determine voluntary feed intake.

In Chapter 4, the practical application of a kinetic model in feed evaluation is presented and discussed, together with other systems that are presently mostly used for feed evaluation in the world. The mathematical formulae and some of the values that are used to calculate voluntary feed intake and animal production are given and discussed. Some of the predictions obtained are compared with actual intakes.

The basic hypothesis is that although voluntary intake is and will probably remain multifaceted, ruminal digestion kinetics do play an important role in determining feed quality and animal production through its effect on voluntary feed intake. The present knowledge on the subject, although not perfect, can be put to good use in establishing a system of feed evaluation that is probably equivalent or even superior to analyses that are currently in use. The model proposed in this section can not be compared to the simulation models (e.g. Dijkstra, 1993, Poppi, Gill & France, 1994), described under sections 1.2 and 4.4.1 since their models are not directly applicable to routine feed evaluation. As Poppi *et al.* (1994) concluded their paper:"As an exercise in the testing of ideas for future research, the model development was successful". Such models are very important when simulating the whole (animal and feed complex). In terms of feed evaluation for formulating diets (e.g. determining the energy value of the feed) feed characteristics should be separated as much as possible from the requirements of the animal, in order not to confound the nutritive value of the feed with the animal's requirements (performance). For that reason the simple rumen digestion kinetics model is very important. It emphasizes digestion kinetics, and present the results in such a manner that it can be used for feed formulation. This is the main objective for presenting the model together with the methods used to estimate the feed characteristics. It also enables the user to apply the principles of rumen digestion kinetics as tools of the trade for ruminant nutritionists. The model it self is not the "gist" of the study, but rather the result of many years of the use of these tools in trying to identify more logical but also practical methods for describing feed quality.

Chapter 2

Developing the first principles of ruminal digestion kinetics and applying them to *in vivo* feed evaluation studies.

Although the broad concepts of ruminal flow- and fermentation dynamics have been associated intuitively with feed quality for many years, the methods used to measure it and the practical mathematical methodology have not been part of the average animal scientist's "tools of the trade" until very recently. Text books such as the "Nutritional Ecology of the Ruminant" (Van Soest, 1982), and more recently "Quantitative Aspects of Ruminant Digestion and Metabolism" (Forbes, 1993) have brought these concepts to the attention of many animal scientists.

2.1 Modelling flow and fermentation dynamics

The experiment which laid the foundation of this thesis regarding the mechanisms of ruminal digestion and flow kinetics commenced in March 1976 and was published in 1980. This simple first order model was adapted in 1989 to accommodate the use of the gamma distribution function when the curves for passage from, and fermentation in the rumen were described. The gamma function allows more flexibility when the curves are fitted. Abstracts of the papers of both the simple first order model and its adaption to accommodate the gamma function are included in the following sections.

2.1.1. Predicting voluntary intake on medium quality roughages[1]

The aim of this experiment was to identify those factors which influence voluntary intake in sheep when roughages of widely different qualities were fed. The diets fed, were selected to elicit a large variation in voluntary feed intake.

A first order model for predicting voluntary intake was applied to sheep fed these roughages of differing quality. Important variables identified in this study were the ruminal capacity of the animals, the first order rate constant for fermentation of the diet, the solubility of the diets and the insoluble fermentable fraction of the diet.

The first order rate constants for the outflow of fermentable and non-fermentable organic matter from the rumen were found to remain constant irrespective of the diet. Using estimates of these variables obtained from cannulated sheep, the intakes of intact sheep fed the same diets were predicted. Results indicated that a relatively unbiased estimate of voluntary intake of diets, including a legume and grass of widely different qualities, were obtained with another group of sheep using this method. Predicted mean intake was 665.9 g/day whilst actual mean intake amounted to 666.5 g/day. The standard error of the predicted means was 4.90 g/day.

The accuracy obtained in predicting voluntary intakes in this manner, indicated that the variables considered were important and probably applicable to a wide variety of circumstances. The diets used, included a legume hay (lucerne), gras hays (*Cenchrus ciliaris*) of different quality, wheat straw and maize residues.

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[1]J.P. Pienaar, C.Z. Roux, P.J.K. Morgan & L. Grattarola
Abstracted from *S. Afr. J. Anim. Sci.* 10, 215-225 (1980)

Some principles were developed and techniques mastered during this experiment. The techniques comprised the measuring of rates of fermentation and rates of passage during steady state conditions (*in vivo*) and when using a pulse dose technique (markers or nylon bag techniques). The *in vivo* method comprised emptying the ruminal contents of an animal, and determining the amount of potentially digestible and indigestible O.M. in the rumen. The Tilley & Terry (1963) *in vitro* method, but with a long (75h) incubation time during the microbial phase was used to separate potentially digestible O.M. from indigestible O.M. When the intake of digestible and indigestible O.M. by the animal are divided by their respective contents in the rumen, rate constants for the disappearance of these two fractions from the rumen can be calculated. The disappearance of potentially digestible O.M. from the rumen by passage can be calculated by measuring the flow of this fraction at the ileum. These techniques were applied in subsequent studies to describe the effects of different dietary treatments on ruminal kinetics *in vivo*.

2.1.2. Use of the gamma function in equations which describe ruminal fermentation and -outflow rates for the prediction of voluntary intake and protein degradation[1]

Both fermentation and outflow from the rumen have been estimated by indirect methods such as the *in sacco* technique (Ørskov & McDonald, 1979), the *in vitro* approach (Mertens & Loften, 1980) and the use of markers (Graham & Williams, 1962). In order to estimate voluntary feed intake from these measurements, the rate constants for fermentation and outflow have to be combined in a single equation, together with other variables, which would allow the situation in the rumen to be described at steady state intake. This was done by Pienaar *et al.* (1980) for first-order equations (summarised in section 2.1.1). However, both outflow and fermentation processes show significant deviations from first-order kinetics

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[1]J.P. Pienaar & C.Z. Roux
Abstracted from *S. Afr. J. Anim. Sci.* 19, 3, 99-106. (1989)

(Mertens & Loften, 1980; McDonald, 1981; Pond, Matis & Ellis, 1982; Mahlooji, Ellis, Mattis & Pond, 1984; Pienaar & Roux, 1984), which consist of a phase of increasing activity at the onset of both the outflow and fermentation processes.

Mertens & Loften (1980) and McDonald (1981) accommodated this deviation from first-order kinetic in their models by including a lag (dead) phase at the onset of fermentation. This correction implies that a phase of no activity immediately precedes a phase of maximum activity, at which first-order kinetics set in.

The gamma function (Law & Kelton, 1982) appears to eliminate the problem of the model of McDonald (1981), since it does not include a lag (dead) phase. Rather, it can accommodate a phase in which activity increases from virtually zero to maximum. This model makes use of two parameters, one describing shape (α) and one the scale (ß) of the curve. These two parameters give this model more flexibility than a simple first-order model. Furthermore, some functional significance may be ascribed to these two parameters. The inverse of parameter ß is similar to a first-order rate constant, whereas parameter α modifies the shape of the first-order curve. When $\alpha = 0$, the equation reduces to that of a first-order curve. As α increases, the effect of a delay (slow starting) phase is superimposed on the parameter ß and disappearance rate is not only slowed down, especially at onset, but is also smoothly carried over into the later stages of fermentation. This description of events would seem to be more appropriate than a lag phase, which is inappropriate to ruminal digestion kinetics.

In this study, the gamma function is fitted to both *in sacco* O.M. disappearance (fermentation) and faecal marker excretion (passage), and the goodness of fit is demonstrated. Other functions, often used to describe fermentation, are compared to the gamma function in terms of accuracy of fit. General equations for intake and protein degradation are also constructed from equations where outflow and fermentation are described in terms of gamma probability distributions.

Five models were studied for goodness of fit to *in sacco* organic matter disappearance. Except for the 24h first order model, all the other models showed an accurate and unbiased fit, although in some cases this was achieved by unrealistic parameter estimates or an increase in the number of parameters that need to be estimated. The 24h first order model showed a biased fit as indicated by a slope which is significantly larger than 1 for the regression between observed and predicted values. Marker passage at the ileum was satisfactorily described by the gamma retention time distribution in 90% of the cases. Only the curve of one sheep, which had a very irregular flow pattern, could not be fitted accurately by this function.

The integer form of the gamma distribution of retention time may be used to combine outflow and fermentation in one equation. This equation describes the disappearance of fermentable O.M. when calculating intake.

2.2 The Application of flow and fermentation dynamics in practical nutrition

Once the principles of ruminal kinetics are understood, they may be used to address problems encountered in practical animal nutrition. Problems such as: what effect does the treatment of roughages with NaOH have on the digestion in the rumen ? Why is voluntary intake normally improved by NaOH treatment, but in some cases no effect is observed on *in vivo* digestibility ? The effect of starch digestion in the rumen on forage digestion- and flow kinetics is another problem that was previously not clearly understood, and could now be addressed with the approach. A problem of low voluntary intakes that is often observed in animals grazing kikuyu pastures was also addressed by studying the ruminal kinetics of sheep grazing kikuyu pastures. From the study with grazing animals it soon became clear that voluntary feed intakes change rapidly with grazing animals. This had serious implications on the results obtained from grazing animals, since most of the methods normally employed to measure intake with grazing animals, have the basic assumption that steady state conditions must exist in the digestive tract

of the animal. This problem was addressed by measuring intake directly on animals in metabolism crates and also measuring intake using the indirect methods available for grazing animals. Measurements were made during situations of constant intake (steady state) and also when rapid changes in intake were induced.

2.2.1 Sodium hydroxide treated wheat straw for sheep[1]

A study of NaOH treated and untreated roughages was undertaken in order to explain the reason for the increased intake observed when roughages are treated with a strong alkali, in this case NaOH. Some of the treated roughage was washed with water to remove excess sodium, while the rest was left unwashed. Washing of the feed would give an indication of the effect of a low versus a high sodium content of the diet on *in vivo* ruminal dynamics and *inter alia* the rate constant describing outflow. Comparison of treated and untreated roughages would give an indication of the alteration in flow and fermentation kinetics caused by NaOH treatment.

Voluntary feed intake, digestibility and ruminal flow- and fermentation dynamics were studied using sheep. NaOH (5 % w/w) treated wheat straw, washed and unwashed, as well as untreated straw were compared.

A comparison of NaOH treated and untreated diets showed a highly significant increase in voluntary organic matter intake with NaOH treatment. This increase could be explained mainly by the faster outflow of O.M. from the rumen and the higher potential digestibility of the diet which resulted in a larger mass of fermentable O.M. in the rumen and a larger mass of O.M. being fermented per day. This larger mass of O.M. fermented per day could not be explained by a change in the rate constant for fermentation, which was not influenced

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[1]J.P. Pienaar, H.S. Hofmeyr, Karen I. Plaut & L.Grattarola
Abstract from *S. Afr. J. Anim. Sci.* 10, 209-214 (1980)

significantly by treatment with NaOH, nor by washing the treated straw. It could only be explained by an increase in the active pool size, i.e. fermentable O.M. in the rumen, caused by an increased potential digestibility of the diet. This increase in the digestibility of the diet resulted in an increased ratio between digestible: indigestible O.M. in the rumen. The increased pool size (mass of fermentable O.M. in the rumen) can be ascribed to this altered ration in the rumen, more than to an actual increase in total rumen fill, which accounted for a small fraction of the actual increase.

Washing of NaOH treated diets did not influence ruminal function significantly, except for voluntary water intake and retention time of a water soluble marker in the rumen. Although washing NaOH treated straw removed some fermentable soluble O.M. from the diet, it did not influence the voluntary intake of digestible O.M. nor *in vivo* digestibility significantly.

2.2.2 Effect of starch fermentation in the rumen on voluntary intake of roughage and kinetics of digestion[1]

The realisation of the negative effect of a concentrate supplement on the voluntary intake of roughage and on digestibility is not new (Hamilton, 1942). The explanation most generally proposed is the well-documented negative effect of a low pH on the activity of cellulolytic microbes (Ørskov & Fraser,1975; Mertens & Loften, 1980; Mould, Ørskov & Gauld, 1983 & 84).

However, there are also other possible causes. The ruminal protozoal population may be stimulated by starch supplementation (Eadie & Mann, 1970). These protozoa significantly depress ruminal bacterial populations and thus indirectly decrease the fermentation rate of

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[1]J. P. Pienaar, C.Z. Roux & G.P. Kühn
Abstracted from *S.Afr.J.Anim. Sci.* 20, 4, 207-215 (1990)

roughage diets. The fact that fibre digestion is sometimes depressed by starch fermentation without a concomitant decrease in pH (Henning, Van der Linden, Mattheysen, Nauhaus, Schwartz & Gilchrist, 1980; Mould, Ørskov & Mann, 1983 & 84) suggested that carbohydrate may specifically affect roughage digestion.

Evidence from the literature indicates that the processing of grains may modify their effect on the voluntary intake of roughage (Ørskov & Fraser, 1975). There is clear evidence also that the processing of grains influences their fermentation rate (Hungate 1966; Ørskov & Fraser 1975; Liebenberg, Meissner & Pienaar, 1979). Therefore, provided that no other nutrients are limiting, the amount and rate of starch digested, and the type of roughage used (Dixon,1986), must be the key to understand the interaction between roughage and concentrate digestion in the rumen.

Very little is known about the effect of concentrate supplementation on the *in vivo* digestion and the outflow kinetics of fibre. The few studies which have addressed this problem (Mould & Ørskov, 1983 and 1984) did not allow the separation of the physical effects of the grain from the effect of carbohydrate fermentation on the passage of roughage and the kinetics of fermentation. Thus, another approach should be followed in order to separate these two factors. Direct application of their *in sacco* results to the *in vivo* situation could also be confounding unless the magnitude of the effects are verified in a kinetic model of rumen function. Since concentrate supplements are normally more readily consumed than roughages, roughage intakes may be suppressed after consuming concentrates. This may be due merely to animal behaviour.

In this study the physical effect of grain as well as the palatability effect of starch concentrates were eliminated by infusing very finely ground starch, which is completely available to microbial attack and has a very short retention time in the rumen. By this method the physical effect of the grain particles on roughage fermentation in and passage from the rumen was

completely eliminated and the effect of carbohydrate fermentation in the rumen could be examined in isolation. Care was also taken to ensure that other nutrients such as minerals, protein, or ruminal ammonia were maintained at adequate levels during the experiment.

The aim of this experiment was, therefore, to study the effects of different amounts of starch, fermented at two rates, on roughage intake and on the kinetics of roughage passage and fermentation.

The effect of starch fermentation in the rumen on the kinetics of roughage digestion was studied using 12 sheep fed 3 roughages (lucerne hay, maize cob leaves and wheat straw). The amount of starch infused daily was increased in steps of 20 $g.d^{-1}$ from 0 to 600 $g.d^{-1}$ over 30d. The amount of starch infused was delivered at two rates, viz the daily amount infused over the first 12h of a day, or infused more gradually over 24h.

The well known negative effect of starch fermentation on roughage intake and digestion was observed when the lucerne and maize cob leaf diets were fed, but not when the wheat straw diet was used. The rate at which starch was infused affected the intake of maize cob leaves in a variable manner. The slow infusion rate led to a very small negative effect, while the fast rate of starch infusion resulted in a large negative effect. Regardless of diet, the negative effects of starch on intake could not be ascribed to a lower ruminal fill, nor to a reduced concentration of ruminal ammonia. Furthermore, pH of the ruminal contents was not lowered. Rate of passage of non-fermentable O.M. was decreased by starch infusion on one diet (maize cob leaves) only, even though the mean retention time (MRT) of water was significantly influenced by starch infusion on all the diets. Although starch fermentation negatively affected many aspects of roughage digestion, the paramount factor appears to be a reduced rate of roughage digestion. The observed inconsistent response to the amounts and rates of starch infused between and within roughages showed that each roughage reacts differently to starch infusion. For accurate estimates associative effects will have to be assessed for each diet individually at the relevant rate of starch fermentation.

2.2.3 Factors affecting the voluntary feed intake of sheep grazing *Pennisetum clandestinum* (kikuyu) pastures. 1. Observations from forage analysis[1].

2.2.3.1 Introduction

Animals invariably perform less well on *Pennisetum clandestinum* (kikuyu) pasture than the *in vitro* digestibility and chemical composition of the material produced by the pasture would suggest (Dugmore & du Toit 1988). Tainton *et al.* (1982), Karnezos *et al.* (1988) and Dugmore (1990) have shown that increasing levels of fertilizer nitrogen reduces the performance of individual steers, but steer performance has also been shown to depend on the stage of regrowth of the pasture (Karnezos *et al.* 1988). The higher the levels of nitrogen applied and the more mature the herbage, the lower the voluntary feed intake of the animals (Dugmore 1990).

The trial was undertaken at two sites - the University of Natal research farm, Ukulinga, and the Irene Animal Production Institute (IAPI). The work reported in this paper concerns those factors which control voluntary feed intake of sheep grazing kikuyu pasture. In particular, we focus on factors relating to the nitrogen nutrition of the pasture, and those that are detrimental to forage quality, such as oxalates and saponins.

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[1]J.P. Pienaar, N.M. Tainton & J.B.J. van Ryssen
Afr J Range For Sci, 10, 3, 140-144 (1993)

2.2.3.2 Materials and methods.

Ukulinga

This investigation was undertaken during January and February 1987, on a monospecific stand of kikuyu yielding 4300 kg D.M. ha^{-1}. The pasture grazed, measured 911 m^2.

At the onset of the investigation, the pasture was fertilized with 300 kg LAN ha^{-1} (84 kg N ha^{-1}). Although the grass was dry when the investigation commenced, 39 mm of rain fell over the first two days of grazing. The grass immediately responded by a flush of new growth. One hundred disc meter readings (Bransby & Tainton 1977) and 10 calibration samples were taken daily on the pasture. The calibration samples were dried and separated into three fractions - leaf, stem and sheath, and dead material. The leaf fractions for each day were analysed for their nitrogen, nitrate and oxalate content. Eight mature S.A. Mutton Merino wethers weighing about 70 kg, fitted with large (83-mm ID) cannulae in the rumen, were used to collect samples of freshly grazed material (masticate). Masticate samples were collected every 1-2 days during the 11 day experimental period (on six occasions in all), by manually emptying and rinsing the rumens with lukewarm water. To limit exposure to oxygen while the digesta was being stored outside the sheep, the surface of the digesta was covered with a plastic sheet. The sheep were then put to grazing with empty washed rumens. Following a period of grazing, the ruminal contents was again emptied to provide the samples for later analysis. The original ruminal digesta was then returned to the rumen and the animals returned to grazing. The whole operation was usually completed within 30 minutes.

IAPI

This investigation was undertaken during February 1989 on a monospecific stand of kikuyu measuring 975 m^2. One month before the investigation commenced the pasture was mown to a height of 2 cm and fertilized with urea (70kg N ha^{-1}). It was irrigated as

necessary. Grazing commenced when the total pasture yield was approximately 5700 kg DM ha^{-1}. Each day material was harvested from 10 randomly placed quadrates measuring $0.164m^2$ and the material separated into the leaf and stem fractions. The stem fractions were discarded and the dry matter content, total nitrogen, soluble nitrogen and oxalate of the leaf fractions determined. In addition, the amount of foam produced by the fresh material was estimated by homogenizing a sub sample of the material in an aqueous ethanol solution.

As with the Ukulinga investigation, samples of freshly grazed material (masticate) were collected at six equally spaced intervals during grazing.

2.2.3.3 Procedure

A macro-Kjeldahl method was used to analyse for total nitrogen (AOAC 1984). Soluble nitrogen was determined by homogenizing 20 g of fresh grass in 100 ml of water for 2 minutes and filtering the homogenized material through a polyester material with 53-μ pore size. The recovered filtrate was weighed and the whole filtrate sample used in the analysis for total nitrogen in the macro-kjeldahl (AOAC 1984). Total soluble nitrogen was calculated by extrapolating from the amount of nitrogen recovered in the filtrate, to the total mass of moisture in both the sample and added water. Soluble nitrogen was assumed to be present in a constant ratio to water throughout the sample.

Foam production was used to indicate the saponin content of the herbage. Fresh leaf material (5 g) was homogenized in 100 ml of a 2% solution of ethanol in water until the maximum volume of foam was produced (ca. 90 seconds). The volume of the homogenate was measured to provide an estimate of the amount of foam produced.

Different plant fractions were analysed for neutral detergent fibre (NDF) using the method of Robertson & Van Soest (1981) and oxalate using the method of Abaza *et al.* (1968). The nitrate content of the grass was determined as described by Gottlieb & Magalhães (1958) while the tannin content of the leaves was determined in two ways. The first method tests for the ability of the material to precipitate protein (Hagermann 1987) and the second method, a vanillin-HCl method is specific for certain flavanols and dihydrocalcones in tannins (Terrill *et al.* 1990).

2.2.3.4 Results and discussion

The quantities of dry matter, leaves, stems and dead material on offer at Ukulinga were estimated each day from their respective relationships with disc height. The linear relationship between the mean disc height and the number of days that the pasture had been grazed is shown in Figure 2.2.3.1 for the Ukulinga site. The relationships between disc height and the total amount of grass and the amount of leaf dry matter for the same data set are shown in Figures 2.2.3.2 and 2.2.3.3 respectively. Both relationships were highly significant, but the correlation coefficients, in the case of total grass yield in particular, were disappointingly low.

These relationships nonetheless allowed the estimation of the total amounts of grass, and of leaf material on the pasture throughout the grazing period (Fig 2.2.3.4). The amount of grass at Ukulinga declined from 4.2 ton ha^{-1} to 3.4 ton ha^{-1}, according to the following equation:

$$y = 4.17 - 0.0530x,$$

where: y represents tons of grass per hectare and,

x the number of days on pasture.

Figure 2.2.3.1. Relationship between disc meter reading and day on pasture at Ukulinga.

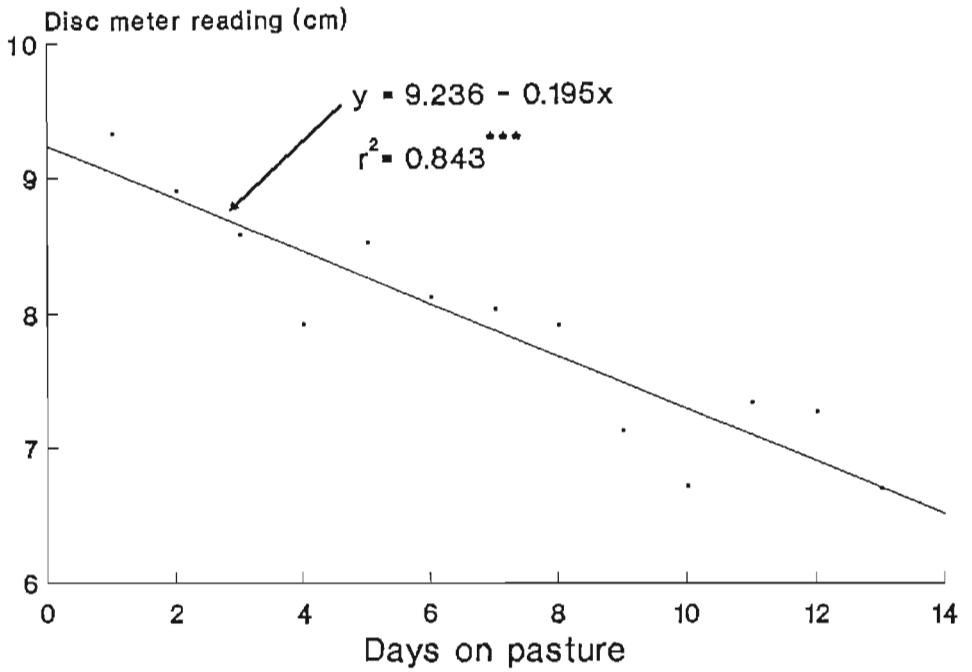


Figure 2.2.3.2 Relationship between disc height and amount of grass on pasture at Ukulinga.

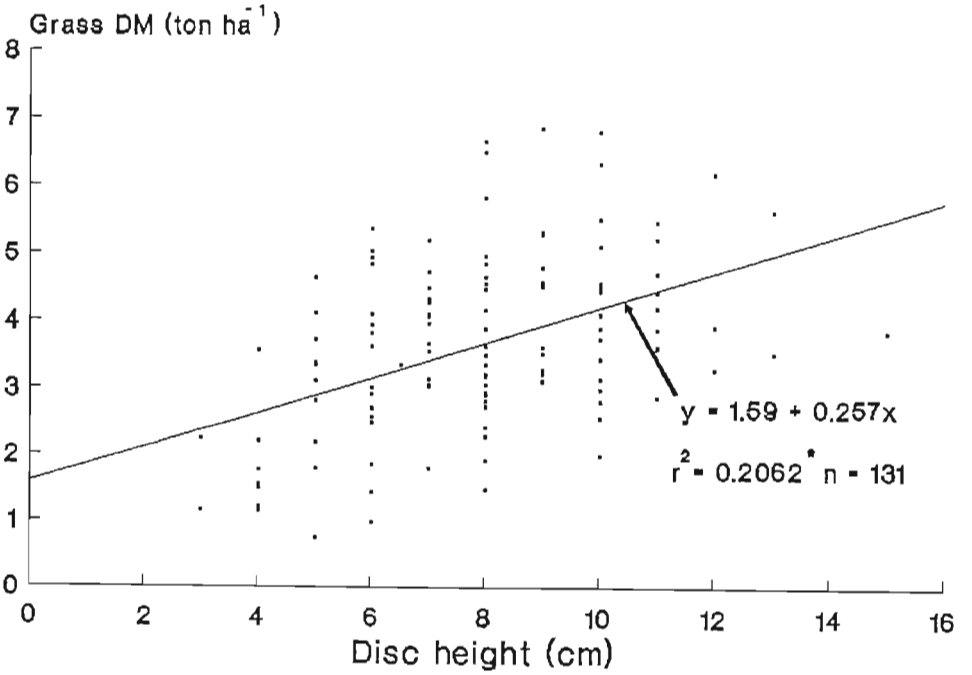


Figure 2.2.3.3. Amount of leaf on pasture versus disc height at Ukulinga.

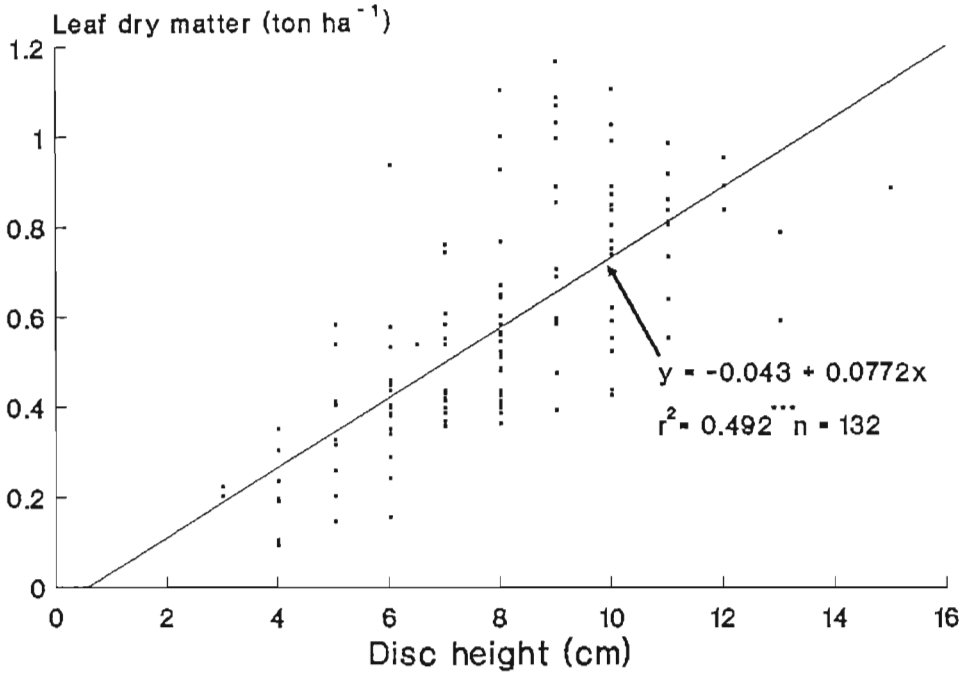
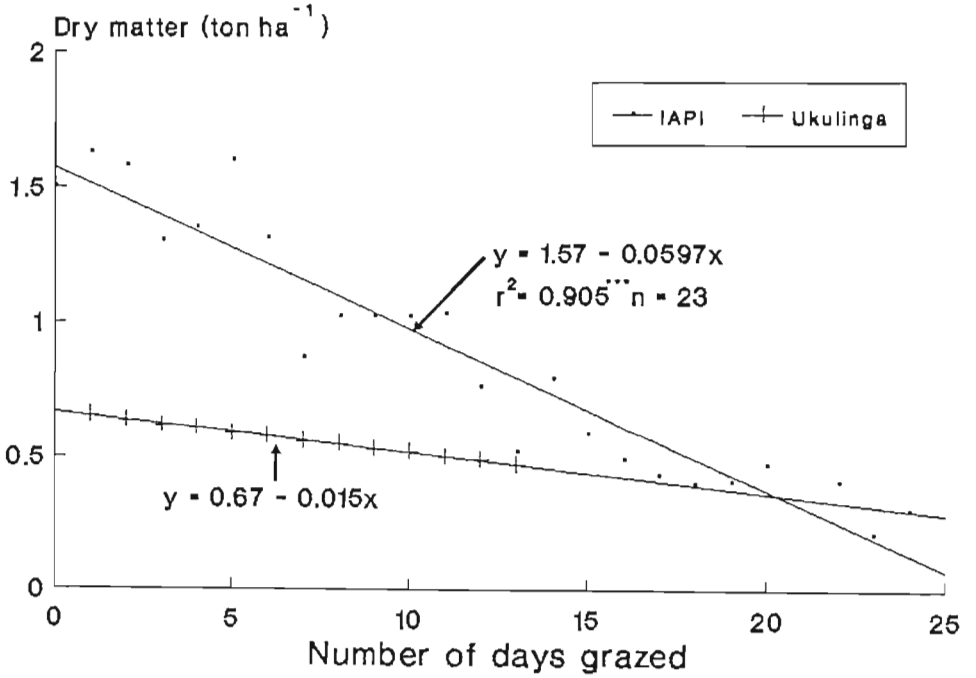
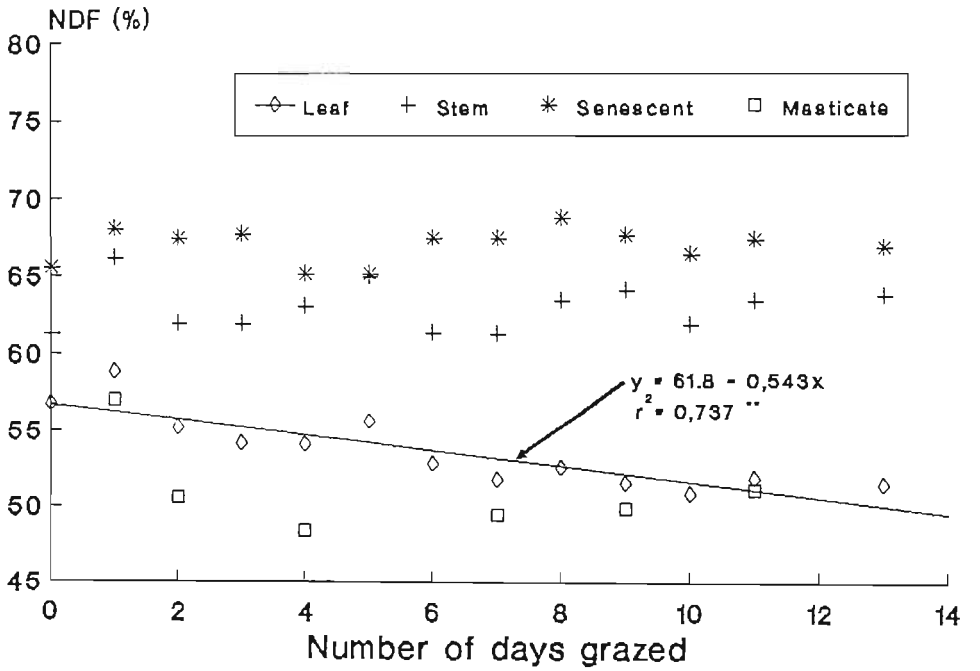


Figure 2.2.3.4. Amount of leaf versus day on pasture at both Ukulinga and IAPI.



The amount of leaf declined from 650 kg ha^{-1} to 470 kg ha^{-1} during the grazing period. Visually, the pasture appeared to have been grazed very short at the end of the grazing period (Figs. 2.2.3.1 & 2.2.3.4) and inspection of the masticate samples (collected via the ruminal fistula) showed that a large amount of stem material was being grazed at this time. The total amount of leaf material available at the IAPI site during the grazing period is also shown in Figure 2.2.3.4. More leaf material was initially available at this site (1 570 kg ha^{-1}) than at Ukulinga, but there was less residual leaf at the termination of grazing (200 kg ha^{-1}). This difference would seem to be associated with the lower growth structure of the kikuyu in the cooler highveld conditions at IAPI, compared to the more upright growth and greater stemminess of this species growing in the warmer conditions at Ukulinga. The flush of growth in the grass immediately following the rain which fell during the first two days of the investigation at Ukulinga, was associated with a near

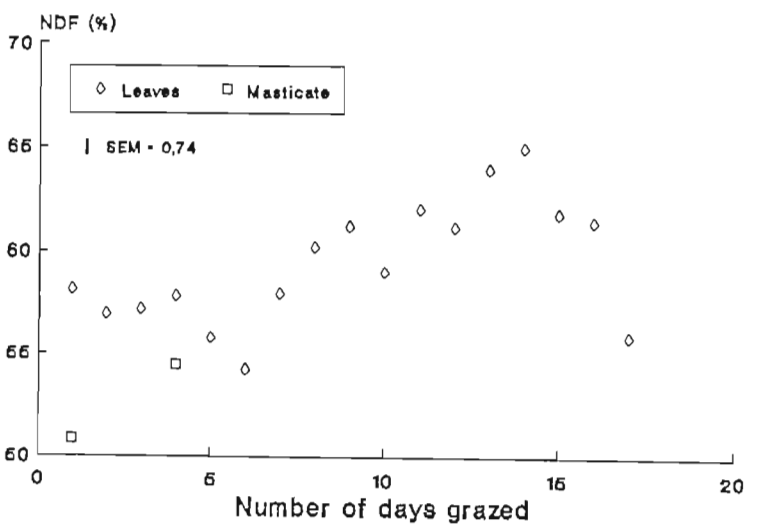
Figure 2.2.3.5. NDF content of the different plant fractions and masticate at Ukulinga.



linear decline in the NDF content of the available leaf material (Fig. 2.2.3.5). The leaf material available to the animals therefore contained progressively less cell wall (fibre) material as time progressed. Also shown in Figure 2.2.3.5 is the NDF content of the other plant fractions and of the masticate collected from the animals. The NDF content of stem and senescent material remained relatively high throughout the grazing period and was considerably higher than that of the leaves, while that of the masticate declined initially but then increased as the amount of leaf material on the pasture declined. This suggests that the animals were forced to graze increasing amounts of stem and senescent material during this stage of the grazing period.

The NDF content of the available leaf material at the IAPI site is shown in Figure 2.2.3.6.

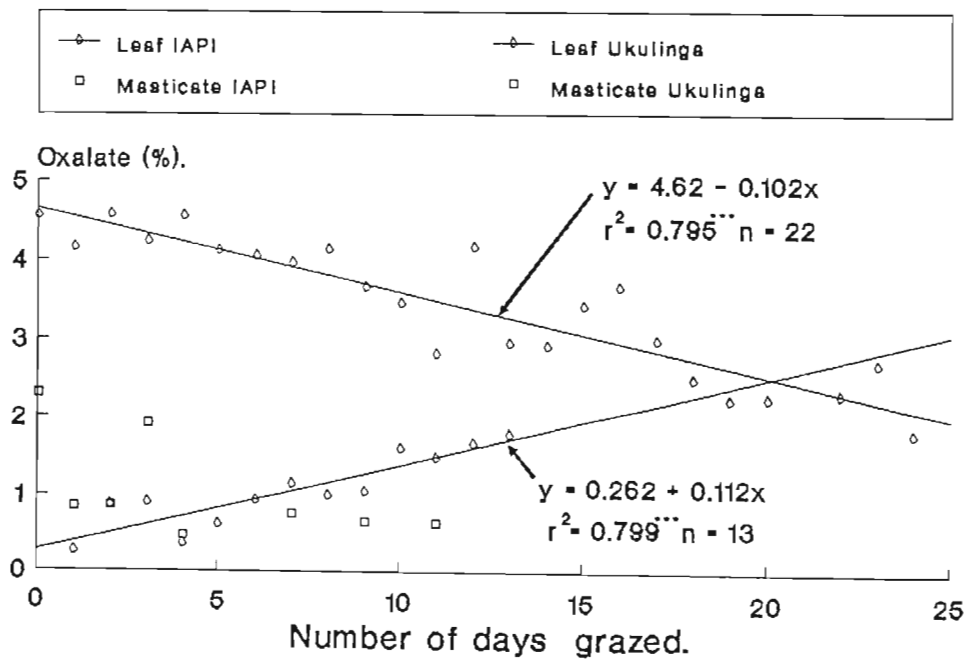
Figure 2.4.6. NDF content of the leaf material and masticate at IAPI.



Here the NDF of the available leaf material increased steadily during most of the grazing period. It is likely that the sheep, having removed most of the material which had accumulated prior to grazing, were forced onto young growth low in NDF during the latter stages of the grazing period. Only two masticate samples were available for analyses at this site (Fig 2.2.3.6) since there was insufficient sample remaining after the extraction of material for use in the nylon bag digestion trial on most occasions. Analyses of both these samples suggest selection of material much lower in NDF than the average of the material on offer.

The oxalate contents of the leaf material on offer and of the masticate for both sites are shown in Figure 2.2.3.7. At Ukulinga the oxalate content of the leaves increased steadily throughout the grazing period (from 0.4% to 2%) as the pasture responded to the

Figure 2.2.3.7. Oxalate content of leaves and masticate at Ukulinga and IAPI.



improved moisture conditions by producing a flush of new leaf material. Such new growth is typically higher in oxalate content than older material (Kipnis & Dabush 1988). At the IAPI site, the oxalate content of the material was initially high (ca. 5%) but declined during the grazing period to reach lower values (ca. 2%) at the end of the investigation. These levels exceeded those quoted by Kipnis & Dabush (1988) as being toxic to animals. However, the fact that it is readily metabolized in the rumen (Allison *et al*, 1977) and only insoluble oxalate was observed in these samples may have prevented poisoning of the sheep. The relatively low oxalate content of the masticate suggests that animals selected material containing lower concentrations of oxalate than the average of the material on offer. The slope of a regression line (not shown) suggests that for each 1% increase in the oxalate content of the leaf material on offer, the oxalate content of the masticate increased by only 0.38 %. However, since this relationship was derived from two separate data sets, it should be viewed with caution. It may also be argued that the oxalate in the masticate was metabolized by ruminal micro-organisms before removal, thus reducing its concentration in the sample that was analysed. However, since the material was frozen in liquid nitrogen within about 30 minutes of it being ingested by the animal, and freeze dried before being analysed, this is an unlikely source of error.

The nitrate content of the leaf material from the Ukulinga site is described by the following quadratic equation:

$$y = 0.0091 + 0.0667x - 0.00438x^2 \quad (r^2 = 0.789)$$

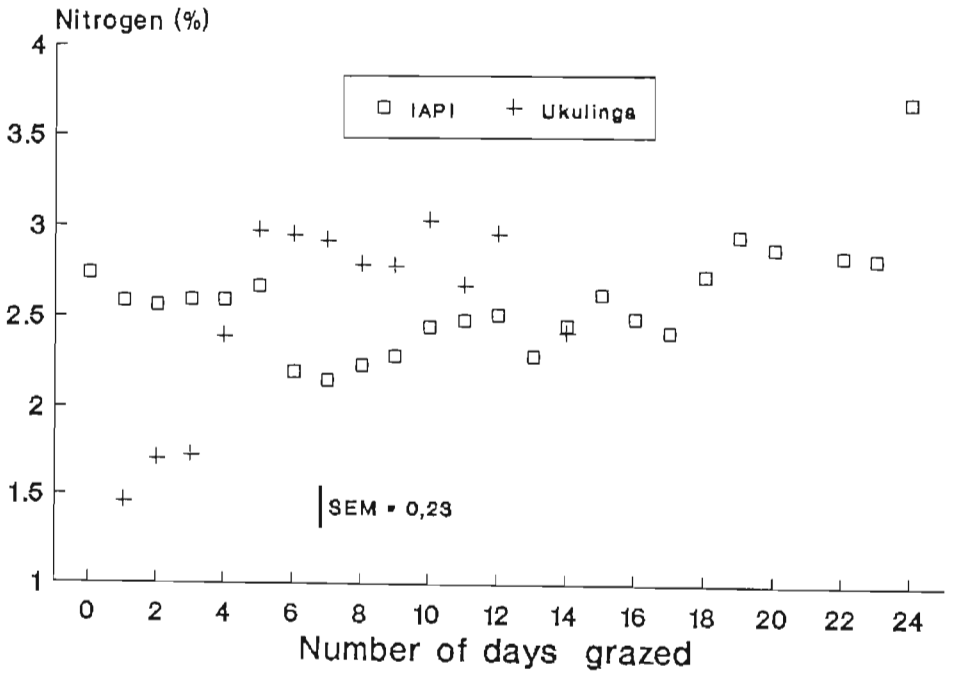
where: y is the percentage nitrate in the leaves and,

x is the number of days of active growth following fertilization.

The nitrate content increased rapidly for the first six days of grazing, but later declined. This pattern is consistent with that produced by Eckard (1990, Personal communication, Cedara college of Agriculture. Private Bag X9059, Pietermaritzburg, RSA.) and suggests that a period of about two weeks of active growth following the application of nitrogen fertilizer is needed in order to minimize the risk of nitrate poisoning in animals.

The total nitrogen content of the leaf material in both investigations is shown in Figure 2.2.3.8. (These levels equate to mean crude protein levels of 19.5% for the Ukulinga site and 15.5% for the IAPI site). These data on total nitrogen content followed the same pattern as nitrate content during the early phase of the Ukulinga investigation, but then remained high for the remainder of the investigation period. If high nitrogen levels in kikuyu have a negative effect on animal performance, as suggested Dugmore (1990), then periods of recovery following the application of nitrogen fertilizer should extend for longer than two weeks. At the IAPI site the total nitrogen content of the leaf material remained high throughout the 24-day investigation period. Of this nitrogen 30.8 ± 11.1 % was shown, on average, to be soluble. Such soluble nitrogen is normally rapidly degraded to ammonia in the rumen (Chamberlain & Thomas 1979) and can therefore be compared to urea in its effects on voluntary intake. This allows for the calculation of an urea equivalent of the material.

Figure 2.2.3.8. Nitrogen content of leaves at Ukulinga and IAPI.



Extensive research has shown that voluntary intake normally declines when the urea content of a complete feed exceeds 1.5% (Wilson *et al.* 1975). This can be ascribed to an ammonia load in the blood in excess of the capacity of the liver to synthesize non-toxic urea from the ammonia. Beever (1989) has shown this to occur with the feeding of some grass silages.

The mean urea equivalent of the kikuyu at the IAPI site was 1.64% (95% confidence limits = 0.37 - 2.91%). Therefore, even the mean value of the soluble nitrogen content of kikuyu monitored at this site is dangerously high. To make matters worse the potential for urea poisoning is aggravated by a relatively high ruminal pH (pH = 6.53 at IAPI) which facilitates the rapid movement of ammonia across the ruminal wall (Bloomfield *et al.*, 1963).

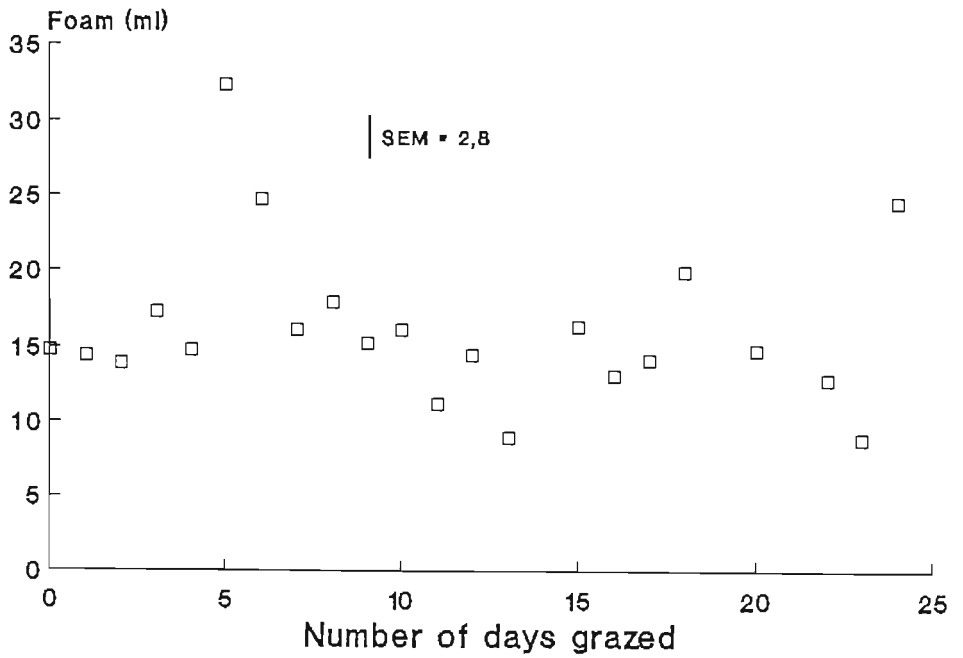
The above results may at least partly explain the negative relationships between levels of nitrogen fertilizer used and both voluntary intake and animal performance reported by Tainton *et al.* (1982), Karnezos *et al.* (1988) and Dugmore (1990). Results by Dugmore *et al.* (1991) suggested a positive selection by steers where the average crude protein content is low ($< 14\%$), but selection against protein on herbage with a high crude protein content ($> 14\%$). These results support this contention by pointing to the apparent negative effect of soluble nitrogen on voluntary intake.

The results of the *in vitro* foam study are shown in Figure 2.2.3.9. The foaming capacity of the material fluctuated widely over the grazing period, but averaged 15 ml of foam for each 5g of fresh material. Note the high value on day five which differs significantly ($P < 0.05$) from the mean.

Foam production on kikuyu compares most unfavourably with, for example, a fresh sample of *Eragrostis plana* which was included in these analyses as a reference, and in which no foam was produced.

No tannins could be detected in the kikuyu leaves using either of the two methods of detection.

Figure 2.2.3.9. *In* vitro foam production at IAPI.



2.2.3.5 Conclusions

A study of the factors in kikuyu that could be implicated in limiting voluntary intake by ruminants suggests that there are at least four factors involved. These include the high soluble nitrogen and nitrate content of the material, its high oxalate content and its considerable potential for foaming, the latter suggesting the presence of saponins. The high fibre content could also limit intake in wilted, dry or heavily grazed and therefore, stemmy kikuyu. The relative importance of these factors appears to change with growth stage, with the amount of nitrogen fertilizer applied and with soil moisture conditions. The factors which are detrimental to forage quality, such as oxalates and nitrates, were higher in the young and actively growing material than in older material, while the fibre content is high in mature and stemmy herbage. However, the *in vitro* production of foam does not seem to be related to these factors. Foam production remained high despite changes in the other factors which are detrimental to forage quality.

A comparison of the composition of the masticate and that of the leaf, stem and senescent material on offer, suggests selection towards low oxalate and NDF contents in the herbage. A similar trend was observed by Dugmore *et al.* (1991) for nitrogen. In the next paper in this series (section 2.2.4), the performance of the sheep grazing the kikuyu will be discussed.

2.2.3.6 Acknowledgements

The following people are thanked for their contributions. Miss L. du Toit of the Grassland Research Centre at Roodeplaat for tannin determinations. Mr. J. Clayton for pasture management and electrical fencing. Mr. S.J. Davie, Mrs. J. Behrens, Mrs. R. Smith and Miss E. Nell for various chemical analysis. Messrs. D. Nkobane, F. Tansha, W. Madutlela and M. Bosoga for handling and care of the sheep. Mrs. M. Odendaal and M. Smith for help and advice with some statistical aspects. **Soli Deo Gloria**

2.2.4 Factors affecting the voluntary feed intakes of sheep grazing *Pennisetum clandestinum* (kikuyu) pastures. 2 Observations in the animal[1].

2.2.4.1 Introduction

In any livestock management programme it is important to be able to predict the performance of the grazing animal given a certain level of available forage of a particular quality. Methods of feed analysis currently employed do not provide data which will allow for the prediction of animal performance when a wide range of forages is used. Estimating forage quality from chemical analysis of oesophageal fistula samples have not yet proved successful since the results of such analyses cannot readily be related to daily animal performance. Pienaar *et al.* (1980) have shown, however, that the voluntary feed intake of sheep can be accurately predicted if ruminal fill and fermentation and flow kinetics are known.

Pienaar, Roux &Cronjé (1989) have shown that *in sacco* estimates of fermentation kinetics agree closely with those obtained *in vivo*. The *in sacco* technique may be used to obtain day by day estimates of fermentation kinetics. However, ruminal fill and the mean pasage time for outflow has to be measured *in vivo* every two to three days while the animals are grazing.

In Section 2.2.3 it was suggested that at least four factors are present in the plant in sufficient quantities to limit voluntary feed intake. In this paper, the possible roles of ruminal fill, digestion kinetics and microbial protein syntheses in the control of intake are discussed.

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[1]J.P. Pienaar, N.M. Tainton, J.B.J. van Ryssen & J.P. Swiegers
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2.2.4.2 Materials and Methods

General experimental procedures adopted in this trial are described under section 2.4.2 and 2.4.3.

Animals

Ukulinga

Three of eight mature S.A. Mutton Merino wethers used in the trial, and fitted with large (83 mm ID) cannulae in the rumen, were also fitted with soft rubber cannulae in the abomasum and terminal ileum. All sheep had been regularly treated for internal parasites and had been provided with a supplement lick containing salt, phosphate and calcium. They were all adapted to being handled and to carrying harnesses and faecal collection bags. The multi-cannulated animals were also adapted to carrying portable infusion pumps, and all had previously been grazing for periods of 14 days on a pasture similar to and adjacent to the experimental pastures.

Masticate samples were collected at six occasions during the 11-day experimental period by manually emptying and rinsing the rumens with lukewarm water every 1-2 days (Figure 2.2.4.4.). To limit the rate of penetration of oxygen while the digesta was being kept outside the sheep, a thick plastic film that was cut the same size than the inside diameter of the bucket, was placed on the surface of the digesta. The sheep were then put to graze with empty, washed rumens. Following a period of grazing, the ruminal contents was again emptied to provide the samples for later analysis. The original ruminal digesta was then returned to the rumen and the animals returned to grazing. The whole operation was usually completed within 30 minutes.

The masticate samples were sealed in plastic bags and quick frozen with liquid nitrogen. The masticate used for the *in situ* study were weighed out into the bags immediately after collection.

IAPI

As in the Ukulinga trial, the mature SA Mutton Merino wethers which had been fitted with large ruminal cannulae (83mm ID) had been pre-conditioned before entering the trial. They had been regularly treated for internal parasites and had been provided with a supplement lick containing salt, phosphate and calcium. Four animals were used for incubating nylon bags for *in sacco* studies while all the sheep were used to collect masticate samples and for the determination of ruminal fill using the procedures identical to those used at Ukulinga.

2.2.4.3. Procedure.

During the measurement of partial digestion at the Ukulinga site, flow of digesta at the abomasum and ileum was measured using radio labelled chromium EDTA and a ruthenium phenantroline complex as soluble and particulate markers, respectively (Faichney, 1975). Portable infusion pumps (Corbett *et al.*, 1976), tied onto the backs of the sheep, were used to dispense the markers. The relative contribution of microbial protein to total protein flow at the abomasum was determined using purine analysis as described by Zinn & Owens (1986).

The method used for *in sacco* fermentation was the same at both experimental sites and is similar to that described by Ørskov & McDonald (1979), except that polyester material

with a pore size of 53 μ was used and one bag was removed from each sheep after ca. 3, 10, 21, 35, 45, 59 and 69 h incubation. The fresh masticate (containing about 3g of DM initially) was incubated in polyester bags immediately after collection. The bags were washed by rinsing them three times with 10 liter clean water. The immediately soluble fraction of the diet was determined as that fraction that disappeared from the *in sacco* bags within the first 3 hours of incubation. The mean retention time model (Pienaar, *et al.* 1989) was used to fit the curves of DM disappearance.

Rate of passage of the indigestible organic matter (O.M.) fraction of the diet was calculated by dividing the indigestible (O.M.) content of the rumen (g) by O.M. excreted in the faeces (g/h). The indigestible organic matter fraction was also determined by incubating ruminal contents *in sacco* for 69h.

The methods used to predict voluntary feed intakes were the same at both experimental sites. The first method (termed the mean retention time (MRT) method) used ruminal flow- and fermentation kinetics as described by Pienaar & Roux (1989a). The second method, termed the faecal excretion (FE) method, used faecal excretion rates (obtained with faecal collection bags), and digestibility of the masticate samples (as determined *in sacco*) to predict voluntary feed intake.

The specific gravity (SG) of the ruminal contents was determined on the material freshly removed from the rumen. The mass and volume of the total ruminal digesta were determined and SG calculated as mass divided by volume. Both the mass and the volume were determined by emptying the ruminal contents into a graduated container and measuring its weight and volume.

Estimated Net Energy (NE) intake was calculated as follows: firstly, voluntary feed intakes were multiplied by the potential O.M. digestibility of the diet, as determined *in sacco*, to obtain digestible O.M. intake. Digestible O.M. intake was multiplied by 0.0184

to obtain digestible energy (DE) intake (MJ/day). DE was, in turn, multiplied by 0.82 to give metabolizable energy (ME) intake (ARC, 1980). The tables of Meissner, *et al* (1983) were used to obtain the efficiency of utilization of ME for maintenance and growth at the respective levels of production as well as the energy requirements of sheep for maintenance and mass gain.

Ruminal ammonia concentration was determined on fresh samples from the mixed ruminal digesta collected immediately after emptying the rumen. The samples were filtered through a nylon cloth, acidified with H_2SO_4 and frozen till analysed. For analysis, the sample was thawed, centrifuged and read on an auto-analyser (Technicon Auto-analyser, 1976).

2.2.4.4. Results and discussion.

The two methods used to estimate voluntary feed intakes by animals grazing kikuyu pasture gave mean intakes, over the 24 day experimental period at IAPI, of 1023 g O.M. day^{-1} (for the MRT method) and 1123 g O.M. day^{-1} (for the FE method). Although differences between methods were small they were consistent over time and statistically highly significant ($P < 0.01$; Figure 2.2.4.1).

The MRT method produced a much more consistent pattern of voluntary intake than the FE method. This is not unexpected since faecal excretion continues as long as passage from the rumen continues, and changes in the rates of faecal excretion will therefore lag behind changes in intake. The MRT method does not suffer from such a pronounced lag phase, and therefore provides a more instantaneous estimate of intake into the rumen. It was therefore the preferred method in this study.

Figure 2.2.4.1 Comparing the mean retention time (MRT) method with the faecal excretion (FE) method for estimating voluntary intake on the IAPI site.

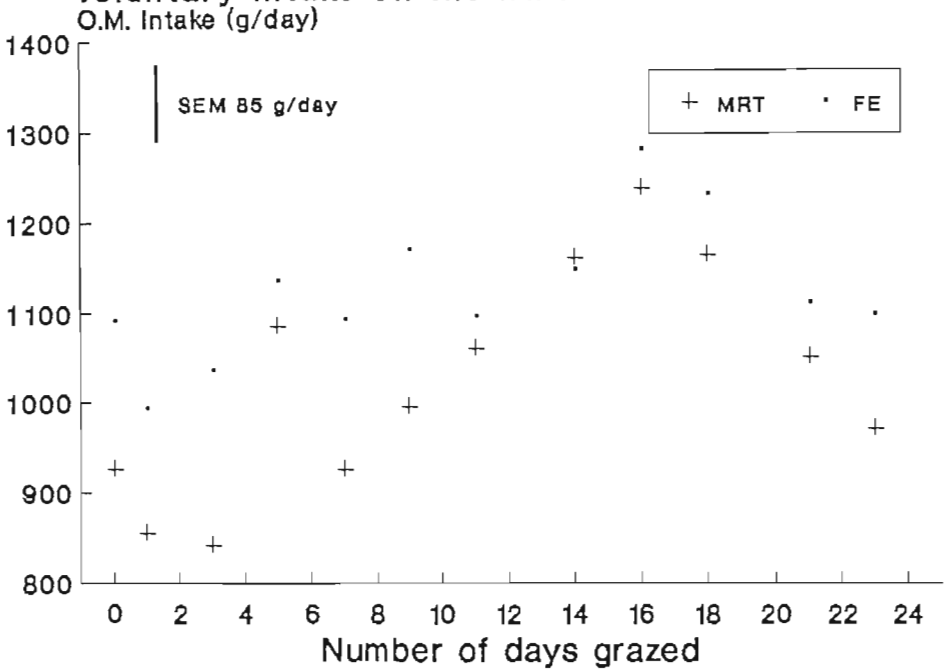
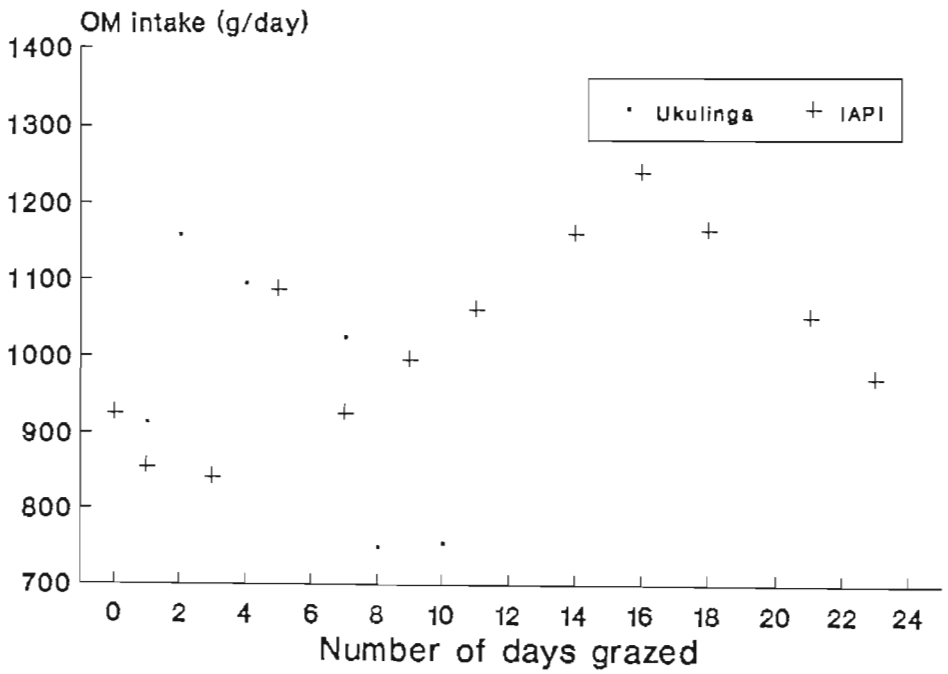


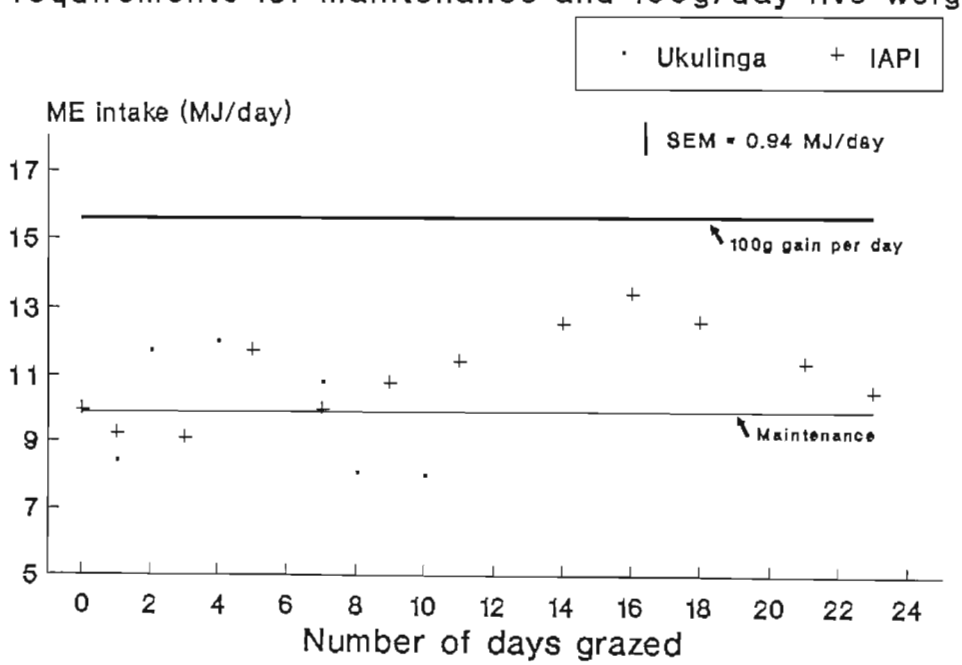
Figure2.2.4.2. Voluntary OM intakes on grazing at the Ukulinga and IAPI experimental sites using the MRT method.



In both trials, voluntary intake initially increased to a maximum and then declined (Figure 2.2.4.2). At Ukulinga this maximum was reached much sooner than at the IAPI site and intake may have been limited by different factors at the two experimental sites. This will be discussed later.

At both experimental sites intakes were just above or below the maintenance requirements of the animals at the beginning of the grazing periods (Figure 2.2.4.3). Intakes then increased to values higher than maintenance which, over the short period of time involved, would not have had any appreciable influence on animal mass and therefore on subsequent maintenance requirement. Intakes then declined to approach maintenance or

Figure 2.2.4.3 Estimated metabolisable energy intake and energy requirements for maintenance and 100g/day live weight gain.



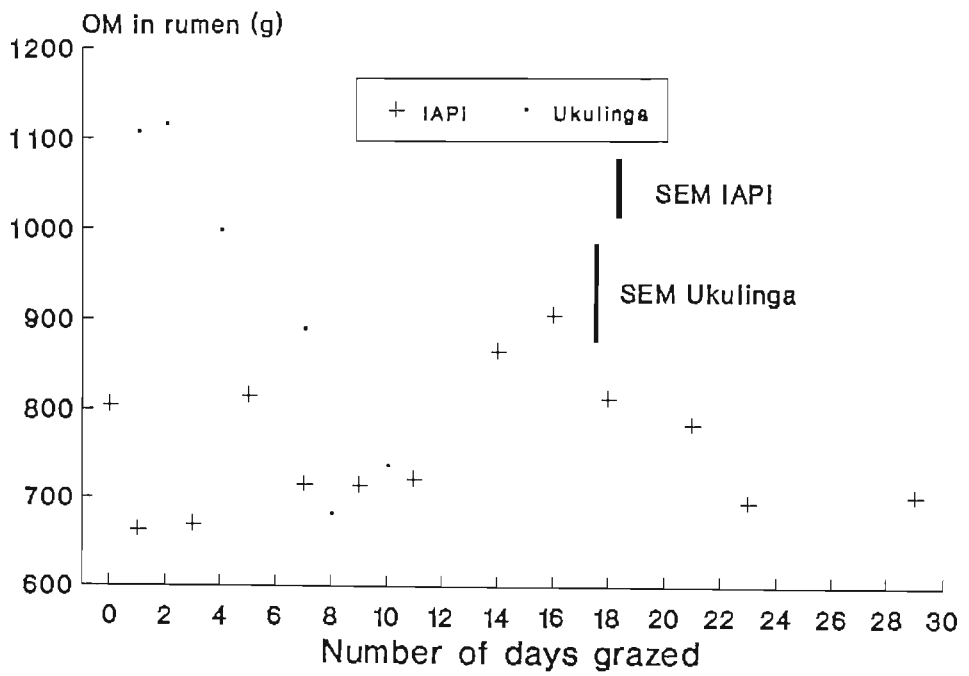
sub-maintenance values. The fact that intake increased while grass was being removed from the grazing at a relatively fast rate, was unexpected. Possible explanations for this will be discussed later in the paper.

Digestibility increased from about 60% to about 70% (SEM = 3.4 %) over the first three sampling periods at the Ukulinga site. It then remained at approximately 70%. This increase was associated with the flushing of the grass following rain, and the subsequent availability of freshly grown material for selection by the animals. Voluntary intake increased at the same time from 914g day^{-1} to 1156g day^{-1}. Thus, if the first two sample values were associated with a high ruminal fill, voluntary intakes may have been limited by ruminal digestion kinetics during this 3-day period. The highest ruminal fill (> 1100g) was observed during these two days at Ukulinga. At the IAPI site, no consistent pattern was found between digestibility and intake. Here the digestibility of the material averaged 71.6 (± 6.2)% and seemed unrelated to intake.

Ruminal O.M. content at the onset of the trial was much higher at the Ukulinga site than at the IAPI site (Figure 2.2.4.4). At Ukulinga it declined almost linearly over the experimental period and here the high values for the first few sampling periods suggest that intake could have been limited by ruminal fill and digestion kinetics for the first two days. As the grass flushed following rain, ruminal fill declined. Thus the increasing voluntary intake associated with the improvement in the quality of grazing was soon offset by decreased ruminal fill. At the IAPI site, ruminal fill was low at the start of grazing but increased to a maximum, before again declining. The two outliers at days 0 and 5 for the IAPI site are conspicuous for their non- conformity with the remainder of the data. The first may be explained as a carry-over from the adaptation period and is associated with a slow rate of passage which was also observed on that day. This is most likely to have been caused by the consumption of kikuyu stems due to limited amounts of leaf material available in the adaptation camp. Because of the slow rate of passage at this time the voluntary intake for that specific day would not have been high. However, on

day 5 the high ruminal fill was associated with a high voluntary feed intake (Figure 2.2.4.2). Inspection of the *in vitro* foam production data (Figure 2.2.3.9.) shows the highest foam production estimates of the period were obtained for that day, and were significantly ($P < 0.05$) higher than the mean foam production. On day six there was also a considerable drop in the mean nitrogen content of the leaves, from 2.66% to 2.19% (Fig. 2.2.3.8). A study of the weather conditions on day six showed a rapid change in wind direction from southerly (200°) to northerly (340°), and cloud cover changed from clear to heavily overcast from 06:00 onward for two days. Relative humidity also increased from 63% on the previous day to 93% and remained at this value for 2 days. Temperatures remained relatively constant. Thus, the high ruminal fill and high voluntary intakes observed at the IAPI site on day five are likely to have been associated with

Figure 2.2.4.4. Organic matter content of the rumen at the Ukulinga and IAPI experimental sites. SEM for each point is 108g and 69g for Ukulinga and IAPI respectively.

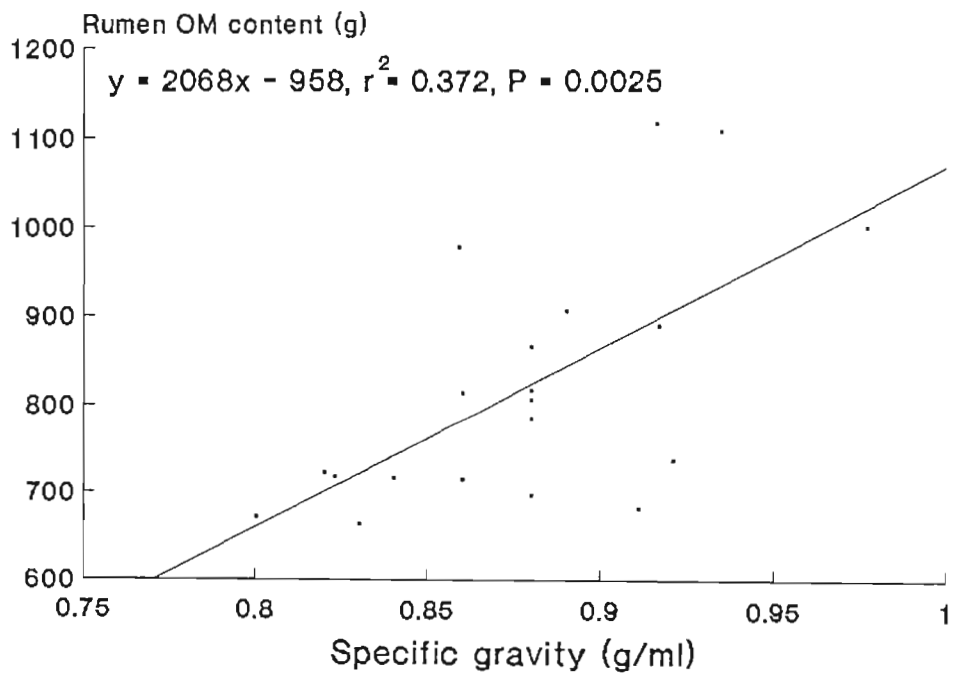


changes in the nature of the forage material on offer brought about by changes in the environment. This observation is confirmed by the change in intake shown for the MRT method and emphasized by a slight change shown in the FE method (Figure 2.2.4.1).

The increased ruminal fill observed at the IAPI site from day 0 to 16 (Figure 2.2.4.4) is associated with an increase in the NDF content of the leaf material. The decreased ruminal fill observed at the Ukulinga site is associated with a decrease in the NDF content of the leaf material caused by the flushing of the grass following the rain (Fig. 2.2.3.6).

The specific gravity (SG) of the ruminal contents gives an indication of its foaminess: the greater the volume of foam the lower the SG. Since a considerable amount of foam was

Figure 2.2.4.5. Relationship between the specific gravity and the OM content of the rumen.



produced from kikuyu leaf material *in vitro* (Fig. 2.2.3.9), it was suspected that ruminal fill could have been limited by foam formation in the rumen (subclinical bloat). The relationship between ruminal O.M. content and the SG of the ruminal contents on the combined data of both the Ukulinga and IAPI sites is presented in Figure 2.2.4.5.

This relationship between ruminal O.M. content and the SG of the ruminal contents is highly significant (P = 0.0025), suggesting that a low SG is normally associated with a low ruminal OM fill. This may be read together with the changes in NDF content of kikuyu (Fig. 2.2.3.6). Ruminal OM fill is low on low NDF kikuyu and high on high NDF kikuyu when intake is not restricted by a limitation of available forage.

In order to establish whether the low mass of the ruminal contents (i.e. low ruminal OM fill) observed with a foamy rumen is caused by a limitation of ruminal volume (i.e. the volume of ingesta plus foam), the SG of the digesta was compared with ruminal digesta volume.

An unexpected positive relationship between SG and digesta volume suggests a tendency for digesta volume to decline as the specific gravity of the digesta declines. If this is so, then the factor which limits ruminal OM fill when ruminal contents is foamy (i.e. has a low SG) is not digesta volume *per se*, but rather something associated with the foamy ruminal content. A high ruminal OM fill with almost no foam was recorded on day five at the IAPI site, despite the fact that *in vitro* foam production was significantly higher than average on that day. This suggests that high *in vitro* foam production (high saponin content of the grass) is not necessarily associated with foamy ruminal contents.

The rate of O.M. disappearance *in sacco* provides an indication of *in vivo* fermentation rate (Pienaar *et al.*, 1989). The latter is an important factor determining voluntary feed intake when ruminal fill limits voluntary intake. No consistent pattern in *in sacco* O.M. disappearance rate during the grazing period was observed at the two experimental sites.

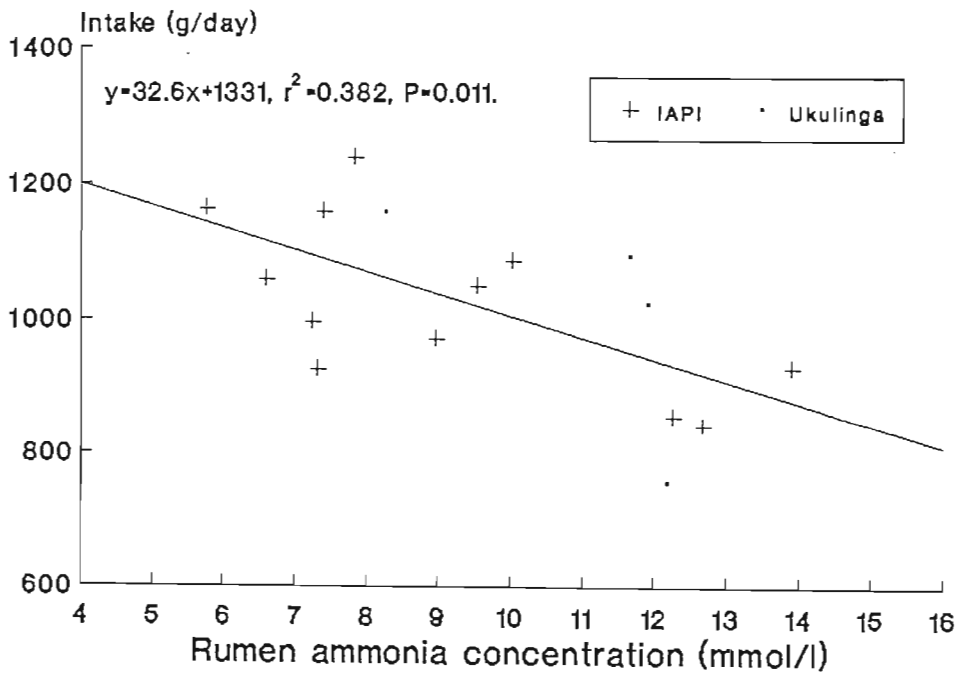
Mean values of 19.63 ± 4.92 and 19.85 ± 2.28 (MFT in hours) were recorded for the Ukulinga and IAPI sites respectively. Thus the changes observed in voluntary intakes were not associated with changes in fermentation rate.

One of the factors which has been shown to influence voluntary feed intake is the level of the immediately soluble fraction in the diet (Pienaar & Roux, 1989a). The higher the solubility, the faster the feed can disappear from the rumen. No significant trend in solubility over time was observed at either of the two experimental sites. However, there was a difference ($P=0.007$) between the means of the two experimental sites. The values for Ukulinga (0.305) were higher than those for IAPI (0.212). This difference is consistent with the difference in voluntary feed intake observed at the two experimental sites. However, the trend observed in the NDF content of the leaves (Fig. 2.2.3.6) is not consistent with the observed solubility of their constituents if it is assumed that a higher NDF content is associated with a lower solubility.

Rate of passage (mean passage time) of the indigestible organic matter fraction (MPT of the I.O.M.) of the diet is also an important determinant of voluntary feed intake (Pienaar & Roux, 1989). However, there seems to be no clear pattern in the rate of passage of the indigestible fraction over time, nor is there a significant difference between experimental sites. An exceptionally high value (55h) on day one at the Ukulinga site could very well be ascribed to experimental error, since it is associated with an unexplained but highly significant lower faeces excretion and an also unexplained but nearly significant lower digestibility of the ruminal contents on that day, both of which would increase the estimate of the MPT of I.O.M. Thus, it does not appear as if the changes in voluntary feed intake can be ascribed to changes in rate of passage. The mean value of 33.11 ± 6.66 (MPT h) could be a useful value for future use on kikuyu pasture.

At no stage during either experiment was ruminal ammonia concentration near the minimum requirement of 3.6 m mol/litre proposed by Satter & Slyter, (1974) for optimal microbial activity. On the contrary, high values recorded at the beginning of the IAPI trial (14 mmol l^{-1}) and during the latter stages of the Ukulinga trial (12 mmol l^{-1}) seem to support the proposal put forward in the first paper of this series (Section 2.2.3.6) that a high load of ammonia on the liver could limit voluntary intakes in some situations. Unfortunately, the concentration alone cannot be used to determine the ammonia flux to the liver, since ammonia concentration is the result of two processes, ammonia production and ammonia uptake. A high ruminal pH results in ammonia being in the NH_3 form which is the form in which ammonia is rapidly transported across the ruminal wall (Bloomfield *et al.*, 1963). Thus ruminal pH must also be taken into consideration. Despite all the limitations of this method a plot of voluntary feed intake vs ruminal ammonia

Figure 2.2.3.6. Relationship between rumen ammonia concentration and voluntary feed intake over both the Ukulinga and IAPI experimental sites.



concentration (Figure 2.2.4.6) revealed a significant association between these two factors ($P = 0.0107$ and $r^2 = 0.382$).

Thus, the possibility that a high soluble nitrogen content of the grass limits voluntary feed intake, as discussed under 2.4.4, seems more than just likely. This conclusion is strengthened by the fact that the large voluntary intake observed on day five was associated with a significant drop in the nitrogen content of the plants at that time. The nitrogen content of the plants at Ukulinga was also very low when ruminal fill was high (Figure 2.2.3.8).

The possibility that a high moisture content of the grazing could have limited voluntary intakes (John & Ulyatt, 1987) was also investigated. No significant relationship between dry matter content and voluntary feed intake could be established at either of the experimental sites. On the contrary, the dry matter content in both experiments was normally higher than the minimum value of about 18% below which most intake problems are usually observed (Du Preez & Meissner, 1991).

In order to establish the periods during which voluntary intakes may have been limited by low availability of pasture, the curves relating ruminal fill, voluntary feed intake and amount of leaf material to the grazing period need to be examined. Figures 2.2.4.2 and 2.2.4.4 show a rapid decline in both voluntary feed intakes and ruminal fill following days seven and 16 for the Ukulinga and IAPI sites respectively. From the data illustrated in Figure 2.2.3.4 it can be seen that 560 kg ha^{-1} and 492 kg ha^{-1} of leaf was present in the paddocks at the Ukulinga and IAPI sites respectively. Expressed as the total amount of dry matter (leaf, stem and dead) available to the animals it amounts to 3820 kg ha^{-1} at the Ukulinga site and, assuming the same percentage leaf (14.7%) at the two sites, an amount of 3344 kg ha^{-1} at the IAPI site. Below these values, intake dropped off rapidly and these

could be considered the upper limits for acceptable levels of sheep performance on such pastures.

Protein intake and partial digestion.

Partial digestion and microbial nitrogen was measured at Ukulinga only. Microbial nitrogen, expressed as a fraction of total non-ammonia nitrogen measured at the abomasum, varied mostly between 0.5 and 0.7, except for day seven when it was between 0.3 and 0.5. Since no explanation can be given for the lower microbial fraction on that day, it is assumed that this difference can be ascribed to experimental error. It is suggested, that for kikuyu pasture, a single value of 0.542 ± 0.082 provides an adequate representation of the microbial nitrogen fraction as a proportion of the non-ammonia nitrogen fraction in the abomasum.

In order to predict the amount of bacterial protein produced from the grazing, it is necessary to know what fraction of the grass was digested in the rumen, compared to that digested in the total gastro-intestinal tract (GIT). The first value (obtained on day 2 at Ukulinga) was considerably higher (68%) than the subsequent values (about 50%). The obvious reason for this is the wilted and dry condition of the grass taken by the animals at the beginning of the grazing period. At that stage, close to 70% of total dry matter digested, was digested in the rumen. As the grass flushed following the rain, the rate of passage increased and relatively more grass was digested lower down in the GIT. The lower values (mean ± SD) agree very well with the value of 0.61 (± 0.049) proposed by the ARC (1984) for fresh grass while the value on day two agrees with the value of 0.7 (± 0.082) which the ARC (1984) have proposed for hay.

The efficiency of microbial protein production was estimated from the flow of microbial nitrogen at the abomasum and the organic matter apparently digested in the rumen. It is expressed as g microbial N per kg O.M. apparently digested in the rumen. No significant change over time was observed in this parameter and a mean value of 43.2 (± 13.3) would seem to be a reasonable average. This value compares favourably with the value of 37.8 (± 10.6) proposed by the ARC (1984) for fresh grass or legume forages. Thus it is unlikely that the explanation for the low voluntary feed intakes sometimes observed in this experiment lies in a protein deficiency caused by a low efficiency of microbial protein production.

2.2.4.5. Conclusions.

The objective of this experiment was to study changes in intake and the concomitant changes in a number of associated factors, rather than to make comparisons between the two sites. This makes it difficult to establish the factor which most limited, and thus controlled, intake at each stage of the experiments. However, many factors could be studied simultaneously in each of the two environments to establish their relationships with voluntary feed intake.

The mean retention time (MRT) method for measuring intake seems to give a clearer and more instantaneous indication of changes in voluntary feed intakes than the faecal collection (FE) method. The obvious reason lies in the fact that ruminal fill is a direct function of intake in the MRT method and it changes rapidly as intake changes in those cases where intake is not limited by ruminal fill.

An examination of changes in ruminal fill showed that intake was not generally controlled by fill and digestion kinetics. In only one case, on a wilted dry pasture at Ukulinga, were the animals' rumens actually full.

A study of the SG of the ruminal contents indicated that a low ruminal OM fill was associated with a low SG (foamy) digesta, but this was not associated with a high digesta volume, suggesting that ruminal volume was not limiting intake. The fact that a very high ruminal OM fill was obtained with a very high *in vitro* foam production in one instance seems to suggest that saponin content is not a rate limiting factor. Only ruminal ammonia concentration had any consistent relationship with voluntary intake, a result which was not refuted by other evidence. All other factors studied, including moisture content, showed no relationship with voluntary feed intake. The total nitrogen content of the leaves (Pienaar *et al.* (1993a) was also negatively related to voluntary feed intakes. Thus, these results seem to confirm those of Tainton *et al.* (1982) that voluntary intakes are negatively related to the nitrogen content of kikuyu pasture, at least above certain nitrogen levels.

2.2.4.6. Acknowledgements.

The authors appreciate the help of Mr. J.D. Davie and the personnel of the laboratory for routine analyses at IAPI with chemical analyses. The help of Messrs. D. Nkobane, W. Madutlela, F. Thansha and M. Bosoga for handling and care of animals are gratefully acknowledged.

SOLI DEO GLORIA.

2.2.5. The accuracy of indirect methods of predicting feed intake for sheep at constant intakes and following abrupt changes in intake[1].

2.2.5.1. Introduction.

Currently a number of methods may be used to determine the voluntary intake of grazing animals. The most widely used ones are probably the faecal collection (FC) and marker-based methods (Kotb & Luckey, 1972). In Section 2.2.4 the development of a model based on ruminal digestion kinetics (RDK) to estimate dry matter (DM) intake on pastures, is described. Intake estimates based on this model were very similar to those obtained from the faecal collection methods. However, the RDK method gave a much more definite pattern, and seemed to follow changes in intakes more closely than the faecal method. It also showed that large and rapid changes occurred in the voluntary intakes of the sheep grazing kikuyu pasture.

All these indirect methods proved to be reliable when feed intakes remained constant and a steady state existed in the rumen (Kotb & Luckey, 1972). However, the large and rapid changes in the voluntary DM intake on grazing (Section 2.2.4) necessitated the evaluation of all these methods under non-steady-state conditions for possible use when following rapid changes in intake. The difference in time between actual changes in intake, and when the same changes are observed in the indirect method, may be estimeted by the calculation of delay times.

Markers are usually introduced into the rumen, either *per os* e.g. chromium oxide tablets, or infused through a ruminal fistula (Faichney, 1975). They could also be infused lower down into the gastro-intestinal tract such as into the ileum, assuming that the smaller the mixing pool, the faster steady state conditions would be reached following a drastic change in

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[1]J.P. Pienaar & J.B.J. van Ryssen
To be published

intake. This would be advantageous if large abrupt changes in intake have to be measured. The same argument would be valid for sampling, viz. that if markers are dosed or infused per rumen, they may be sampled immediate post ruminally, such as in the abomasum. This should eliminate the hind-gut as a potential mixing pool, and decrease the measured response times following changes.

The objective of this study was to determine the reliability of methods of predicting voluntary intake, both under steady state conditions and in situations of drastic change in feed intake. The effect of site of sampling and marker concentration on accuracy of prediction was evaluated as well.

2.2.5.2. Methods.

Animals, diets and experimental plan.

Eighteen mature South African Mutton Merino wethers weighing ca. 70 kg were used. The sheep were fitted with large (83 mm ID) cannulae in the rumen and simple cannulae made from soft silicone rubber in the abomasum and terminal ileum. The sheep were randomly allocated into three groups, each group receiving a different experimental diet, viz. wheat straw, lucerne or maize cob leaves, grounded in a hammer mill (12 mm sieve size). The feeds were minimally supplemented with salt, urea and fishmeal to meet N.R.C. (1975) requirements for nitrogen and mineral requirements. These supplements were mixed with the diets, together with 3% molasses as a binding agent. The sheep were individually penned in metabolism crates. They were adapted to the diets for a period of two weeks at *ad lib* intakes. After adaptation, total daily faecal collections commenced while the sheep were fed *ad lib* for a further 7 days, and daily faecal collections continued for the duration of the trial. Subsequent to feeding the sheep *ad lib* for 7 days, intakes were regulated as follows: restricted to 50% of *ad lib* for 6 days; *ad lib* for 8 days; limited to 50% of *ad lib* for 11 days; increased

to *ad lib* for 8 days; limited to 75% of *ad lib* for 6 days. During the days on 75% of *ad lib*, the voluntary intakes of the sheep continued to drop, and the experiment was terminated six days after that.

Marker infusions, sampling and counting.

Radio labelled chromium EDTA and a ruthenium phenantroline complex (Faichney, 1975) were infused into the ileum and rumen respectively to serve as flow markers. Infusion started six days before faecal collections and spot sampling from the ileum commenced. Spot sampling from the abomasum commenced 20 days after faecal collections started and continued for 28 days.

Glass tubes for gamma counting were filled with wet faeces by stuffing the pellets tightly into the counting tubes, using a glass rod. The abomasal sample was filtered through, and manually squeezed in a nylon cloth with a 53μ pore size. The liquid was discarded and the solids stuffed into the counting tube. Ileal samples were mixed with "Ready Value" in the counting tube, before counting for ^{103}ruthenium and ^{51}chromium in a Compugamma model 1282 with a multi isotope option (LKB Wallac, Finland). Ready value is a gelling agent, supplied by Beckmann Instruments.

Standards of faeces were prepared by mixing known amounts of the infusates containing the two radio-active markers into faeces from sheep consuming the experimental diets but without the chromium and ruthenium.

each deliberate change in intake the differences were included again, enabling a value for delay time to be calculated for each sheep for each method. These values were used in an analysis of variance.

The marker-based and faecal collection methods produced many more estimates of intake for calculating delay time than the method based on digestion kinetics. This influenced the estimates of delay and was corrected for by calculating another set of delays for the excretion of marker in the faeces, but using only those points where estimates were available for digestion kinetics.

Since all the estimates for all the methods used were obtained simultaneously on the same set of sheep, this could have caused a correlation between methods, which would have caused the normal assumptions of analyses of variance to be invalid. Thus the estimates obtained with the different methods were plotted against one another, to see if such a relationship actually existed. In none of the cases the r^2 was larger than 10%.

When the effect of the hind-gut was studied, samples were not taken from the abomasum until 20 days after the first collections were commenced. Thus the comparison between delay times in the ruminal marker at the abomasum and faeces was made only over the period when abomasal samples were available.

2.2.5.3. Results and discussion.

The estimates of digestibility which are used when calculating intakes with either the marker or faecal collection methods, are critical for the accuracy (bias) of the final predictions. Digestibility values obtained both *in sacco* and *in vivo* are presented in Table 2.2.5.1. Both methods gave very similar results with the wheat straw diets, but with both the lucerne and maize cob leaf diets, the *in sacco* values are much higher than the *in vivo* values for

digestibility. The reason for this is that the *in sacco* values were not corrected for the passage of potentially fermentable material that would occur *in vivo*. The *in sacco* values corrected for passage from the rumen, but not for fermentation in the hind-gut, are also presented in Table 2.2.5.1. This correction brought the average potential digestibility much closer to the *in vivo* values.

Table 2.2.5.1. Digestibility (%) of three diets determined by in sacco or in vivo methods.

	Wheat straw	Lucerne	Maize cob leaves
In sacco	46.2	64.0	78.5
In sacco corrected	40.0	60.4	67.9
In vivo	45.5	54.0	66.0

The mean voluntary intakes obtained with the different methods during the first 7 days of steady state conditions, and calculated using both the *in vivo* and the *in sacco* estimates of digestibility, are presented in Table 2.2.5.2. When *in vivo* digestibility was used for calculating intakes, only the mean of the RU method was significantly lower than actual intakes. As can be expected the mean of FC method was almost identical to mean observed intake. This was to be expected since digestibility was calculated from intake and faeces excretion, and the only remaining source of error is differences between sheep in digestibility. When the *in sacco* digestibility values were used, voluntary intakes on both the IL and RU methods as well as the FC method was grossly overestimated. When presenting the plots of predicted vs observed intakes in order to study the accuracy of the different methods, only the predictions obtained with the *in vivo* digestibility were used where applicable.

Table 2.2.5.2. Estimates of intakes obtained using four different methods and compared with actual intakes.

Method	*In sacco* digestibility		*In vivo* digestibility	
	Intake (g/day)	SE	Intake (g/day)	SE
Ruminal marker	1185 $_{ab}$	92	904 $_{a}$	50
Ruminal digestion kineti	1029 $_{a}$	80	1029 $_{ab}$	80
Actual intake	1081 $_{a}$	67	1081 $_{b}$	67
Faeces collection	1444 $_{b}$	138	1082 $_{b}$	68
Ileal marker	1414 $_{b}$	138	1095 $_{b}$	85

$_{ab}$Means within columns with different subscripts differ significantly $P \leq 0.05$

The accuracy of the RDK method in predicting intake is demonstrated by the regression shown in Figure 2.2.5.1. This regression has an intercept that does not differ significantly from 0 and a highly significant slope which does not differ significantly from 1. It shows a close fit with an $r^2 = 0.796$. Thus the RDK method gave both an unbiased (mean) and accurate fit to intake during steady state conditions. The accuracy is demonstrated by a non-significant difference from the line which shows the theoretical perfect fit which would have an intercept of 0 and a slope of 1.

Figure 2.2.5.1. Intakes predicted by rumen digestion kinetics

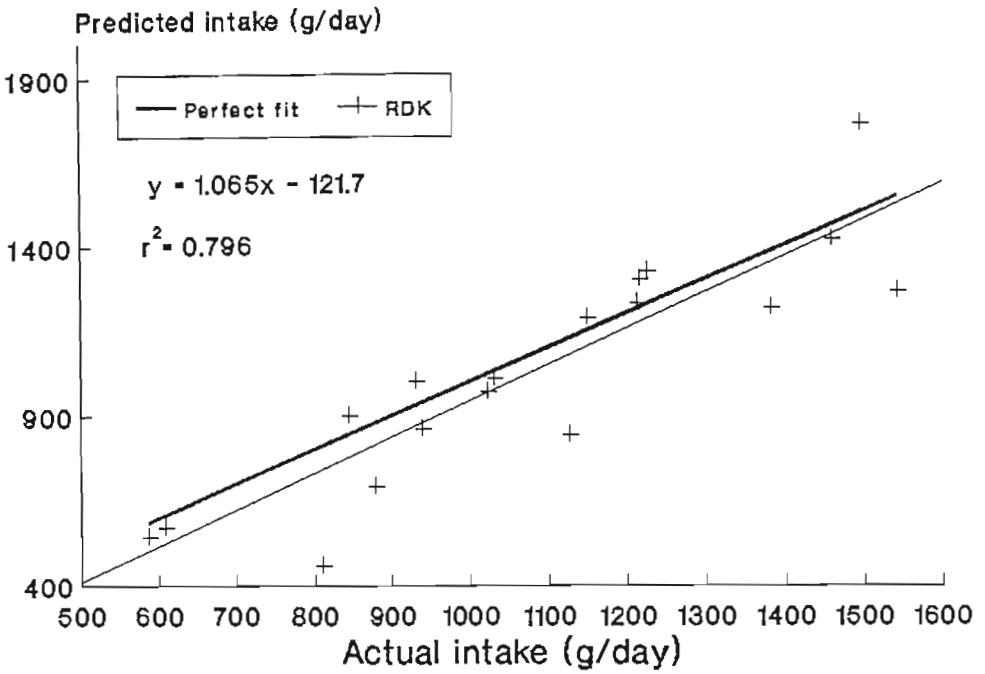
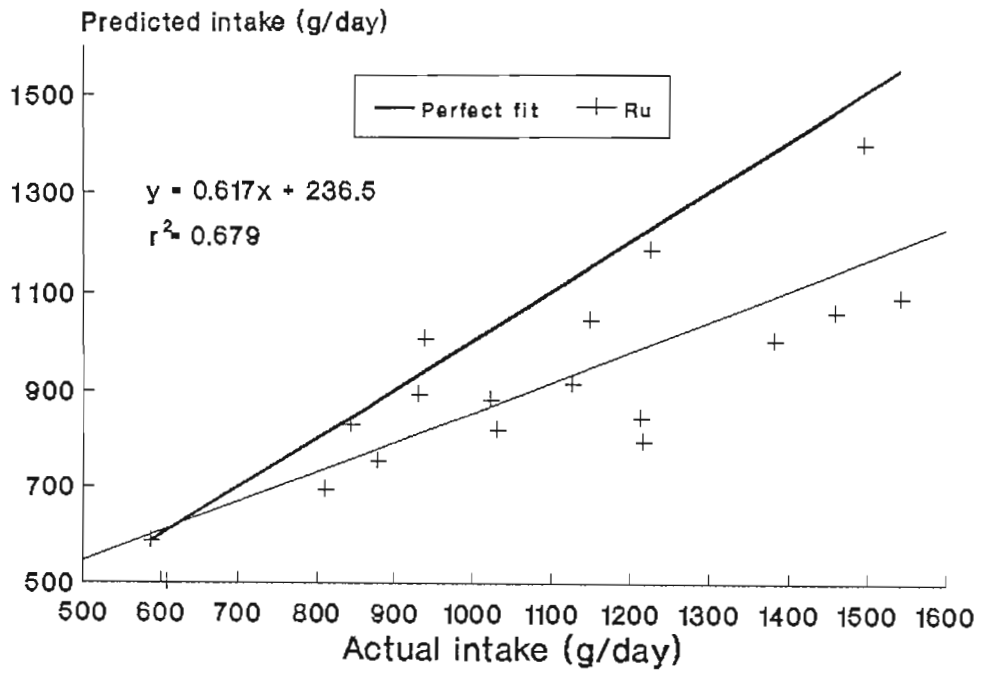


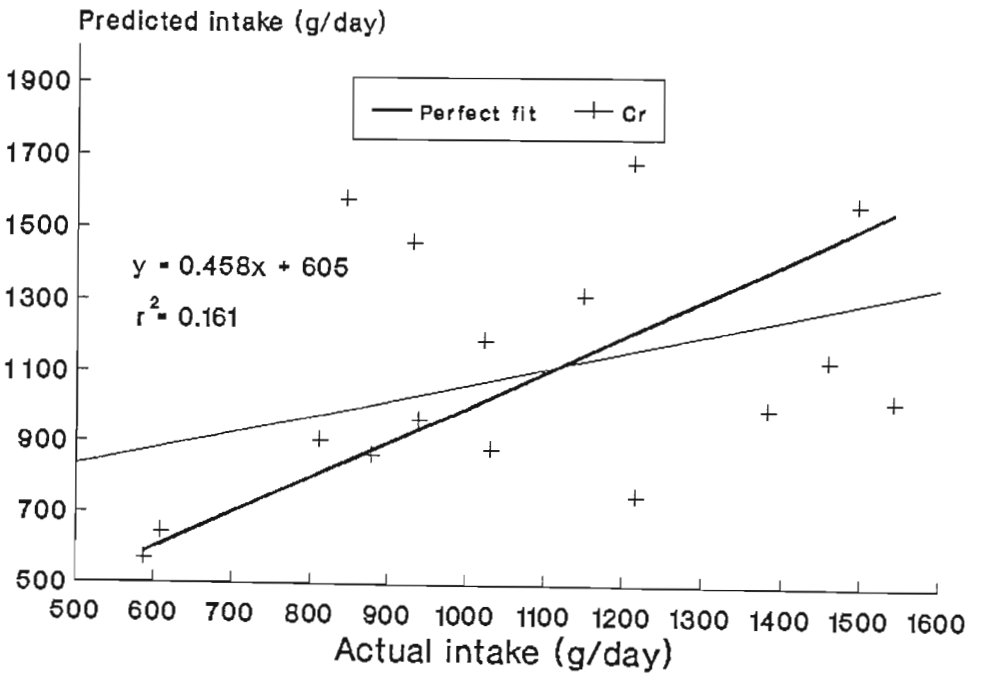
Figure 2.2.5.2. Intakes predicted by the rumen marker.



The regression of the ruminal marker method, shown in Figure 2.2.5.2, has an intercept which is significant at the P = 0.06 level and a slope which differs significantly from 1. The fit is reasonably close with an r^2 of 0.679 . Thus the significant difference between means is confirmed by a significant deviation from the theoretical line of perfect fit and thus from actual intake.

The ileal marker method, shown in Figure 2.2.5.3, has a large but non-significant intercept and the slope is, though smaller than for the RDK method, not significantly different from 1. However, in this case the fit is very poor (r^2 = 0.161). Thus the ileal marker method gave estimates where the means were not significantly biased, but with no significant regression between actual and predicted intakes. The reason for this poor regression should not be sought

Figure 2.2.5.3. Intakes predicted by ileal marker.



in the marker used, but rather in the location where the marker was infused since chromium EDTA has been adequately verified as an accurate flow marker (Faichney, 1975). Why such a poor accuracy was obtained when the marker was infused at this location, is not clear.

The regression of the faecal collection method shown in Figure 2.2.5.4 has a very close relationship between observed and predicted intake (r^2 = 0.892) as well as a non-significant intercept and a slope which does not differ significantly from 0. This was to be expected, since digestibility was originally calculated from faeces excretion and voluntary intake. If faeces excretion and the *in vivo* digestibility are used, the original intakes should be produced. The only source of variation in this regression is the differences in digestibility between individual sheep since a mean value for *in vivo* digestibility was used. The pooled standard error of *in vivo* digestibility was 1.6% which indicates a large variation between sheep in this measurement.

Figure 2.2.5.4. Intakes predicted by faecal collections

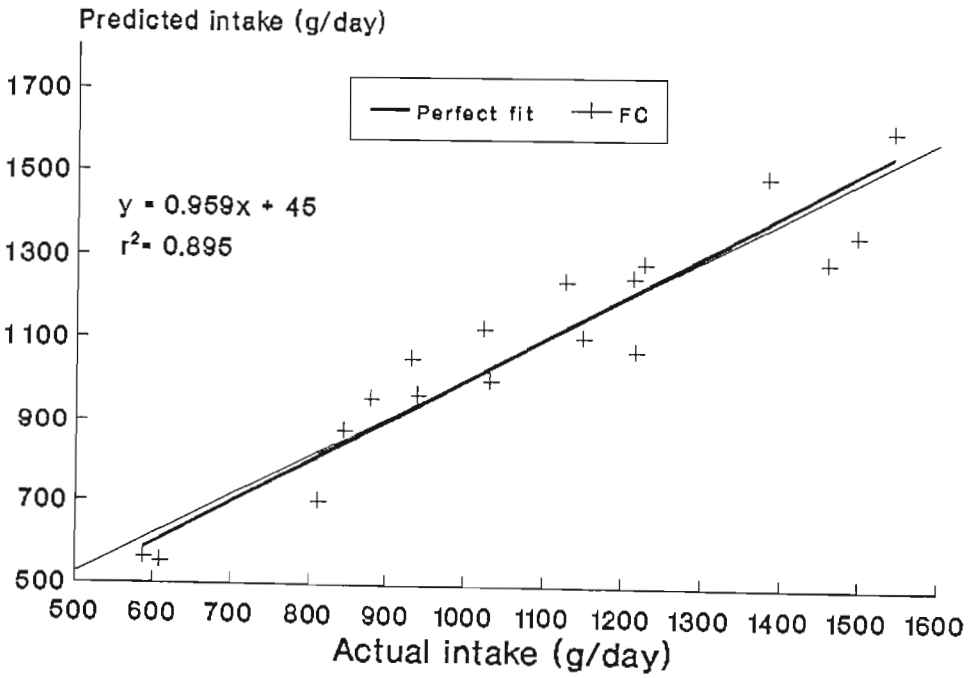


Table 2.2.5.3. Delay times obtained with four different methods for estimating intake.

Method	Delay (hours)	Standard error
Digestion kinetics	36.4 $_a$	2.4
Faeces bags	38.8 $_a$	1.1
Ruminal marker	48.5 $_b$	1.6
Ileal marker	50.1 $_b$	1.6

$_{ab}$ Means with different subscripts differ significantly $P \leq 0.05$

The values obtained when calculating delay times are shown in Table 2.2.5.3. The first column shows the delays obtained when a limited number of points were used. These points correspond with the times when values were available for the RDK method which are shown in Figure 2.2.5.5. The Table shows the mean values, statistical significance of the differences and standard errors. From these it can be seen that the shortest delays were obtained with the RDK and FC methods. Both the marker based methods gave significantly longer delays than the other two methods. A comparison of delay times of the ruminal marker, collected at the abomasum or in the faeces, gave mean times of 44.5 and 35.9 hours for the two sites respectively. The difference was significant on the $P < 0.10$ level and had a standard error of the difference of 4.1 hours. The difference between the two methods, although not highly significant shows the direction which the results tended to follow. The direction was rather unexpected, since the samples collected at the abomasum gave a longer delay time than those collected from the faeces. It was expected that the elimination of mixing in the hind-gut would decrease the delay time but it actually increased it. The plots of the standardised values

Figure 2.2.5.5. Changes in standardised intakes as predicted by rumen digestion kinetics.

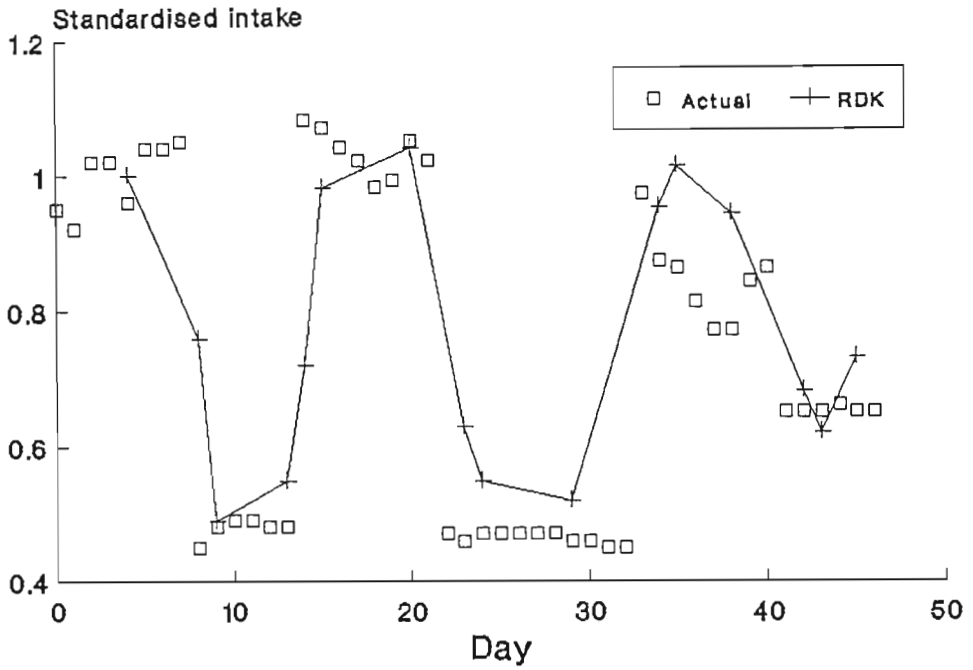
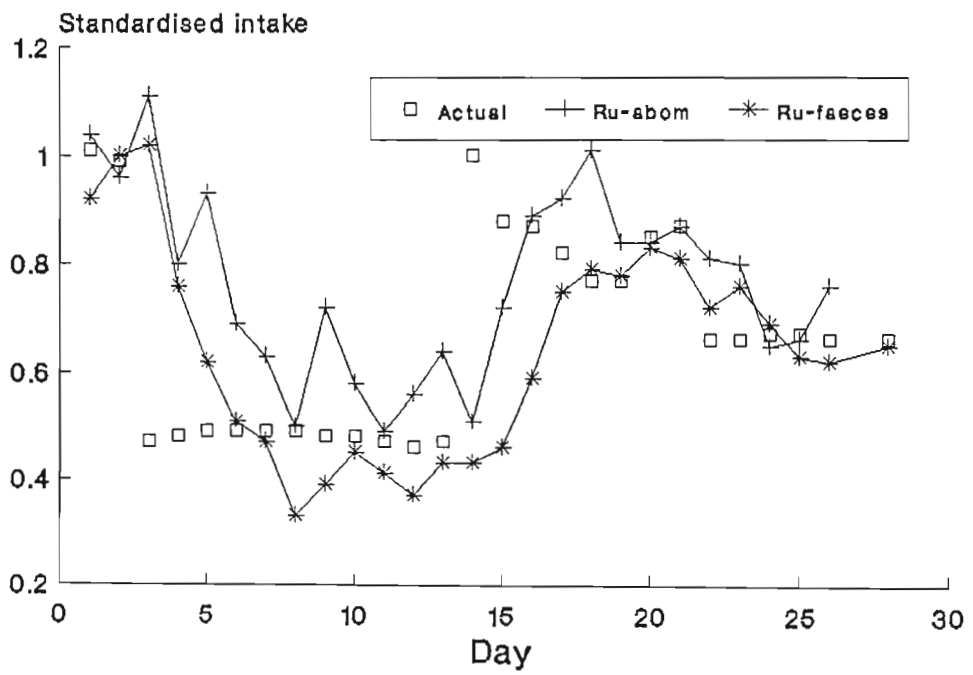


Figure 2.2.5.6. Changes in standardised intakes measured at the abomasum or faeces.



obtained with the two methods are shown in Figure 2.2.5.6. The values obtained at the abomasum tended not to go below the observed intake, but those in the faeces did. The same tendency was observed with the method employing digestion kinetics, but all estimates which did not exclude the hind gut showed a tendency to go below actual intake after a drastic change. This was observed in both the rumen (Figure 2.2.5.6) and ileal (not shown) markers. It indicates that the effect of the hind-gut is probably responsible for the fact that these methods gave estimates of intakes which are lower than actual intakes after a drastic change in intake. Figure 2.2.5.6 also shows that although abomasal sampling eliminated a significant bias from the estimates, the values were much more variable than with faecal collections.

2.2.5.4. Conclusions

It may be concluded that under steady-state conditions there were relatively small differences between actual intake and predicted intakes provided that digestibility was estimated *in vivo*. If digestibility was estimated by other methods, such as by *in sacco* methods, the result showed a large bias in the marker based and faecal collection methods, and especially so if the *in sacco* digestibility value was not corrected for rate of passage.

The two methods which eliminated the effect of the hind-gut (ruminal digestion kinetics and sampling at the ileum) had the advantage of not underestimating actual intake as in the case of sampling from the faeces. However, sampling at the ileum did not decrease the delay time as had been expected.

The longest delays were observed using the marker-based methods. The order of magnitude of these delays showed that unless intake remains relatively constant on grazing, which apparently it does not, all estimates of intake will be rather inaccurate, and will lag behind actual intake with a mean delay time of about 2 days. The marker based methods estimate a longer delay than faecal collections or ruminal digestion kinetics.

2.2.5.5. Acknowledgements

The authors gratefully acknowledge the assistance of the following people: Dr. C.Z. Roux for advice on the statistical analyses, Messrs. D Nkobane, W Madutlela and F Thantsa for technical assistance.

Soli Deo Gloria

Chapter 3

Finding indirect methods to estimate the variables that determine voluntary feed intake.

In order to use the principles of ruminal digestion kinetics for routine feed evaluation, the variables which determine voluntary feed intake *in vivo*, have to be estimated by methods which can be executed relatively easily, within a reasonable period of time and at as low cost as possible. The time and cost involved in *in vivo* experiments precludes the use of animals for the purpose of routine feed analyses. In the following sections, the possible use of an *in sacco* and a manometric *in vitro* technique for this purpose, is examined.

3.1. Comparison of *in vivo* and *in sacco* methods to estimate mean retention time of fermentable organic matter in the rumen[1].

Different *in vitro* and *in situ* methods for approximating the *in vivo* fermentation process in the rumen are available. Probably the best known of these methods is the *in sacco* artificial bag technique (Ørskov & McDonald, 1979). This method has some advantages, the most obvious being simplicity and the number of samples which may be handled simultaneously.

The aim of this experiment was to compare estimates of the disappearance of fermentable organic matter from the rumen, expressed as mean retention times (MRT) obtained by the steady state *in vivo* method (Pienaar *et al.*, 1980) and various *in sacco* estimates. Different models were used to describe *in sacco* fermentation. MFT is ideally suited to the comparison of different models, which are otherwise difficult to compare since they all use different parameters. The concept of mean retention time has to my knowledge, been used in this

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[1] J.P. Pienaar, C.Z. Roux & P.B. Cronjé
S. Afr. J. Anim. Sci., 19, 2, 71-75 (1989)

context for the first time by Minson in the mid nineteen sixties (Minson, 1967). The concept, as he defined it, included both fermentation and passage of feed from the rumen. Following his exposition, it was decided to use the term mean retention time (MRT) when an estimate includes both fermentation and passage. When only fermentation is described the term mean fermentation time (MFT) can be used, when only passage is described the term mean passage time (MPT) is appropriate. When gas production is used to describe fermentation rate, the term mean gas production time (MGT) can be used.

The calculation of MRT or (MFT) has other convenient aspects described by Roux & Meissner (1984). It was decided to compare fermentation distributions using mean fermentation times (MFT) since it allows for the comparison of estimates from different models by calculating a single comparable value for a model.

A group of mature South African Mutton Merino wethers (n = 6), fitted with large ruminal cannulae (83 mm ID) and weighing 68.2 ± 7.7 kg, were used in this study.

The methods to calculate fermentation rates included a first-order model calculated over 24 h, a first-order model calculated over 48 h, a first-order model incorporating lag time, a moment-generating function of a gamma distribution and the calculation of mean fermentation time (MFT) by a discrete approximation to the mean of a continuous random variable. A comparison of *in vivo* and *in sacco* fermentation showed, in general, good agreement between the two methods. Care should, however, be taken when selecting a method to calculate *in sacco* estimates, as some methods induce large differences between the *in vivo* and *in sacco* estimates. *In sacco* and *in vivo* O.M. disappearances are in close agreement when *in sacco* MFT of fermentation is calculated according to the method based on the discrete approximation of a continuous random variable, and *in sacco* MFT is corrected for the outflow of fermentable O.M.

3.2. The use of *in vitro* gas production to estimate rate and extent of digestion for ruminant feeds[1].

Manometric methods for estimating exchange of gases have been used in the study of both chemical and biological reactions in science for many years (Umbreit, Burris & Stauffer,nd). The measurement of gas production under constant volume, has been known since 1902 (Barcroft & Haldane, 1902). Although its use to estimate feed quality for ruminants has been known for long (Hungate, Fletcher, Dougherty & Barrentine, 1955), it has never been as widely used as the *in vitro* digestion method of Tilley & Terry (1963).

More recently Menke, Raab, Salewski, Steingass, Fritz & Schneider (1979) proposed a method based on the Tilley & Terry (1963) method where the volume of gas produced in syringes over a 24 hour period, can be used to estimate the metabolisable energy content of a feed. However, their proposal has not been accepted widely, probably because on its own, the volume of gas produced cannot be used to predict the rate of passage of a feed in the animal, nor can it be used to estimate digestibility with any degree of precision (Menke *et. al.* 1979). Theodorou, Williams, Dhanoa and McAllen (1991) used head-space gas pressure to calculate kinetic parameters for forage digestibility, but not to the extent of predicting roughage intake.

A method in which gas production is monitored with the aid of a computer was presented by Pell & Schofield in 1993. This method corresponds well with a method used by Pienaar & Kühn (1991), except that Pell & Schofield (1993) used much smaller samples and used syringe fittings instead of Schott bottles and lids. They also did not use their measurements for the prediction of feed intake, but to determine the effect of fat on ruminal fermentation (Pell, 1993).

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[1] J.P. Pienaar & J.B.J. van Ryssen
To be published

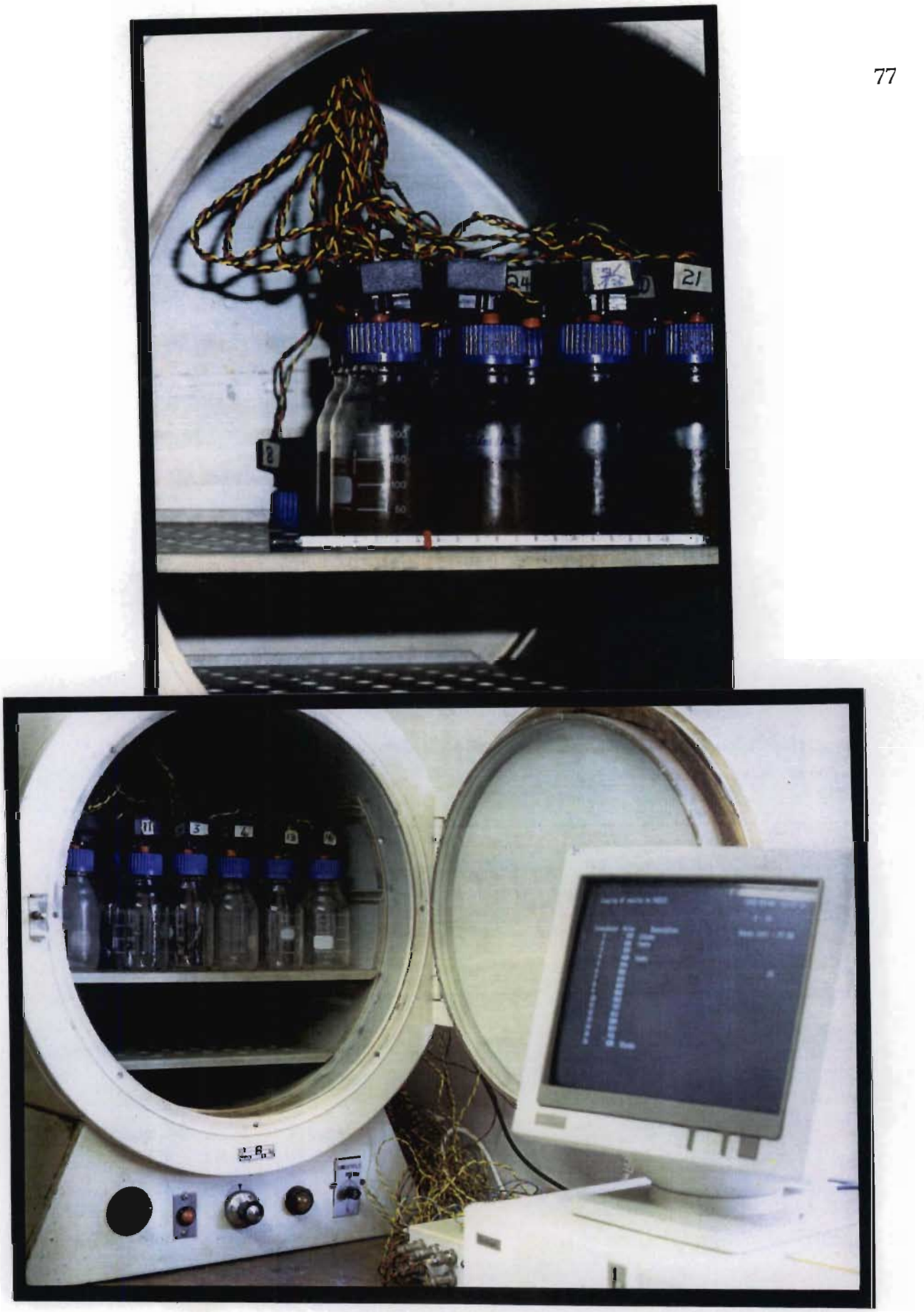
Kinetic mathematical models of ruminant digestion are also not new (Waldo, Smith & Cox, 1972), and have been used to predict feed intake (Pienaar *et al.*, 1980, and also summarized in section 2.1.1). A well-known method to estimate some of the parameters of these kinetic models, is the *in sacco* or artificial bag technique (Ørskov & McDonald, 1979). A major disadvantage of the *in sacco* method is the large variation between replications. A large number of bags (42), and at least 3 rumen cannulated animals are required to obtain a reasonable estimate of the digestion rate of one feed sample. This makes the method labour intensive and expensive and can hardly be seen as a practical method for routine feed evaluation. However, the method is often used as a research tool.

The use of a combination of the Tilley & Terry *in vitro* technique with automated measurement of gas production and the mathematical modelling of ruminal digestion kinetics and voluntary feed intake, are evaluated as a possible tool for feed evaluation. Since gas production from feed samples for these purposes is not well established as an analytical procedure yet, questions such as the relationship between the rates obtained *in vivo, in sacco* and by *in vitro* gas have to be answered. Also, the correct sieve size for grinding the samples before analyses and the effect of feed particle size on fermentation rate have to be established.

3.2.1. Methods

3.2.1.1. The *in vitro* technique.

The basic technique of Tilley & Terry (1963) was followed. Urea was supplemented at a level of 10 mg nitrogen per 0.5g of sample as recommended by Engels & van der Merwe (1967). Some modifications were introduced to allow the measurement of gas production. The samples were incubated for 72h at 39°C for the microbial phase.



General layout of *in vitro* gas production equipment, showing the data logger and the incubator in the bottom, and a close-up view of the fermentation flasks with mounted pressure sensors in the top.

Fermentation is not conducted in a test tube, but in a 250ml or 100ml Schott bottle, and the bunsen valve in the lid is replaced with the normal cap and a tight sealing rubber ring. In the cap a hole (12mm ID) is drilled, which is sealed with a rubber stopper. A pressure sensor is mounted onto the rubber stopper with the sensing pipe and orifice pushed through a tight fitting hole in the stopper into the inside of the lid. Both the lid and the pressure sensor are glued to the red rubber stopper using super glue (Cyanoacrylate glue).

A ratio of about 0.67:1 between the volume of the head-space relative to the volume of the buffer is recommended for the 250 and 100ml bottles. This gives a large enough pressure change to allow effective pressure measurements, but without the pressure rising to a level where the bottle may explode. In the 250ml Schott bottles this means 150ml buffer and ruminal inoculum and 1.5 g of sample, the same ratio between buffer and sample stipulated by Tilley & Terry (1963). If Schott bottles larger than 250ml are used, relatively more head-space should be allowed since large bottles are not as strong as the smaller ones.

3.2.1.2. Experiment 1. Gas pressure and weight of carbon dioxide.

About 55g of McDougals saliva was weighed accurately in a 250ml Schott bottle and CO_2 burbled through until no increase in weight was observed, indicating that the solution was saturated with CO_2. The weight was recorded and the bottle closed with the lid containing the pressure sensor. Different quantities of CO_2 was then injected into the vessel through a needle, which passed through the rubber stopper into the vessel. After each injection the pressure and weight were recorded in order to establish whether a linear relationship existed between gas produced and pressure in the vessel.

3.2.1.3. Experiment 2. Stirring the fermentation vessels.

Tilley & Terry (1963) stipulated regular stirring of the incubation tubes. It was hypothesized that this would be even more important when rates are determined. The problem was solved by using programmable magnetic stirrers which would stir the vessels slowly, every four minutes for thirty seconds. In the vessels that were not to be stirred, the stirrer bars were merely left out. The feeds used in this study were lucerne (rapid fermentation) and *Eragrostis curvula* (slow fermentation).

The experiment was conducted over two consecutive runs. In the first run, gas production was measured in 9 vessels (5 stirred and 4 not stirred) and in the second run in 8 (4 stirred and 4 not stirred). Both diets were present in each run. Data analysis was done using Harvey's mixed model least-squares and maximum likelihood computer program (Harvey, 1988). Fermentation rates were expressed as mean gas production times. These were calculated as described by Pienaar & Roux (1989a).

3.2.1.4. Specifications for Electronic Equipment.

The system is equipped with a 12 bit A ---→ D converter which uses a successive approximation. A low cost commercially available A ---→ D converter card for a personal computer is used.

Low cost Pzieso electric pressure sensors are used with conditioning electronics. These electronics are situated close to the sensor in order to amplify the signal to an acceptable level before transmitting it to the converter. They also allow the adjustment of the sensor to read full scale at a minimum pressure of 2.8 Bar, which is much lower than the maximum of 5 Bar for which the sensor is rated. This increases the sensitivity with which the readings can be made.

An average of a number of pressure readings, specified by the user, can be taken at any specified time (up to 9999 but typically between 100 and 1000 readings for a mean). The software, supplied with the system, logs the results (mean of the readings) to disk at intervals as specified by the user. It allows a maximum of ten different settings for intervals and an unrestricted number of readings per interval.

The system is supplied by:

Strike Technologies
21/48 Richards drive
P.O. Box 1810
1658 Halfway House
Republic of South Africa
Tel 011 315 0815

It was developed in collaboration with researchers of the Irene Animal Production Institute.

3.2.1.5. Experiment 3. The effect of particle size on rate of digestion.

Five forages were each ground through four sieve sizes (0.5mm, 1mm, 2mm and 5mm) in a Wiley mill. The forages were teff hay, three different qualities of *Cenchrus ciliaris* hay and lucerne hay. Feed particle size was determined by sieving a sample of the feed through seven test sieves on a test sieve shaker. Mean particle size was calculated as the mean of the sieve apertures weighed for the amount of material on each sieve.

Rate of gas production was determined. Since only 4 samples could be analysed for gas production at a time, a 4 x 5 rectangular lattice design (Cochran & Cox, 1968) was used, and treatments were analysed in blocks and periods, with four pressure sensors and five repetitions per block.

3.2.1.6. Experiment 4. The comparison of rates obtained with *in vivo* *in vitro* and *in sacco* methods.

Rates of O.M. disappearance *in sacco* were determined as described by Pienaar *et al* (1989) in Section 3.1. This *in sacco* method is similar to the one described by Ørskov & McDonald (1979) but the gamma function was used to describe the fermentation curves. Bags with a pore size of 53μ were used. Bags were removed after 3, 6, 9, 12, 24, 48 and 72 h of incubation. Only feeds ground through the 5mm sieve were used in the *in sacco* study.

The *in vivo* rate of fermentation was determined as described briefly in chapter 2.1. This method requires that the ruminal contents of an animal be removed, weighed and sampled for the determination of dry- and organic matter (O.M.) content, and the potential digestibility of the ruminal contents. The latter was determined using the Tilley & Terry *in vitro* method, but with a long (72h) period of microbial digestion. The digestibility of the feed is also determined in this manner, and the ratio between the digestible O.M. content of the rumen (numerator) and the intake of digestible O.M. (denominator), gives the mean retention time of the digestible O.M. in the rumen. This mean retention time is the result of both fermentation rate and passage rate of digestible O.M. If an estimate of passage rate is available, rate of fermentation alone may be calculated (Section 4.3).

$$\text{MFT fermentation} = (1/\text{MRT combined} - 1/\text{MPT passage})^{-1}$$

This equation is true if first order kinetics are assumed, which is reasonable for this case since the steady state technique, which was used in this case to obtain *in vivo* estimates of fermentation rate, does not give an indication of the type of kinetics followed during fermentation and passage. So the simplest type of kinetics was assumed.

3.2.1.7 Experiment 5. Measurement of the total amount of gas produced.

Sixty seven diets differing from pure concentrate to pure roughage and mixtures were used. These included silages from different sources, protein sources like fishmeal and carcass meal, chicken manure, cassava pellets and by-products from the brewery and milling industries and many more.

The quantity of gas produced was derived from the change in gas pressure inside the fermentation vessel at constant temperature. Since the low cost pressure sensors give electronic readings rather than pressure in kPa, all the sensors were calibrated to kPa. This allowed gas production to be related to total amount of O.M. digested *in vitro*. The results of this experiment is reported as changes in pressure in a 250ml fermentation flask when a certain weight of O.M. was digested.

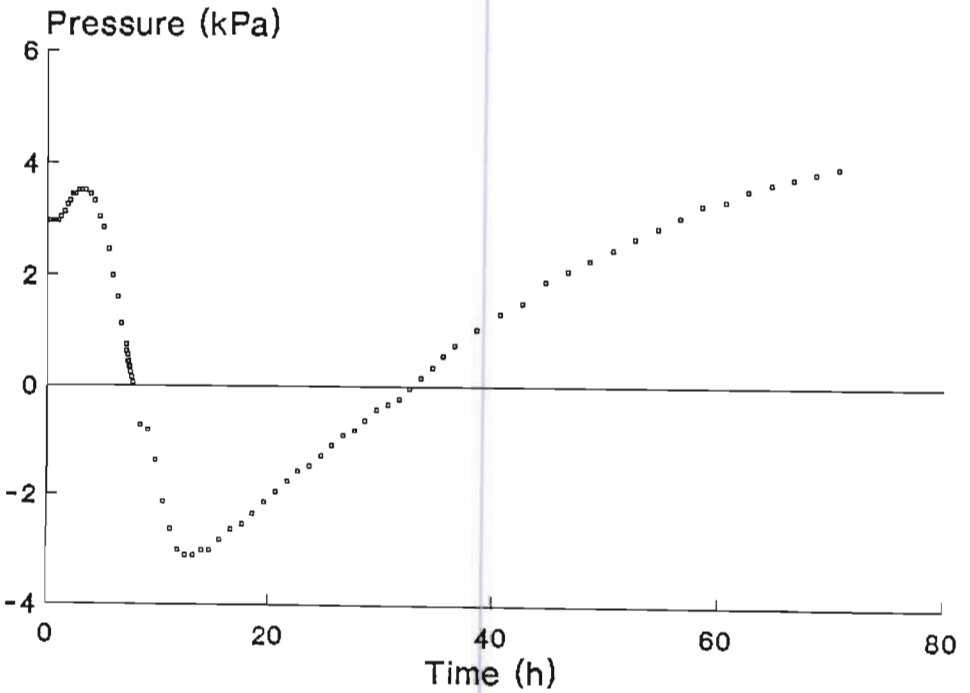
3.2.2. Results and discussion.

3.2.2.1. Gas pressure curves.

The results of changes in gas pressure in the fermentation vessels of both a blank and silage sample are shown in Figs. 3.2.1 & 3.2.2. The decrease in pressure observed in the blank,

which contained only the ruminal inoculum, urea and McDougals buffer is typical of what we regularly observe. A possible explanation for this decrease in pressure could be the change from a slightly aerobic type of fermentation, to an anaerobic one. During slightly aerobic conditions, hydrogen gas is produced instead of methane (Wolin & Miller, 1988). When all oxygen has been depleted by the facultative anaerobes, methane production can commence, and hydrogen and carbon dioxide may be metabolized to methane. The stoichiometry of this reaction indicates that 4 moles of hydrogen and 1 mole of carbon dioxide is metabolized to 1

Figure 3.2.1. Gas production from blank

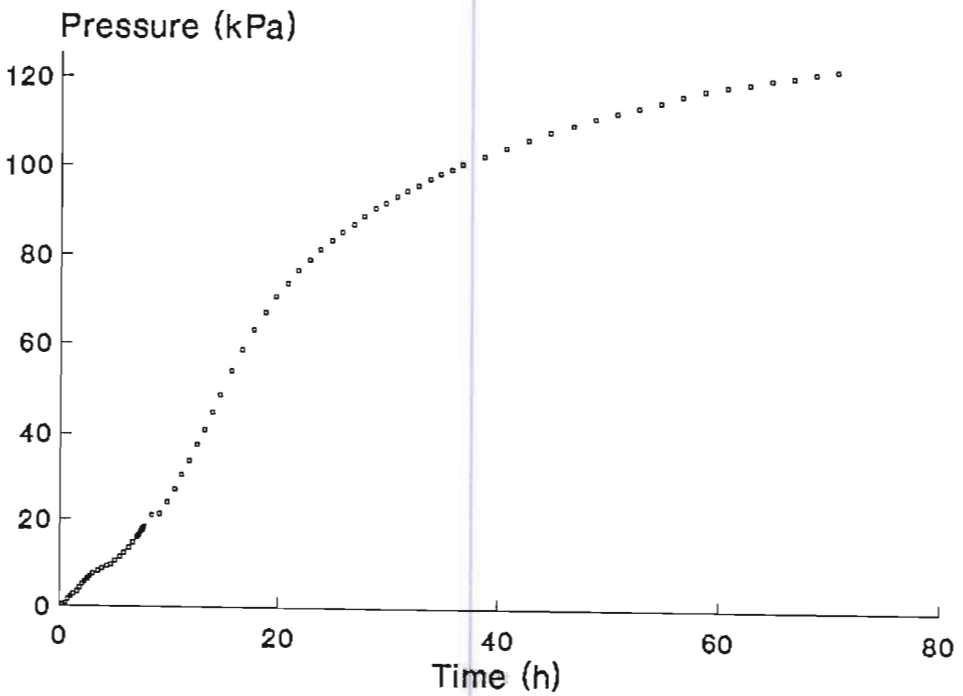


mole of methane and 2 moles of water. By this reaction 5 moles of gas are changed into 1 mole of gas.

$$4H_2 + CO_2 \dashrightarrow CH_4 + 2H_2O$$

This could easily explain the decrease in pressure often observed in the blank vessels, but sometimes also in samples during the early phases of fermentation. In the example shown here, a slight dip in pressure was also observed at about the same time that it occurred in the blank. However, it is not as obvious here because of the much smaller scale of this graph, and probably also because of the rapid rate of gas production that occurred in the sample. From this example, it is obvious that even though the measurements of gas production are taken very rapidly at the onset of fermentation, no dead phase was observed as is sometimes postulated

Figure 3.2.2. Gas production from silage sample.

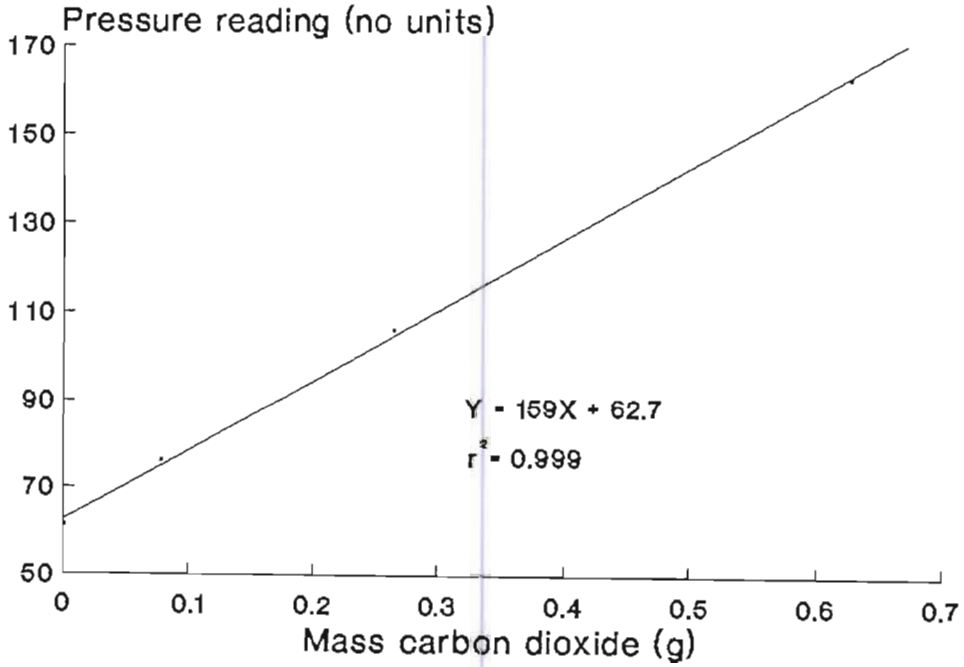


(McDonald, 1981). This supports the hypothesis put forward in section 2.1.2 that, instead of using a first-order reaction with a lag (dead) phase to describe the fermentation curve, a delay (slow starting) phase such as can be accommodated by the gamma function, is more appropriate.

3.2.2.2. Experiment 1. Mass of carbon dioxide and pressure in the vessel

It is important to know whether a linear relationship existed between gas produced and pressure in the vessel. Figure 3.2.3 shows a very close fit, with a linear relationship between mass of carbon dioxide in the vessel, and the pressure in the vessel. This relationship was established before the pressure sensors were calibrated in terms of kPa, so only the electronic

Figure 3.2.3. Relationship between weight of carbon dioxide and pressure reading after saturation of buffer solution with carbon dioxide



readings are given. The electronic readings are linear with pressure in terms of kPa.
The linear relationship between gas pressure and weight of carbon dioxide was observed only after the buffer solution has been saturated with carbon dioxide. It took 0.161g of CO_2 to saturate 55.308g of McDougals saliva. It should be noted that in the Tilley & Terry *in vitro* method, the buffer solution is saturated with CO_2 before ruminal fluid is added. Thus, it may be concluded that the changes observed in gas pressure actually reflect the amounts of gas produced in the vessel.

3.2.2.3. Experiment 2. Effect of stirring on fermentation rate.

Stirring the fermentation vessels actually decreased *in vitro* rate of gas production (increased mean gasproduction times) from 15.1 to 15.9 h, with a pooled standard error of 0.107 h. This difference, though small in practical terms, was highly significant (P = 0.0021). Although these results were rather unexpected, they are in agreement with a personal communication by Kistner (1993)(Irene Animal Production Institute) who said that earlier work has shown that stirring the samples could hinder the adhesion of micro-organisms to feed particles and thus interfere with digestion rate.

Pell & Schofield (1993) found that stirring their fermentation vessels reduced the variability between replicates. This hypothesis could be tested with our results as well. The variances of the mean gas production times were 21.3h and 22.18h for the stirred and unstirred samples respectively. When an F-ratio was calculated it was 1.04, which is not statistically significant. Could the positive response to stirring the samples found by Pell & Schofield (1993) be due to the small sample they use in their apparatus ?

Since stirring the samples actually reduced fermentation rate, and did not reduce the variance significantly, it was concluded that stirring the fermentation vessels was not desirable under our conditions.

3.2.2.4. Experiment 3. The effect of particle size on rate of gas production.

Grinding the samples through sieves of different sizes resulted in the mean particle sizes shown in Table 3.2.1. An analyses of variance showed that, using the same sieves, all feeds were ground to the same mean particle size, but different sieve sizes resulted in large and significant differences in mean particle sizes.

Table 3.2.1. Mean particle sizes of forages after grinding the forages through different sieve sizes.

Sieve size (mm)	Mean particle size (μ)	Standard error (internal)
.5	176	8.4
1	201	8.8
2	288	14.5
5	422	11.7

3.2.2.6. Experiment 4. A comparison of rates obtained with *in vivo, in vitro* and *in sacco* methods.

In Table 3.2.2, rates of gas production are compared with the rates calculated from the *in sacco* and *in vivo* methods, as well as the effects of particle size on the rates of gas production. Only the *in vivo* corrected for outflow differed significantly from the means of all the other methods. The large differences between the standard deviations within methods should also be noted. Obviously each method has its own error structure. The largest standard deviations were observed with the *"in vivo"* - and *"in vivo* corrected for outflow" methods. The reason for this large variation may lie in the fact that only one estimate of ruminal fill was obtained at

Table 3.2.2. The rate of digestion of five roughages determined *in vivo, in sacco* or by *in vitro* gas production. Results are expressed as mean fermentation times (h).

	Teff	Cench 1	Cench 2	Cench 3	Lucerne	Mean	S.D.	n
Gas 0.5mm	21.9	24.0	23.0	18.0	11.9	19.8 [a]	4.7	15
Gas 1mm	22.9	23.0	22.1	20.6	13.9	20.5 [a]	3.6	15
Gas 2mm	23.6	23.9	22.8	21.2	14.1	21.1 [a]	4.4	15
Gas 5mm	25.2	24.9	24.1	22.9	13.6	22.1 [a]	4.7	15
in sacco	25.4	22.8	22.9	23.0	9.9	20.8 [a]	6.6	30
in vivo	23.0	28.5	26.5	24.7	11.2	23.2 [a]	8.0	47
in vivo corrected for outflow	33.0	43.2	40.6	36.6	13.3	34.2 [b]	15.7	47

[ab] Columns with different superscripts differ significantly ($P \leq 0.05$)

slaughter. If these rates can be calculated from a mean of a number of estimates of ruminal fill, such as can be obtained from emptying the rumen through a ruminal cannula, a mean with a much smaller standard deviation can be expected. The next largest standard deviation was obtained with the *in sacco* method. Although the mean is calculated from n = 30 values, these values were each calculated from a curve obtained from seven bags, representing 210 bags in all. This shows the large inherent variability in this method.

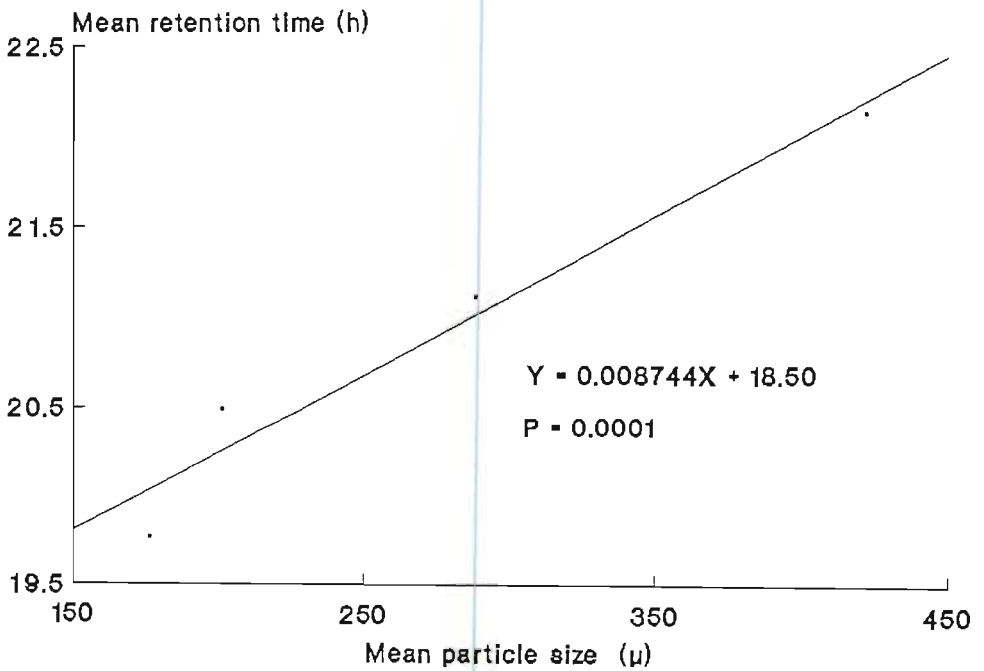
The difference between the *in vivo* corrected for outflow and *in sacco* estimates of fermentation rate is conspicuous. In previous work (Section 3.1), the means of these two methods were nearly identical. No obvious reason can be given for this discrepancy, except that the animals' rumens were obviously very full, and mixing and rumination could have been restricted somewhat by the excessive fill. Because of this, the value of 82h MPT used to estimate the passage of fermentable O.M. from the rumen could also have been too fast, and an over-correction could have been made, resulting in an underestimation for fermentation rate.

The results of the *in vitro* gas production and *in sacco* methods were in good agreement. It was not unexpected when the *in sacco* results agreed best with those of the smaller particle sizes of the gas production method, since some small particles are lost from the *in sacco* bags. These are not lost with gas production. Thus disappearance from the bags should be faster than gas production on the same particle size feed, due to the loss of small particles from the bags.

Particle size had a significant effect on the rate of *in vitro* gas production, shown in Table 3.2.2. Although the means did not differ significantly, there was a significant regression between sieve size and rate of gas production (Figure 3.2.4). Rates of gas production had the smallest standard deviation, and, therefore, tests between means within this method have a greater sensitivity than tests which included the methods with the larger standard deviations.

When the means are plotted against mean particle sizes, the relationship appears to be curvilinear. However, when all the data points are included in the fit and the main effects diets and blocks have been eliminated with analyses of variance, the data are best fitted by a straight line. The second order polynomial is not significant. Thus, although a change in particle size from 176μ to 422μ caused only a small decrease in fermentation rate, from about 20h to about 22h, there was a significant linear relationship between particle size and rate of fermentation.

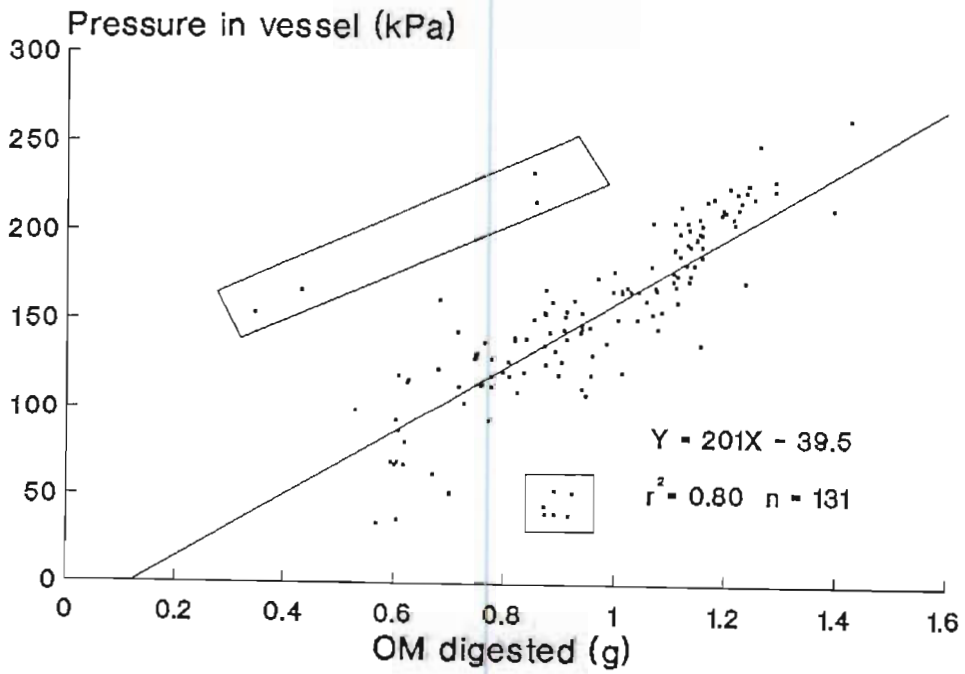
Figure 3.2.4. Relationship between mean particle size and fermentation rate (MRT).



3.2.2.6. Experiment 5. *In vitro* organic matter disappearance and gas production.

The measurement of total gas production *in vitro* is relative cheap, and can easily be fully automated. Digestibility obtained from the two-stage *in vitro* method of Tilley & Terry (1963), is considered to give the most accurate estimation of digestibility outside the animal (Golding, Moore, Franke & Ruelke, 1976). The accuracy of the relationship between these two methods would indicate whether gas production alone can be used for estimating both rate and extent of digestion, instead of using both gas production and the two-stage *in vitro*. The relationship is shown in Figure 3.2.5. It is not a very close fit, and also includes some obvious outliers (blocked) both above and below the line. These outliers show some important aspect of *in vitro* gas production and digestibility.

Figure 3.2.5. Relationship between organic matter digested and gas produced.



The very low gas production on samples with a relative high digestible O.M. content, is what could be expected on feeds with a relatively low ruminal degradability and a high digestibility in acid pepsin. When investigated, these samples turned out to be fishmeal samples. The carcass meal samples did not have a high digestible O.M. content, but also gave a very low *in vitro* gas production. The highly significant negative intercept of -39 kPa indicates that some digestibility occurs without gas production.

The outliers that were blocked above the line indicate samples that had a high gas production relative to OM digested. These were pure maize meal. Two quantities were fermented c. 0.5g and 1.0g. A possible explanation for this high gas production, is the low digestible protein content of the meal. Since no ammonia is produced as an additional buffer CO_2 is produced from the reaction between acids produced during fermentation, and the bicarbonate buffer. Since this fraction is not constant over feeds, it is probably responsible for a significant part of the variation around the line.

The obvious conclusion from these results is that gas production probably gives some indication of ruminal fermentation, but not of digestion in the small intestine. Thus, the second stage of the Tilley & Terry *in vitro* has to remain an essential part of the process, and may not be replaced by gas production alone.

A question which often arises is whether the "high" (c. 200 k Pa.) pressure that may develop in the fermentation vessels does not inhibit microbial activity. Due to the frequent manner in which this question arises, the problem is now being addressed by conducting a ring test in collaboration with other laboratories. Some of the participating laboratories have equipment which is designed to release gas after each reading.

The question may be asked why the effect of pressure wasn't studied right at the beginning of this work. The reason for this is that micro-organisms are apparently insensitive to pressure since they exist on the deepest ocean floor and on the highest mountain tops. About ten years ago it was established in this laboratory that fermenting a sample in a closed fermentation

vessel does not effect the extent of digestion adversely when feed samples are fermented for 48h as is usually done in a "Tilley & Terry" *in vitro* study. I would be surprised if the results of the ring tests do indicate that pressure influences rate of fermentation.

3.2.3 Conclusions

Measuring gas production during fermentation is non-destructive, cheap, rapid and can be largely automated. It is accurate enough to record small changes in the fermentation process, but changes from a semi-aerobic, hydrogen producing type fermentation to a strictly anaerobic type of fermentation could cause changes in gas pressure that are not necessarily related to disappearance of the solid substrate. Fortunately, these changes are small and usually only occur during the start of the fermentation process.

Since stirring the samples during the fermentation process had no beneficial effect on the fermentation rate, it was concluded that stirring the samples was not necessary. Changing feed particle size from very fine to coarse, resulted in a small but significant decrease in rate of fermentation. The rate of fermentation determined by gas production, agreed well with the results obtained with the *in sacco* nylon bag technique, but a largely unexplained discrepancy was found between the *in vivo* results and the other methods, when the latter was corrected for rate of passage. This is in contrast to previous work which showed a good relationship between *in sacco* and *in vivo* fermentation rates.

In vitro gas production should be related to fermentation in the rumen, rather than to digestibility in the whole digestive tract, which also includes pepsin digestion.

Despite the discrepancy observed in the rates obtained with the *in vivo* method and the other methods, we still considered the gas production method a viable proposition for estimating some dynamic and static parameters of ruminal digestion kinetics.

3.2.4 Acknowledgements

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Soli Deo Gloria

Chapter 4

Application of a kinetic model in feed evaluation.

4.1 A critical evaluation of different laboratory methods used to determine the available energy content of feeds.

4.1.1 Introduction

Feed evaluation systems have a much wider significance than the formulation of adequate rations to achieve the desired animal performance. They are used by the farmer and feed industry and also for the management of least-cost strategy for the feeding of farm animals as well as for the purchase-policy of feedstuffs for the least-cost formulation of concentrate mixtures. Moreover, they help to find the best systems of grassland management and fodder conservation (Van der Honing & Steg, 1990).

Since the Weende analysis was developed in 1860, it was assumed that the digestibility and voluntary intake of feeds would be determined using the animal. Dividing a feed into its different chemical components was never intended to replace the evaluation of a feed with animals. The result of the numerous digestion trials which followed was the compilation of tables of feeding values. The ARC, NRC, INRA tables and the European "starch equivalent" system are probably the best known of these. Many "local" translations and modifications of these have also seen the light in South Africa and other countries.

As a consequence of the fact that feeds have been analysed chemically, an impression has developed that the true nutritive value of a feed should be derived from its chemical composition. This has captured the imagination of many agricultural chemists and numerous studies have seen the light in which about every imaginable chemical component has been related to a production parameter and/or an energy value of some sort that was obtained *in vivo*. Many different types and kinds of analyses have been proposed, but the results have

been rather disappointing in the majority of cases (Weiss, 1993). The two main approaches which are used today, are the Tilley & Terry *in vitro* analysis and the detergent methods of analysis (NDF, ADF & ADL (Van Soest, 1982)).

The detergent methods have the advantage of a relative short time needed for analysis, and the fact that no donor animals are required to supply live micro-organisms. The *in vitro* is strictly speaking not a chemical analysis since it uses live micro-organisms to digest the feed. However, it is a very practical, relatively simple laboratory method, needing very small samples for analyses. Consequently many studies have also included the *in vitro* in their comparisons. In the majority of studies reported, the *in vitro* has performed consistently better than all the approaches based on chemical analyses when predicting digestibility. In the few cases where the detergents gave a closer fit within a small group of samples, the *in vitro* method gave a better overall fit over all samples (Givens, Moss & Adamson, 1993).

4.1.2. Analyses in the United Kingdom

ADAS, in the United Kingdom, supply a feed analysis service to farmers, and have tested various methods for estimating the ME values of feeds (Givens *et al.*, 1993). ADAS proposes the method of Thomas (1990) for predicting ME in compound feeds, but not in roughages. This method uses the following analyses to predict ME:

$$ME = 0.25 \times OIL + 0.14 \times NCD$$

Where:

ME = metabolisable energy (MJ/kg DM)

OIL = oil as determined by acid extraction (g/kg DM)

NCD = cellulase digestible organic matter in the dry matter following neutral detergent extraction (g/kg DM)

Givens *et al.* (1993) of ADAS concluded that "the accurate prediction of the ME contents of silages for extension purposes is not possible from normal laboratory measurements...", since not one chemical method is suitable for all types of samples. Every method has to be used within the class for which it has been calibrated. In order "to avoid the need to maintain animals as donors for ruminal fluid" they propose that the *in vitro* technique is not suitable for laboratory analyses . However, their work (Givens *et al.* ,1993, Table 5) showed the *in vitro* to be the one analysis which gave a regression between actual and predicted digestibility in which the intercept did not differ significantly from 0 and the slope did not differ significantly from 1 !

It appears as if ADAS is moving to the concept of fermentable ME (Phelps, 1993) using near infrared reflectance spectroscopy (NIRS) to separate the energy that would digest in the rumen from the fraction that would digest post ruminally. By doing this they seek to accommodate differences in the amount of microbial protein synthesized during ruminal fermentation. This concept was introduced in 1992 to their clients at "The UK Dairy Event". The NIRS approach produces results virtually immediately, which is very important for extension purposes. However, it is also reputed to be inaccurate when moving outside its range of calibration. The calibration curves vary from season to season and from area to area. It is population dependent and calibrations have to be established for each type of crop and for each method by which the crop is conserved. NIRS is especially useful when reasonably homogenous batches containing large numbers of samples have to be analysed. In that case, a number of samples are chosen from each batch. These are also analysed chemically and are used to calibrate the NIRS for each batch (Fisher, D.S., 1992, North Carolina State University, Department of Crop Science, Box 7620, Raleigh, North Carolina, 27695-7620, USA).

The use of NIRS could be impractical for certain situations in South Africa. For our laboratory a batch normally consists of small numbers of samples which may range from paprika to raisins and from rye grass to cassava, with everything in between. In order to give a

reasonable service over such a wide range one has to use a method which is both versatile and accurate. The fact that donor sheep must be kept for ruminal fluid is considered a minor limitation.

4.1.3 Analyses in the United States of America

The concepts of cell walls, cell contents and the *hotel theory* (Van Soest, 1982) as obtained from the detergent method of analyses have been rooted deeply in the thoughts of present day nutritionists, especially in the USA. Even their earlier modelling has been in terms of neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin (Waldo, Smith & Cox, 1972).

The ability of various methods of feed analysis to predict the available energy content of feeds has been studied and reviewed by Weiss (1993). He concluded that the results of these analyses are population dependent when used as single components in regression equations. In other words, an equation developed for one set of feeds will not be suitable for another set. Even when more than one component is used in empirical regressions, such as the components of the proximate analysis (crude protein, ether extract, crude fibre and nitrogen free extract) or even NDF and crude protein, the results remain population dependent and are not really more accurate than the single component analyses.

Weiss (1993) proposed a more robust method based on theoretical rather than empirical relationships for the prediction of the available energy content of a feed. His method is based on the analysis of nitrogen free NDF, crude protein, ash, fatty acids and lignin. It is said to be accurate for grasses, legumes, by-product feeds and concentrates. He emphasized that all these reflect only the chemical composition of the feed and indicated other factors affecting the available energy content, such as rate of digestion, rate of passage, and particle size should also be considered.

The Cornell Net Carbohydrate and Protein System (CNCPS) (Sniffen, O'Connor, Van Soest, Fox & Russell, 1992) uses equations that estimate fermentation and passage of feed carbohydrate and protein fractions. Their equations are also based on the detergent method of analysis. They include NDF, lignin, total nitrogen, soluble protein, nitrogen insoluble in neutral detergent, nitrogen insoluble in acid detergent, ash and fat. Nonstructural carbohydrates are calculated by difference. Their model is a very comprehensive one, which encompasses ruminal fermentation of carbohydrates and protein, microbial protein production and degradation and digestion in the small intestine. Apparently, they do not consider it "field-usable" yet, and see it as being "a beginning structure to predict ruminally degraded carbohydrate and nitrogen fractions and absorbed amino acids from microbial and feed protein".

However complicated and impressive a model might appear to be, one should not be fooled by the fact that the basic building block of the model remains the detergent methods of analysis. The combinations and applications of the analysis are original, and warrant a fair chance to demonstrate their accuracy in practice.

4.1.4 The importance of using the concept of rates in feed evaluation.

Since digestion in the rumen consists of a number of kinetic processes which are in most cases the ones that limit the rate of animal production, it seems rather futile to describe animal production from feeds, if the rates of these processes are not also considered. The importance of this was realised many years ago when Crampton, Donefer & Lloyd (1960) stated that the intake of nutrients is more important than their actual concentrations in the diet. Garnswothy & Cole (1990) concluded that the effects of feed ingredients on intake are very important and the intake modifying characteristics of feedstuffs should be included in their evaluation. The

importance of this concept is appreciated by most researchers today. The Cornell Net Carbohydrate and Protein System, mentioned previously, describes all the digestive processes in the rumen in terms of rates. They assume first order kinetics to describe the rates.

As soon as rates are measured, the problem which immediately arises is, how does one express digestibility together with rate, both which determine the available energy content of a feed, to give one meaningful term which expresses the feeding value of the feed. An example of the type of problem encountered is: when both maize grain and cassava pellets are analysed, maize has a slightly higher digestibility, but a slower rate of digestion than cassava. Which has the highest potential as a concentrate supplement ? From which one will the animal be able to obtain the most energy for production per time unit ? The only logical manner in which to express this is to combine both terms in the intake equation. The answer may then be given in terms of energy intake or animal production. This can be a theoretical value for certain diets, since maize or cassava may not be fed as the sole component of a diet. However, when analysed, a value for potential energy intake and milk production is given for each item. This is not a prediction of actual energy intake from maize or cassava, but a calculation of theoretical potential intake in terms of digestibility and rate of digestion. It expresses the combined effect of digestibility and rate of digestion in terms of something which is easily conceivable and useful for the animal scientist and the producer.

The intake equation accounts not for only rate of digestion and digestibility, but also for rate of passage and ruminal fill. Since no indirect methods are available yet to estimate rate of passage and ruminal fill accurately, published values (constants) are used. This may be a source of error, since they may differ from animal to animal, and from feed to feed.

4.2 The present uses of *in vitro* gas production techniques.

Although the importance of rates in digestion kinetics have been realised for a long time, scientists have been hesitant to use them regularly to evaluate feeds in practice since, as Weiss (1993) stated, "they are difficult to obtain and are variable". However, with the development of computerised monitoring of gas production to measure feed digestion *in vitro* (Pienaar & Kühn,1991; Pell & Schofield, 1993) the determination of rates of digestion has become a viable proposition. Hillman, Newbolt & Steward (1993) used it to determine the relative contributions of bacteria and protozoa to cellulase activity.

Blümmel & Ørskov (1993) related rate and extents of gas production as well as their estimates of digestion rate and extent, obtained with the nylon bag technique, to voluntary intake and animal production. They used multiple linear regression of the following type:

$$y = a + b_1x_1 + b_2x_2 + b_3x_3 + \ldots b_ix_i$$

The immediately soluble fraction, the rate constant for fermentation and the potential digestibility, single or in different combinations were used as x-values to predict dry matter intake, digestible dry matter intake and growth rate (y-values).

Initially Ørskov, Reid & Kay (1988) and Blümmel & Ørskov, (1993) concluded that rate of fermentation does not play a significant role in the determination of voluntary intake. This conclusion was correct for the approach used, but the approach used was not correct for the problem addressed. If a dynamic or mechanistic model were used, it would have been apparent that fermentation rate varies in importance, depending on the digestibility of the material under discussion. In a highly digestible material such as maize grain, changes in fermentation rate have a large effect on potential intake and animal production. In poorly digestible material, such as wheat straw, it has a much smaller effect simply because fermentation rate is a function of the digestible fraction of the diet only. Thus a simple multiple linear regression cannot identify, nor accommodate the interaction between the relative importance of fermentation rate and the digestibility of the diet. When the experiment was repeated using

better quality feeds such as lucerne, clover and rye grass (Khazaal, Dentinho, Ribeiro and Ørskov, 1993) a different conclusion was reached, namely that "multiple regressions using the kinetics of degradation resulted in the highest accuracy for predicting intake and apparent digestibility". However, their method still represent a static approach to a dynamic process and the results should be applicable only for the immediate situation where they were developed.

A more correct approach would be to use the mechanistic model which has all the variables in the correct relationship to one another:

$$\text{Intake} = \frac{\text{Ruminal fill}}{\text{Mean retention time}}$$

To accommodate the effect of digestibility, mean retention time is divided into the mean retention times of the digestible and indigestible fractions giving:

$$\text{Intake} = \frac{\text{Ruminal fill}}{p_1 t_1 + p_2 t_2}$$

Where p_1 = the digestible fraction

p_2 = the indigestible fraction

t_1 = MRT of the digestible fraction

t_2 = MPT of the indigestible fraction

By varying t_1 and p_1 the actual effect on intake can be demonstrated for feeds with different digestibilities using the intake equation.

4.3 Calculation of voluntary intake and animal production[1].

4.3.1 The principles concerned with calculating intake.

Voluntary feed intake is calculated using the general formula of Pienaar & Roux (1989a). In their approach, use is made of MRT to describe both fermentation and passage rates.

In the general equation, voluntary feed intake is written as;

$$\text{rate of feed intake} = Z/\sum_{i=1}^{n} p_i t_i$$

With Z = the total amount of O.M. in the rumen

p_i = the proportion of the i th component in the feed

t_i = the MRT of the i th component

n = the total number of components.

For the purpose of ruminal digestion kinetics, dietary O.M. may be divided into two components. A potentially fermentable fraction p_1 with MRT $= t_1$ which disappears through both fermentation and passage and an unfermentable fraction p_2 with MPT $= t_2$ which disappears only through passage. These fractions may be estimated by either *in sacco* (Section 3.1) or *in vitro* (Section 3.2) methods.

The curves obtained from both the *in sacco* or *in vitro* fermentations have to be described mathematically and expressed as MFT's. The gamma distribution was chosen for reasons explained in Section 2.1.2.

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[1] J.P. Pienaar & C.Z. Roux
Compiled from: *S. Afr. J. Anim. Sci.* 1989, 19, 3, 99 - 106
and Technical Communication No 223. Department of Agricultural Development. RSA.

Briefly stated, the gamma function describes a curve of substrate disappearance, using two parameters α and ß, being respectively the shape and scale parameters of the gamma function. A larger (>1) shape parameter (α) is usually associated with a delay (slow starting) phase during fermentation or passage. A value for α of 1 indicates the absence of a delay phase and suggests simple first order kinetics. The product of α and ß gives mean fermentation time (α * ß = MFT).

In vivo O.M. disappearance (t_1) includes disappearance by both fermentation and outflow, both acting independently on the same pool of potentially fermentable O.M. Therefore, when *in sacco* or *in vitro* estimates of O.M. disappearance have to be used to estimate *in vivo* disappearance (retention time), the two gamma functions may be combined under the assumption that they act independently on the same pool. The general form of mean retention times for two integer gamma functions acting independently on the same pool, is as follows:

$$t_1 = [(\alpha_0 - 1)!\beta_0^{\alpha_0}]^{-1} \sum_{x=0}^{\alpha_1 - 1} 1/x!(1/\beta_1)^x(x+\alpha_0)!(1/\beta_0 + 1/\beta_1)^{-(x+\alpha_0+1)} +$$
$$[(\alpha_1 - 1)!\beta_1^{\alpha_1}]^{-1} \sum_{y=0}^{\alpha_0 - 1} 1/y!(1/\beta_0)^y(y+\alpha_1)!(1/\beta_0 + 1/\beta_1)^{-(y+1+\alpha_1)}$$

Where it is assumed that $0! = 1$

and α_0 and β_0 are the parameters of the gamma function of the curve which describes the passage of potentiality fermentable O.M. α_1 and β_1 are the parameters of the gamma function of the curve which describes the fermentation of potentiality fermentable O.M.

The general form of the mean retention time model can be solved for all cases of α and ß. For instance, when $\alpha_0 = 1$ and $\alpha_1 = 1$ the first order situation applies, since both outflow and fermentation follows first order kinetics. The general equation then solves to

$$t_1 = (1/\beta_0 + 1/\beta_1)^{-1}$$

This is the simplest situation, and is equivalent to the other equations published for first order reactions (Pienaar *et al.* 1980, Allen & Mertens, 1988). It also demonstrates that the equation is applicable to all the situations where the gamma function describes passage and outflow from the rumen accurately.

For the calculation of voluntary intake an estimate of ruminal fill (Z) is needed. This may be obtained from the relationship between ruminal O.M. contents and live mass. A reasonable relationship can be expected only if ruminal fill is the first limiting factor controlling voluntary feed intake, as is the case under certain conditions with roughage diets (Roux & Meissner, 1984 pp 675 equation 6).

Estimates of the ratio between ruminal O.M. contents and live mass reported in the literature, may vary between 6 g of O.M. per kg live mass (W) for sheep grazing white clover, to 32 g/kg W for growing sheep fed for an extended time (2-3 months) on a low quality dry roughage, and which scarcely satisfied their energy requirements for maintenance .

The following values for ruminal O.M. content are recommended for the prediction of intake:

For sheep grazing high quality pasture such as rye grass or clover, 6-9 g O.M./kg W (Thompson, Cruickshank, Poppi & Sykes, 1985);

For lower quality grazing like kikuyu, values of 13-17 g O.M./kg W are appropriate;

For dry roughages values of between 15 and 25 O.M. (g/kg) have been recorded, but a value of about 20 g O.M./kg W (Poppi, Minson & Ternouth, 1980) is recommended;

For lactating sheep the value is about 18% higher than for dry ewes;

For young growing sheep fed low quality diets on maintenance or sub-maintenance requirements for long periods (2-3 months), values of 32-33 g O.M./kg W are appropriate;

For cattle, the values reported range from 12 to 23 g O.M./kg W, but an average value of 19 g/kg W (Poppi *et al*, 1980) is recommended for dry cows. The same adjustment as for

lactating sheep (18%) is also recommended for lactating cows, although values of up to 30% have been reported (Williams *et al*,1989). This difference is explained by a further increase in ruminal fill when energy demand significantly exceeds the energy supply to lactating cows. Thus, ruminal fill would increase from 19 g/kg W at calving to 25 g O.M./kg W at 100 days *post partum*. From 100 days *post partum* for the rest of the lactation it would decrease linearly to about 20 g O.M./kg W. The increased ruminal fill caused by a severe energy deficiency would not be possible during the last part of pregnancy, due to ruminal fill being limited by the foetus.

A correction for limiting feed intake on rumen capacity or on energy intake is not new. Work by Fisher, Burns & Pond (1987) reports on this. Their work incorporates energy as a feedback throughout the range of diet digestibilities. Energy feedback is interpreted if the level of distension is known. It modulates the chemostatic influence based on the ratio of actual to nominal distension, and also the other way round. In other words if the level of energy demand is known, then the level of distension that will be tolerated can be calculated. The dairy animal is a good example. The large increase in intake as lactation begins can be explained partly as increased capacity because of the birth but also the lactation causes a large increase in demand for energy and the level of rumen fill increases substantially.

However in this thesis aspects of modeling rumen digestion kinetics are emphasized. Not so much the animal factors (such as rumen fill), which were not studied in depth. Animal factors are needed in order to compare feeds in a realistic environment. However feed evaluation is more simple and realistic if animal factors can be kept as constant as possible. When formulating feeds, an estimate of the energy requirements of the animals is usually available. After analysing the feed, the rate at which energy is supplied from the feed, may be compared with the animal's requirements. This was considered sufficient for the purposes that this model was intended for.

There is no *in vitro* or analytical method yet available by which the MPT for passage of unfermentable O.M. (t_2) may be estimated. It must be determined *in vivo*. However, the work of Sutherland (1987) and also Wattiaux Mertens & Satter (1992) suggested possible ways to develop *in vitro* methods to estimate this parameter. Until these methods have been developed, it is suggested that reliable estimates of t_2, which are available in the literature, be used. The values used should be specific to the diet and type of animal concerned (Meissner *et al.*,1979; Pienaar *et al.*,1980). However, estimates of t_2 should be carefully chosen since Ehle (1984) has shown that some estimates may be biased because of the method used to label the feed. Retention times of between 25 to 50 hours are not uncommon. Course roughages have higher values than finely ground roughages. A t_2 value of 48h is recommended for coarsely ground (> 25 mm sieve) or chopped dry roughages and about 27h for finely ground (6 mm sieve) dry roughages (Pienaar & Roux, 1989b). For green roughages a value of 35h is recommended.

An estimate of the MPT for passage of potentially fermentable OM from the rumen (t_0) on roughage diets was obtained from the steady state method (Pienaar *et al.*, 1980). Only a mean value was obtained and no estimates were made of either α_0 or $ß_0$, being the shape and scale parameters respectively describing the outflow of potentially fermentable O.M. It is suggested that the process follows first-order kinetics (Pienaar & Roux, 1989a, equation 8), thus giving values of $\alpha_0 = 1$ and $ß_0 = 81.7$h. No other estimate of t_0 is available as yet and it is expected that lower values would be applicable with animals grazing green pastures. The value of 82 hours has produced satisfactory results when predicting intakes on dry roughages, while an arbitrarily chosen value of 60h produced satisfactory results for sheep grazing kikuyu (Pienaar & Roux, 1989b).

When the values for α_0, $ß_0$, α_1 and $ß_1$ are known, t_1 may be calculated. If α_1 is a non-integer value between 1 and 2, t_1 will have to be calculated for both $\alpha_1 = 1$ and $\alpha_1 = 2$ from the equation MRT = $\alpha * ß$.

For $\alpha_0 = 1$ and $\alpha_1 = 1$, which is the situation when only first order kinetics apply:

$$t_1 = (1/\beta_0 + 1/\beta_1)^{-1}$$

When $\alpha_0 = 1$ and $\alpha_1 = 2$

$$t_1 = (1/\beta_0 + 1/\beta_1)^{-1} + 1/\beta_1(1/\beta_0 + 1/\beta_1)^{-2}$$

Since α_1 lies between 1 and 2, the actual value for t_1 may be obtained by interpolating the two answers for t_1

Voluntary intake can then be calculated using the following equation:

Feed intake (g/h) = $Z / (p_1t_1 + p_2t_2)$ if t is expressed in hours.

Once intake has been calculated, an estimate of *in vivo* digestibility is needed if potential animal performance needs to be predicted. This can be calculated from the digestion kinetics of the feed, similar to the calculation of protein degradability. However, that only describes digestion in the rumen and does not include digestion in the small intestine and hind gut. For the gamma function the following equation can be used to estimate digestion in the rumen.

$$\text{digestibility} = p_3 + p_1[1/(\alpha_1\beta_1)]t_1$$

Where p_1, α_1, β_1 and t_1 are as explained above, and p_3 is the immediately soluble and fermentable fraction.

Until methods for estimating hind gut fermentation have become available, *in vivo* digestibility is estimated for the practical situation by digestion up to 48 hours. From the gas production curves, the fraction of gas produced at 48 hours as a fraction of total gas production is calculated. This fraction is used to correct total (72h) digestibility (which also includes pepsin

digestion).

4.3.2. A step by step example of the calculation of intake.

1. Use the following equation to calculate intake

$$\text{Intake} = \frac{\text{Ruminal fill}}{\text{Mean retention time}}$$

$$\text{Intake} = \frac{\text{Ruminal fill}}{p_1 t_1 + p_2 t_2}$$

Where p_1 = the digestible fraction

p_2 = the indigestible fraction

t_1 = MRT of the digestible fraction

t_2 = MPT of the indigestible fraction

2. Obtain an estimate of ruminal fill as described under Section 4.3.1. If it has to be calculated from live weight for a sheep weighing 60 kg at 19 g O.M./kg W it amounts to 1140g O.M.

3. Determine the digestible fraction (p_1) of the feed using a "Tilley & Terry" *in vitro* with a 72h incubation time in the microbial phase. In this case for lucerne it is 0.64 or 64%.

4. Calculate the indigestible fraction (p_2) of the diet by difference 1.00 - 0.64 = 0.36.

5. Obtain an estimate of the MPT of the indigestible fraction from Section 4.3.1. In this case t_2 is 48 hours for chopped lucerne.

6. Obtain an estimate of the MPT for passage of the digestible fraction from Section 4.3.1. In this case it is 81.7 hours with $\alpha_0 = 1$ and $\beta_0 = 81.7$ hours.

7. Obtain an estimate of the MFT for fermentation of the digestible fraction by using *in vitro* gas production as described under Section 3.2 or by using the *in sacco* technique described in Section 3.1. Use an appropriate statistical computer package or the method described by Law & Kelton (1982) to fit the gamma function to the data, to obtain values for α_1 and β_1. In this case $\alpha_1 = 1$ and $\beta_1 = 10$ hours.

8. Combine the estimates for flow and fermentation of the digestible fraction as described in Section 4.3.1. In this case the following equation is appropriate, $t_1 = (1/\beta_0 + 1/\beta_1)^{-1}$ which solves to $(1/81.7 + 1/10)^{-1} = 8.91$ hours.

9. Substitute the values in the following equation:

$$\text{Intake} = \frac{\text{Ruminal fill}}{p_1 t_1 + p_2 t_2}$$

and obtain Intake (g/h) = $1140/(0.64 \times 8.91 + 0.36 \times 48)$

= 50 g/h

or 1190 g/day

4.4 Testing the use of ruminal kinetics for predicting voluntary intake.

Testing the results obtained with ruminal kinetics do not imply the validation of the principles concerned, since the principles used are the basic ones functioning in ruminants, and these can hardly be altered. The testing rather concerns the adequacy or not of the methods adopted to describe the kinetic processes and the assumptions made concerning the control mechanisms involved. The last word concerning these have obviously not been spoken, since the field is relatively new, and the processes involved are complicated. However, an idea of the accuracy with which the processes are described, is needed in order to determine whether the results obtained are useful for feed evaluation. The validation is usually done by comparing the results of the modelling with the actual results obtained with animals.

Pienaar *et al.* (1980) compared observed intakes with intakes predicted using a first-order approach. Model parameters were obtained with mature cannulated sheep and predictions were made for both the cannulated sheep and for young intact sheep fed either a lucerne or a wheat straw diet. The only model parameter obtained with the young sheep was ruminal fill at slaughter. The results are presented in Table 4.4.1.

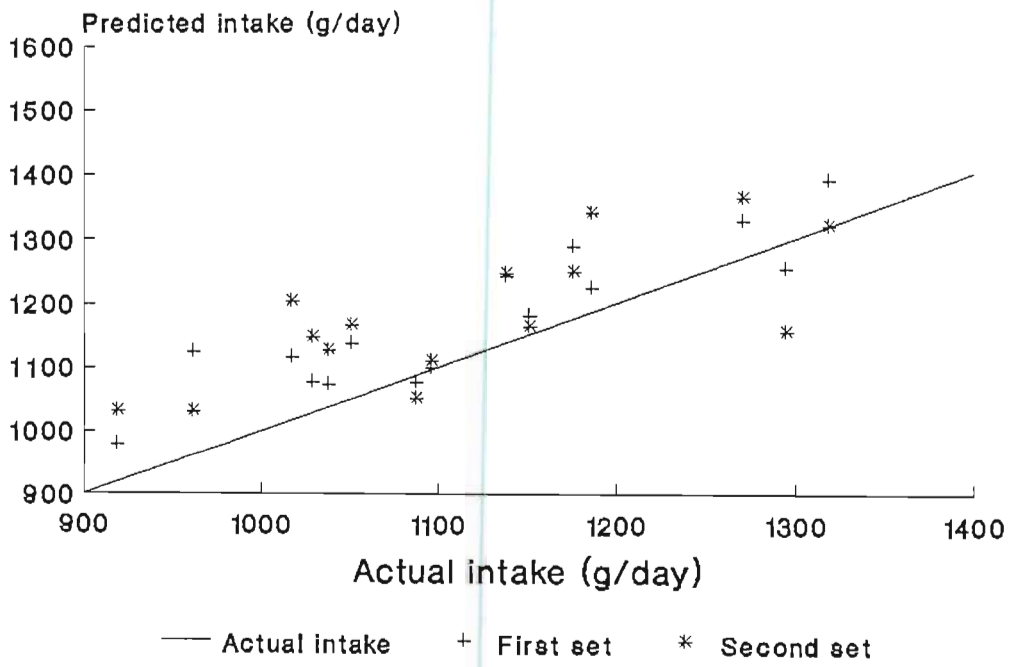
Table 4.4.1. Observed versus predicted intakes (g/day) for cannulated and intact sheep fed either a wheat straw or a lucerne diet.

Type of Animal	Predicted	Observed	r	SE
Cannulated	856	848	0.68	36
Intact	666	667	0.95	19

Table 4.4.1 shows predicted values to be very close to observed values. This suggested that the model parameters are the ones that were actually concerned with voluntary intake. A crucial aspect that must be borne in mind when comparing the results from the validations, is the method by which the parameters of the method have been estimated. If all the parameters were obtained from animals, as was the case with the results reported in Table 4.4.1, a high accuracy can be expected. The fact that these parameters were determined with sheep eliminates many possible sources of bias from the determinations, but in terms of feed evaluation it is rather impractical to have to feed a feed to an animal before being able to predict its voluntary feed intake. A more practical approach for feed evaluation would be if the parameters can be estimated by indirect - or laboratory methods.

The second example (Figure 4.4.1 and Table 4.4.2) shows the accuracy where *in vitro*

Figure 4.4.1. Predicted versus actual intakes using two sets of model parameters.



methods were used to estimate some parameters, and some parameters were obtained from previous experiments. Fifteen different qualities of *E curvula* were chopped and each fed to six Merino type wethers. Voluntary feed intakes were determined. Samples of these feeds were analysed for solubility in McDougal's saliva (Pienaar *et al.* 1980), *in vitro* digestibility, and rate of *in vitro* O.M. disappearance. Estimates of rate of passage, and ruminal O.M. contents per kg live mass were obtained from the experiment reported in Table 4.4.1 (first set of model parameters) or determined using *E curvula* hay and another set of sheep, older than those for which intakes were predicted (second set of model parameters). The ratio between live mass and rumen OM content was 2.76% and 1.78% the first and the second set pespectively while the MPT for passage (mean passage time) was 48h and 22.02h for the first and the second set respectively.

Table 4.4.2. Observed versus predicted intakes (g/day) for sheep fed fifteen *E curvula* diets calculated with two sets of model parameters.

Model parameters	Predicted	Observed	r	SE
First set	1172	1115	0.90	49
Second set	1180	1115	0.74	71

The predicted mean obtained with the first set of model parameters corresponded well with actually observed mean intake. Also the close distribution of the points to a straight line is reflected in the correlation coefficient of $r = 0.90$. The predicted mean obtained with the second set of model parameters was similarly close to the observed mean but with a correlation coefficient of only $r = 0.74$. The plot of actual intake versus actual intake (Figure 4.4.1) also shows the line of perfect fit. Both sets of model parameter resulted in a relatively unbiased

prediction of intake. However, the most accurate estimates (Table 4.4.2) were obtained with the first set of parameters where ruminal fill and rate of passage were estimated from the same type of animal than those for which intakes were predicted. The higher voluntary feed intake resulting from the higher rumen fill used in the first set of parameters was offset by a lower rate of passage for the indigestible fraction. This resulted in similar mean intakes for the two sets. However since the effect of the rate of passage would be influenced by the size of the indigestible fraction of the diet, the faster rate of passage used in the second set would result in relative higher intakes on diets with a lower digestibility. Due to the lower rumen fill, intakes would be lower on diets with a higher digestibility in the second set. Thus the accuracy of the prediction and in this case not so much the bias would indicate which set of parameters would be the most appropriate for the situation.

In the third example (Table 4.4.3) voluntary feed intakes were predicted for Merino type wethers, fed either wheat straw, wheat straw treated with 10% NaOH or wheat straw treated with 10% NaOH and 25% H_2O_2 (Meeske, 1989, unpublished). In this example the live masses of the animals, the potential digestibility, the rate of digestion and solubility of the feed as determined *in sacco*, were used to predict voluntary intake.

Table 4.4.3. Observed versus predicted intakes (g/day) for sheep fed wheat straw (W S) or wheat straw treated with alkali or hydrogen peroxide.

Diet	Predicted	Observed	r	SE
Wheat Straw	855	1042	0.95	39
W S + NaOH	1980	1718	0.61	243
W S + NaOH + H_2O_2	2225	1388	- 0.03	419

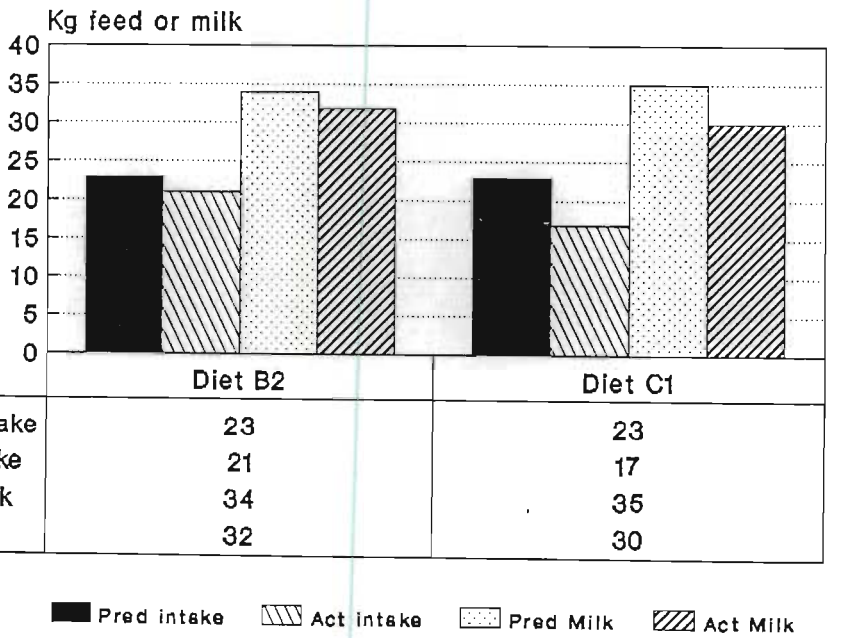
The results are presented in Table 4.4.3. Table 4.4.3 show predictions which are not as close to the actual intakes as the ones shown in the two previous examples. A smaller group of sheep (6) for each mean than in the previous treatments may explain this. Also in this experiment older sheep (68 kg) were used together with younger sheep (53 kg). For example, by eliminating only one sheep from the group fed wheat straw the observed and predicted means changed to 894 and 806 g/day respectively. This change improved the prediction considerably and emphasizes the fact that model parameters such as ruminal fill per unit body mass and rate of passage which could be applicable to older sheep are not necessarily valid for younger sheep. The fact that the model predicted much higher intakes on the NaOH and H_2O_2 diets demonstrates the next point. The predictions of intake obtained in the last two examples are based on the assumption that animals would fill their rumens to capacity and voluntary intake would be equal to ruminal clearance rate. If this assumption is not true, as it wasn't in this case, predictions would be biased. The rumens were not filled to capacity, especially on the H_2O_2 treated diet and thus voluntary intakes were overestimated. The reason for the rumens not being filled, most likely lies in the fact that excessive foaming was observed in the sheep's rumens on the H_2O_2 treated feed. The amount of foaming observed was so excessive that it most probably prevented the sheep from eating to capacity.

Figure 4.4.2 shows the results of two diets fed by Ferreira (1993) to two groups of dairy cows at the IAPI Dairy Section. Rate and extent of fermentation was determined using the Tilley and Terry *in vitro* described under Section 3.2. The other parameters were from published data given under Section 4.3.1. The first problem was to decide for what stage of lactation to predict the intake. It was decided to do it at peak lactation, which is an easily definable point. Figure 4.4.2 shows a close prediction of both intake and milk production on Diet B2. On diet C1, actual intakes and milk production was considerably lower than predicted intakes. When Ferreira was questioned on this point, it was found that the two diets were formulated to have the same energy content, but they differed in terms of their protein

contents. Diet C1 had very little true protein, with protein being mostly supplemented in the form of NPN. It is clear that the predicted intakes and milk production showed no difference between diets B2 and C1 since intakes were calculated from the energy values, and not the protein contents of the diets. It is interesting to note that on these diets no diet selection was possible, since the animals were fed a pelleted complete diet.

Figure 4.4.3 shows the results of feeding 4 forms of Smuts finger grass (*Digitaria eriantha*) to mature Merino rams. The material was either preserved as hay, or as three forms of silage. The prediction was consistently lower than the actual intakes, and although the hay which realized the lowest intake was also predicted the lowest, and the inoculated silage which

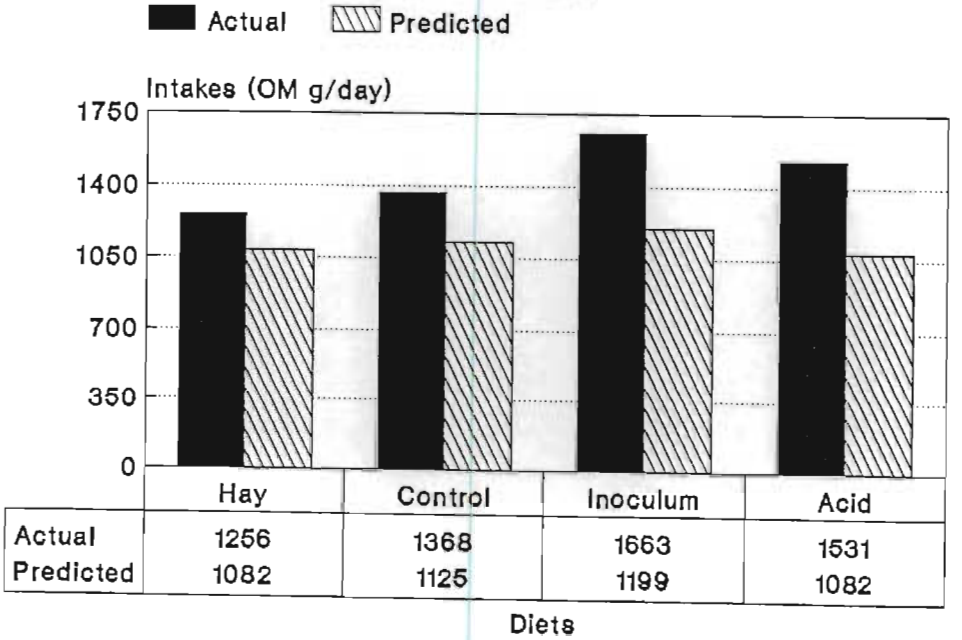
Figure 4.4.2. Predicted versus actual intakes and milk production at peak lactation.



	Diet B2	Diet C1
Pred intake	23	23
Act intake	21	17
Pred Milk	34	35
Act Milk	32	30

showed the highest intake, was also predicted the highest, the acid preserved silage which realised the second highest intake, was rated the same as the hay. A possible explanation for consistently lower predicted intakes on all the diets, is the fact that sheep, especially Merino sheep, are well known for their ability to select a diet that differs significantly from what they are offered (Cilliers & van der Merwe, 1993). Since they were fed at a level of *ad lib* +10% or more orts, they have most probably selected a diet which was better than the representative samples which were analysed on all three diets. The fact that the underprediction was larger on silages than on hay, probably lies in the fact that silage is well soaked in water, and therefore a silage particle would pass much faster from the rumen than a dried particle such as hay (Sutherland, 1987).

Figure 4.4.3. Actual versus predicted intakes of sheep on silage and hay diets



Another factor which can influence the accuracy of predictions, can be found in the body condition of the animals. Since the ruminal fill of an animal is predicted from its live mass, a relatively thin animal would cause an under prediction of ruminal fill, while the opposite is also true.

Table 4.4.4 shows the prediction of the voluntary intake and milk production of four cows fed four diets in a 4X4 latin square design, conducted by Erasmus (1993).

Table 4.4.4. Predicted versus actual intakes and milk production for cows in an experiment by Erasmus (1993)

	‹----Feed-----›		‹---------- 625kg cow ----------›			
Sample Name	MFT h	ME MJ/kg DM	DM intake kg/day	TDN intake kg/day	ME intake MJ/day	Milk production Litre
8430 B						
Predicted	15.3	11.0	17.5	11.4	193	23.5
Actual		10.7	23.1			32.9
8550 K						
Predicted	14.7	10.9	17.7	11.4	193	23.6
Actual		10.9	23.8			31.5
8550 M						
Predicted	15.7	11.0	17.4	11.3	191	23.2
Actual		10.8	22.3			32.7
8605 G						
Predicted	16.2	10.9	17.3	11.2	190	23.0
Actual		10.9	23.6			32.8
Predicted mean (4 diets)	16.8	10.9	17.5		191	23.3
Actual mean (4 diets)		10.8	23.2		251	32.5
*Adjusted mean prediction	16.8	11.1	22.7	14.8	251	34.8

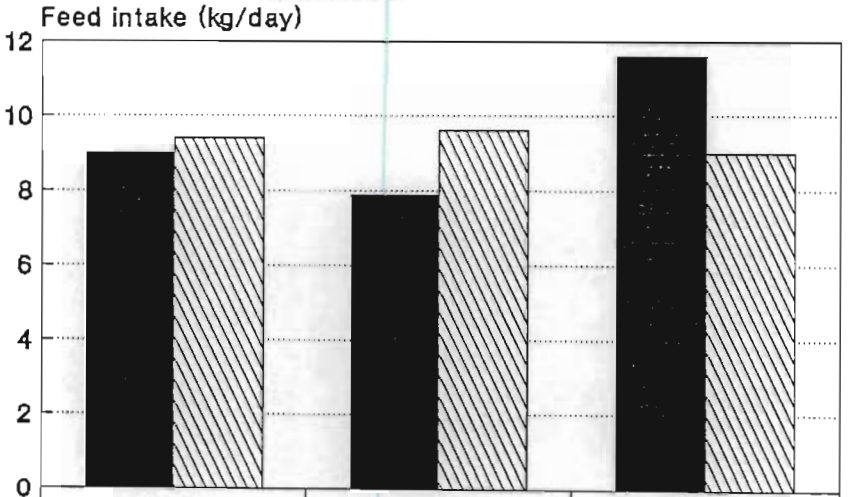
*Adjusted for a fast rate of passage as for a finely ground feed (27h instead of 48h)

The similarity between the actual and the predicted energy concentrations of the four diets is striking, as well as the lack of difference between the four diets, both for predicted and actual values. However actual intakes and milk production on the four diets were much higher than

the predicted values. The reason for this is the fact that when predictions are made for the first time, it is always assumed that cows are in mid lactation and that the feed is fed chopped or coarsely ground. This assumption allows feeds to be compared on a common basis. The results of feed analyses are not comparable if they are calculated for a different set of animals every time. However, in practice, each group of animals is different from the other. Once the analyses have been completed, adjustments for a another set of animals can easily be done.

The values are corrected to suit other situations when more information is supplied. It turned out that the diets were fed finely ground and the experiment was conducted close to peak lactation. The necessary adjustments in rate of passage were made according to Section 4.3.1 and adjusted intakes are also shown in Table 4.4.4. The adjusted intake was then much closer to actual intakes.

Figure 4.4.4. Actual and predicted intakes of bulls at Irene



	Mean	340 kg	500 kg
Predicted	9	7.9	11.6
Actual	9.4	9.6	9

Figure 4.4.4 shows the means of actual and predicted intakes for 20 bulls from the phase C test at the National Performance Testing Centre at Irene. The bulls were from two age groups, some starting a test and some completing the test. When the means over all bulls are compared, predicted intakes were close to actual intakes. However, when the intakes of the younger cattle (340 kg) are studied separately from that of the older cattle (500 kg), it becomes clear that voluntary intakes were under-estimated in the younger cattle and over-estimated in the older ones. It can be seen that actual intakes actually decreased from 9.6 to 9 kg while the animals grew from 340 kg to 500 kg. Since intakes are always related to live mass when predicting intakes, it is quite clear why intakes were biased for the two groups. It was expected that the heavier group would eat more, which they did not. The fact that the mean over all groups was reasonably closely predicted, shows that the assumptions were reasonable for an "average" animal.

4.4.1 A critical evaluation of the use of ruminal kinetics for feed evaluation.

In order to decide whether the approach proposed here would be valuable for feed evaluation, the following questions may be asked:

Would the measurement of ruminal kinetics be useful in feed evaluation ?

The very first models of ruminal function (Baldwin, Lucas & Cabrera, 1970), have included ruminal kinetics. This was to be expected since ruminal kinetics are a quantification of the processes by which the digestion of a feed occurs. It is the only terms in which feed characteristics can be logically expressed. Even though those early models, and the ones that followed later (Baldwin, Thornley & Beever, 1987 and also Dijkstra, 1993) were never applied directly to feed evaluation, they emphasize the importance of kinetics in the digestion of feed in the rumen.

In order use ruminal kinetics in feed evaluation, a simple model with accessible inputs is needed. The outputs of the model should be in terms that are comprehensible to everybody and should also be useful for feed formulation. Thus it was decided to express the final outputs in terms of voluntary energy intake and animal production. It should be specified very clearly what inputs were actually measured (e.g. rate and extent of digestion) in order not to leave the receiver of the results in any doubt about what he can expect from the results he is receiving.

The bounds of the model are determined by the purpose the model is to be used for. This model was developed mainly for the purpose of determining the energy value of feeds. If these values have to be used to formulate feeds, it is logical to set the bounds of the model without the restriction of energy control on intake, in order to obtain the potential energy intake of feed components such as maize meal or cassava meal. Any nutritionist knows that these components should not be fed alone or *ad libitum* to ruminants. However if these components have to be used in a mixture with roughages their potential value have to be known. When a feed is formulated the potential energy intake of that feed has to be known. If it exceeds the amount of energy the animal will be able to utilize, the feed may be reformulated or the expected intake may be calculated from the potential rate at which the feed can deliver energy when the animal fills its rumen to capacity, and the ability of the animal to utilise the energy.

In order to make rumen digestion kinetics useful and logical for feed evaluation, the simple model proposed here is needed.

Is the theory behind the use of rumen digestion kinetics robust ?

It is not without reason that the first paper reported in this thesis, is the one by Pienaar, Roux, Morgan & Grattarola (1980). In this paper, the concepts of ruminal digestion kinetics used in a first order model, were reported. This model, was developed mathematically by C.Z. Roux on request from J.P. Pienaar. After developing the concept it was found that a similar model

has already been published by Waldo, Smith & Cox (1972), but they used cellulose as the basic entity for modelling. The principles to be used for the calculation of fermentation rate *in vivo* and *in vitro* were shown and afterwards applied in many published and unpublished experiments. The pertinent ones are summarised in this thesis. While measuring fermentation rate it soon became apparent that first order kinetics do not fit the disappearance from the nylon bag perfectly. Thus other methods were searched to describe the curves which describe the fermentation process. The gamma function was chosen for reasons given in section 2.1.2. While working on the use of the gamma function and how to combine its equations into a model of rumen digestion kinetics, Roux came across the work of Zierler(1962). Zierler showed that the concept of mean retention time can be used in many situations, independent of the shape of the curves which describe the particular processes. Thus the first-order model, described by Pienaar *et al* (1980), was generalised to the simple and well known concept that was described by Minson (1967) which states that the mean retention time of components is equal to the content of that components in the rumen, divided by the intake of those components. Thus it may be concluded that the theory behind the model is founded on the work of a number of researchers in different parts of the world. The final and simplified version of the kinetic model was not proposed at the beginning of the work, but is the result of the integration of more specific but complicated models into a general model of rumen kinetics.

The quantification of the restrictions which rumen digestion kinetics place on voluntary intake is a critical but also most difficult aspect of feed evaluation. The basic theory proposed here concentrates on this aspect of the model and not on the general prediction of intake. Only, the fact that factors other than rumen kinetics do play a role in determining voluntary intake should always be kept in mind.

Is the pattern of response following changes in the kinetic parameters sensible ?

When using such a simple mathematical model, the pattern of response following changes in the kinetic parameters are very predictable. The question is, are these responses always sensible in terms of animal performance ? The examples presented in section 4.4 compares predicted intakes with actual intakes. These demonstrate a number of important points. Firstly, if an animal does not fill it's rumen to the extent that it was expected, the observed intake would be lower than predicted intake. This phenomenon can have two reasons, the one being that the amount of energy the feed can potentially supply exceeds the energy requirements of the animal. If an estimate of the energy requirements of the animal can be made, this can be compared with the actual intake, and the hypothesis excepted or refuted. If it is obvious that the actual intake is lower than the predicted intake and also lower than the energy requirements of the animal, it indicates that there is some factor, other than the digestion kinetics or energy requirement of the animal, was limiting intake. This observation has been put to good use as shown in sections 2.2.4 and 4.4. In section 2.2.4 it was shown that in most instances the intake was probably controlled by the high soluble nitrogen content of the grass. Only when the grass was wilted and dry did rumen digestion kinetics control intake. The same applies to the foamy rumen contents on H_2O_2 treated straw. However unless the calculations of rumen digestion kinetics and energy requirements have been made, it would not have been possible to decide whether a response obtained was what could be normally expected from the animals. Even in the example shown with the dairy cows fed diets low or high in NPN content, calculation of the rumen digestion kinetics helped to show that the effect obtained on the treatments was not due to a difference in the energy content of the two diets, but to some other factor, in this case the experimental treatments.

The observations made on the silage and hay diets (shown in Fig 4.4.3) are more difficult to explain. Firstly if animals consume consistently more than the predicted amount, it shows that the problem does not lie with the feed but with parameterisation of the model. The

explanation of a faster rate of passage for soaked particles, the extreme dietary selection of merino sheep as well as the effect of body condition on rumen fill, are all well known phenomena. However, which one or more of these were operative in this case is difficult to say since no measurements other than voluntary intakes were available on the sheep. This merely indicates that for this specific case the matter will have to be researched in more depth and the apparent anomalies explained by actual observations on the animals. As has been said in this thesis before, this work has so far mainly concentrated on quantifying factors in the feed which would influence animal performance. Even so, not all the feed factors can be estimated with indirect methods yet. Factors in the animal which could interact with feed quality have only been touched upon. Even in the last example discussed, which yielded the worst predictions so far, the broad tendencies were in the right direction.

Is the magnitude of the response following changes in the kinetic parameters sensible ?

The magnitude of the response following changes in the kinetic parameters are dependent on the correct parameterisation of the model since the responses in changes of all parameters are interdependent. Only rumen fill is directly and linearly related to intake as can be seen from the following equation:

$$\text{Intake} = \frac{\text{Ruminal fill}}{\text{Mean retention time}}$$

$$\text{Intake} = \frac{\text{Ruminal fill}}{p_1 t_1 + p_2 t_2}$$

Where p_1 = the digestible fraction

p_2 = the indigestible fraction

t_1 = MRT of the digestible fraction

t_2 = MPT of the indigestible fraction

This implies that if all else remains constant, a 1% change in rumen fill will always result in a 1% change in intake or vice versa. All the other parameters are inversely related to intake, and the effect of for example a change in the mean passage time (MPT) of the indigestible fraction on voluntary intake will be determined by the size of that fraction in the diet. In order to demonstrate that point a sensitivity analysis was done on the parameters of the model using three different diets. Each parameter was increased individually by 10% and the effect on energy intake reported. The results are presented in Table 4.4.1.1.

Table 4.4.1.1. A sensitivity analysis of the parameters used in the model. Intakes calculated for a 600 kg dairy cow in mid lactation.

Sample Name	Crushed Maize		Lucerne bales		Chicken Litter	
	ME intake MJ/day	change %	ME intake MJ/day	change %	ME intake MJ/day	change %
Normal analysis	314		172		92	
Potential digestib+10%	440	39.94	218	26.55	107	16.64
Rumen fill + 10%	346	10	189	10	101	10
MFT + 10%	298	-5.17	168	-2.21	89	-2.69
MPT IDOM + 10%	303	-3.68	160	-6.96	86	-6.12
MPT DOM + 10%	312	-.61	171	-.22	91	-.61
α_1+10%	313	-.34	172	-.06	91	-.29

The three diets were, crushed maize, lucerne and chicken litter. The model output from the calculation of rumen digestion kinetics from the crushed maize may be considered unrealistic. An ME intake of 314 MJ/day with a potential milk production from the feed of 47.4 liter on a diet of pure maize can never be realised. However these dietary characteristics are needed when formulating feeds and to give an economic value to the feed components. Thus the user of the model should understand exactly what he is doing. The other two feeds, lucerne and chicken litter gave model outputs that may be considered realistic, with energy intakes of 172 and 92 MJ/day respectively, giving potential milk yields from the energy digestion of 20 liter and 4.5 liter each respectively. In none of these cases were the effects protein or minerals included. These may be corrected during diet formulation.

The sensitivity analysis showed that an increase in 10% in the potential digestibility had the largest effect on energy intake. By increasing the potential digestibility of maize from 87,9% to 96,7%, energy intake was increased by nearly 40%. By increasing the potential digestibility of lucerne from 67,6% to 74,3% energy intake was increased by about 26%. For chicken litter energy intake was increased by about 16% by increasing potential digestibility from 58,5% to 64,3%. The next largest effect was observed when rumen fill was increased by 10%. As could be expected this resulted in an increased intake of 10% on all diets. The next biggest effect was observed in either MFT or MPT for indigestible OM. On the highly digestible maize diet, MFT had the largest effect, about 5%, while MPT had the biggest effect, between 6% and 7% on the other two diets which were not so highly digestible. Changing both the MPT for digestible OM and the shape parameter for MFT with 10% resulted in changes smaller than 1% in energy intake. Thus it can be safely said that assuming first order kinetics to describe MPT of digestible OM, will not have a significant effect on the prediction of energy intake. At least as long as MPT remains in the region of about 80 hours.

A sensitivity analysis may also be conducted by using an error term to calculate the amount a parameter must be changed when studying its effect on the output of the model. In this case reliable error terms were not available for all the parameters. The size of the error term will also vary, depending on the method used to estimate the parameter (*in vivo, in vitro or in sacco*), thus it was decided to use a constant 10% change for all the parameters.

The means of most of the comparisons between predicted and actual intakes shown above were remarkably close. This was really astonishing if it is borne in mind that accurate predictions could only be expected where rumen flow and fermentation kinetics were the rate limiting factors. This might indicate that many diets formulated for ruminants are in this area. Another factor which contributes to making the magnitude of the responses sensible is the fact that all the parameters of the model were originally measured *in vivo*. Methods to estimate these parameters outside the animal were only developed later.

Are the outputs from a model of rumen digestion kinetics realistic ?

The objective of this model of ruminal digestion kinetics must be considered in order to decide whether the outputs are realistic. The example of the analysis of maize used above may even be used to prove that model outputs are not realistic. However the objective of this model has never been to replace the scientist's knowledge of animal nutrition during feed formulation. In the extreme case it may be stated that the model merely supplies a mathematical basis from which the different parameters that are known may be combined to determine the energy value of a feed. Energy value in this case not meaning the DE, ME or NE concentration in a feed, but the actual rate at which available energy is released from a feed during digestion. These "unrealistic" values may be used to formulate a feeds that actually produce very closely to what they were formulated to do. This proves that when used correctly, the model is a tool in the modern nutritionists hands. It allows him to apply rumen digestion kinetics to everyday situations and that the model outputs are not unrealistic in terms of what they are needed for.

4.5 Conclusions

The accuracy of the relationships between all chemical methods of feed analysis with *in vivo* results, depend on how homogenous the feeds are that have to be analysed. A relationship should be established separately for each type of feed. Some analyses are more sensitive for this aspect than others. The Tilley & Terry *in vitro* analysis seems to be more closely related to ruminal digestion, than any chemical method. In all studies conducted so far, it is the analysis that is the least population dependent.

Comparisons between actual and predicted values from *in vitro* studies show large effects of animal types, when differences are corrected only in terms of live weight. However, very often the predictions are not far out when predicting for an "average" animal. In most cases, the estimates of the ME content of the feed from *in vitro* digestibility were close to actual or expected values.

Factors such as rate of passage and condition of the animal play an important role in feed intake. Our further research is aimed at finding a suitable laboratory method to quantify rate of passage, and to make a correction for animal condition. At this stage the best we can hope for when only analysing rate and extent of digestion is to determine differences between feeds. Deviations between actual and predicted intakes should be expected. However by analysing only these two parameters in the feed the objective is often realised with a remarkable degree of success.

Chapter 5

General Conclusion

The study of ruminal digestion and flow kinetics provides a useful approach towards understanding some of the processes that control voluntary feed intake in ruminants. Since voluntary feed intake is not always controlled by these processes it cannot always be used to predict intake accurately. However, an *in vivo* study of the flow and fermentation kinetics very soon reveals whether these processes are actually controlling intake in the specific situation. It is shown by measuring the ruminal fill of the animal. As was shown under Section 4.1.5 the ruminal fill, expressed relative to live mass, can vary considerably, depending on many different factors. However, a high ruminal fill indicates that ruminal flow and fermentation dynamics are the first limiting factors controlling voluntary intake. An example of such a situation is described under Section 2.3 where the effect of starch fermentation in the rumen on the digestion of forages was studied in the animal. In this case ruminal fill remained high, despite the fact that voluntary forage intake was low under certain conditions.

A low ruminal fill is indicative of a situation where the intake of animals is limited by factors other than flow and fermentation kinetics. Such an example is described under Sections 2.4 and 2.5, where the factors limiting voluntary intake on kikuyu were studied. The low ruminal fill observed on lush green kikuyu was shown to be caused by a high soluble nitrogen content causing problems in the metabolism of the animal.

The kinetics of ruminal flow and fermentation may also be estimated by methods outside the animal and by *in situ* methods. Relative to the *in vivo* methods, these methods have a huge advantage in terms of time and the costs involved to obtain estimates of feeds' flow and fermentation characteristics. In order to make the application of the concepts of fermentation and flow kinetics useful in practical animal nutrition, it is imperative that methods be developed by which ruminal flow and fermentation kinetics may be estimated at reasonable

cost. Both the Tilley & Terry *in vitro* and the *in situ* nylon bag technique have been used for this purpose, but the amount of time and work involved to estimate the fermentation rate of a single sample makes both methods rather impractical for purposes of routine feed evaluation.

The development of a system which logs the progress of *in vitro* fermentation automatically on a computer (Pienaar & Kühn, 1991; Pell & Schofield, 1993) has made the estimation of rate and extent of digestion an economically feasible proposition. Although these two variables are not the only ones concerned in ruminal flow and fermentation dynamics, they are two very important ones. Their inclusion in a mechanistic model of voluntary feed intake and animal production definitely improves the prediction of animal performance from the analyses of feeds.

In vivo evaluation of estimates of rate and extent of digestion obtained with *in vitro* and *in sacco* methods showed good correspondence between the *in vivo* and *in sacco* estimates obtained in the first experiment (Pienaar, Roux & Cronjé, 1989). In the second experiment (Pienaar, & Kühn, 1991) good agreement was found between the *in vitro* and *in sacco* estimates, but the *in vivo* estimates differed considerably from these. The reason for this difference is not clear and the experiment should be repeated to obtain a better understanding of all the factors which might influence *in vivo* estimates of fermentation rate.

The ideal conclusion for all the research reported so far in this study, would be the culmination of all the ideas into a new method of feed evaluation, which should be better than all previous methods. Some comparisons have been made between actual animal intakes and animal performance and the corresponding predicted values. The predictions have varied from highly accurate and unbiased to some estimates which were relatively biased. The main source of this bias was attributable to differences between animals, mainly in terms of ruminal fill and possibly also in the rate of passage values that were used.

The main advantage of this approach in terms of feed evaluation can be found in a relative unbiased estimate of the available energy value of the feed and also the inclusion of rate of digestion in the evaluation of feeds. The effect of rate of digestion and digestibility is expressed in terms of its mechanistic effect on voluntary feed intake and animal production.

The use of ruminal digestion kinetics as proposed here can be considered the culmination of many years of research into a new method of feed evaluation. The method expresses feeding values in terms of dynamic processes and methods of analysis by which these processes can be measured in a laboratory on a routine basis are also proposed. When the efficiency with which available energy is converted to animal products is combined with the intake and available energy values obtained from laboratory analyses, reliable estimates of animal performance in terms of growth and milk production can be expected.

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