DEVELOPING AN ENERGY EVALUATION PROTOCOL FOR HORSE FEEDS IN SOUTH AFRICA

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DECLARATION

I declare that this dissertation is my own work, except for assistance that is acknowledged or where due reference is made in the text. The results contained in this dissertation have not been submitted, in whole or in part, for a degree at another University.

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December 2004

MARION YOUNG.
SUPERVISOR

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"Do not go where the path may lead, go instead where there is no path and leave a trail"

Ralph Waldo Emerson

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ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
ADL	Acid detergent lignin
AEE	Acid ether extract
AIA	Acid insoluble ash
AA	Amino acids
ADF	Acid detergent fibre
CF	Crude fibre
CP	Crude protein
dE	Digestibility of energy
DCF	Digestible crude fibre
DCP	Digestible crude protein
DC	Digestion coefficient
DE	Digestible energy
DEE	Digestible ether extract
DM	Dry matter
DMD	Dry matter digestibility
DMI	Dry matter intake
DN	Digestible nutrients
DNFE	Digestible nitrogen free extract
DOM	Digestible organic matter
ED	Energy digestibility
EE	Ether extract
FFU	Fattening feed unit
FRGP	Fractional rate of degradation
GIT	Gastro-intestinal tract
G/L	Glucose/Lactate
GP	Gas production
HC	Hemicellulose

INRA	Institut Nationale de la Recherche Agronomique
	(French Net Energy System)
KJ	Kilojoule
Km	Efficiency of ME utilization for maintenance
Kp	Kilopascals
Kw	Efficiency of ME utilization for work
LCFA	Long chain fatty acid
Mcal	Megacalorie
MJ	Megajoule
ME	Metabolizable energy
MRT	Mean retention time
N	Nitrogen
NSC	Non-starch polysaccharides
NDF	Neutral detergent fibre
NFE	Nitrogen free extract
NE	Net energy
NIRS	Near infrared reflectance spectroscopy
NSC	Non-structural carbohydrates
OM	Organic matter
OMD	Organic matter digestibility
ScFU	Scandinavian Feed Unit
TDN	Total digestible nutrients
UFC	Horse feed unit
VFA	Volatile fatty acid

ABSTRACT

The purpose of this study was to find the most accurate and reliable method available in South Africa to evaluate equine diets on an energy basis. Currently South African horse owners purchase food according to the crude protein content of the diet, not knowing the energy density of the diet, which they are feeding their animals. Energy is one of the most important measures of an animal feed, as the energy density determines how much of a diet needs to be fed to meet an animal's requirement. The level of feed intake determines the concentrations of all other nutrients in the diet, therefore one cannot formulate a diet correctly without knowledge of its energy content. Through domestication, there has been an increased demand for horses to perform under circumstances that require energy greater than that provided by its natural diet of grass alone. This has therefore lead to the inclusion of cereal grains and their by-products. These large grain meals can overwhelm the digestive capacity of the horse thus leading to various types of digestive disorders such as colic. Therefore by the development of an energy evaluation system, one could provide the horse with the correct amount of energy from the appropriate source without compromising its digestive system. Predicting digestibility of a diet is the basic step for energy evaluation of horse feeds. Currently horse diets in South Africa are formulated using ruminant total digestible nutrient (TDN) data. As large horses are difficult to work with in digestibility trials, a preliminary experiment was designed to see how accurate it would be to use miniature horses as predictors of digestible energy for large horses. By comparing the digestibility data with that of overseas predictive equations, where large horses were used, the results were found to be highly comparable. As it was established that miniature horses were a perfect pilot animal for digestibility studies on large horses, the next step was to determine the rate of passage in miniature horses so as to determine if the length of the collection period, in a digestibility trial, proposed by overseas researchers for large horses, was enough time to clear the digestive system in a miniature horse of the diet under investigation. An experiment was carried out using Celite® as an insoluble marker to determine rates of passage via the acid insoluble ash method. A mean retention time of 66.64 hours was obtained, therefore assuring that a collection period of 5

days, as recommended by overseas researchers, was sufficient time to clear the miniature horse's digestive system of the test diet.

Following the preliminary trial, a digestibility experiment was designed to investigate the accuracy of using ruminant data to formulate equine diets as well as using rabbits as a possible pilot animal in horse digestibility trials. The trial involved five miniature horses, four male sheep and ten rabbits. Four commercial horse diets were investigated. From these results it was found that rabbits proved difficult too work with and did not favour the experimental conditions and therefore gave digestibility results very different to that of the equine. Ruminants proved accurate predictors of the digestibility of the fibre components for horses but not for the other digestible nutrients. Significant differences were found between the diets given only to the horses and diets that should have provided a higher digestible energy did not. This accentuates the need for the development of an energy evaluation protocol, so that equine diets can be formulated more precisely and thereby ensure that the energy requirements of the horse are met.

In vivo digestibility results were compared to the same feeds incubated *in vitro* and significant differences ($P<0.05$) were found between the results obtained by the two methods. A possible reason for this could be the method employed for removing supernatant between the two stages of the Tilley and Terry (1963) method, leading to an overestimation of digestibility for feeds containing hay and incubated *in vitro*. No significant differences ($P>0.05$) were found between digestibilities, rates and maximum gas production between the sources of inoculum used. Significant differences ($P<0.05$) were found between digestibilities obtained by incubating concentrates alone or in an 80:20 ratio with hay. Further investigation is needed here as it was felt that the supernatant removal method contributed significantly to inaccurate *in vitro* results.

This experimental work centres on discovering and developing the best method available to the South African feed industry for predicting digestible energy contents of horse feed, so as to improve defined performance within an equine discipline and reduce nutritionally-induced disorders.

CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

The equine industry has rebounded from a low point in the 1950's to become a thriving multi-billion dollar industry, and the industry shows strong signs of continuing to thrive (Hintz, 1985).The fact that speed and endurance records on the track have not changed very much over the past 80 years (Ensminger, 1971) while production levels for dairy cattle and poultry have increased over 100% in the same time period, shows that horses have been victims of "fads, foibles and trade secrets" (Ensminger, 1971). The National Research Council, published in 1966 the recommended nutrients requirements of horses, but at the time much of the recommendations were based on experimental work with cattle. Better understanding of the nutrition of the horse, and a need to define its nutrient requirements as near to exact as possible is needed.

In the wild, the horse will eat a mainly fibrous diet, and this is very efficiently utilized. In an all hay diet over 70% of the horse's energy requirement is derived from hind gut fermentation. Stabled horses are usually given three meals a day and these will pass rapidly through the gut leaving insufficient time for all the soluble material to be digested by the enzymes in the small intestine. The remaining soluble material passes into the caecum, which is wasteful and can cause severe digestive upsets. If we could design a model with which we could determine the energy value of the feedstuffs so as to provide food energy as close to the horses' requirements as possible, then we could minimise the occurrence of digestive upsets by ensuring that we do not over-supply the animal with excess energy.

One of the most important measures of a horse feed's value is its digestible energy content. Energy density determines how much feed must be fed to meet an animal's energy requirement. Level of intake in turn dictates the concentration of all other nutrients in the feed. Therefore, horse feeds cannot be properly formulated without knowledge of their energy contents. There are various ways that energy content can be estimated and this has led to a great deal of confusion about how much energy is actually in horse feed (Pagan, 1997).

In order for the stud breeder, horse nutritionist and horse owner to obtain the best balance of nutrients at the lowest cost, the quantity of nutrients contained within the ration must be known. As energy is one of the most expensive nutrients comprising the equine diet, it is very important to correctly determine the energy concentration in a feed so as to prevent the over-feeding of expensive energy. Energy is the first limiting nutrient in most animal production systems, thus the need for an accurate and precise feed rationing system that can budget energy balance, and predict responses, will remain.

The aim of the following study was therefore, to evaluate the various methods available to the feed industry in South Africa, to thereby quantify the energy supplied in commercial horse rations and thereby move horse nutrition forward in this country.

1.2 Energy Content of Feedstuffs

Energy from food sustains life. A large part of the food energy intake is required for maintenance purposes, with a varying but much larger proportion required for productive purposes. In equine nutritional terms, energy, whether it is derived from carbohydrate, fat or volatile fatty acid sources, can be broken down into four separate fractions (Figure 1).

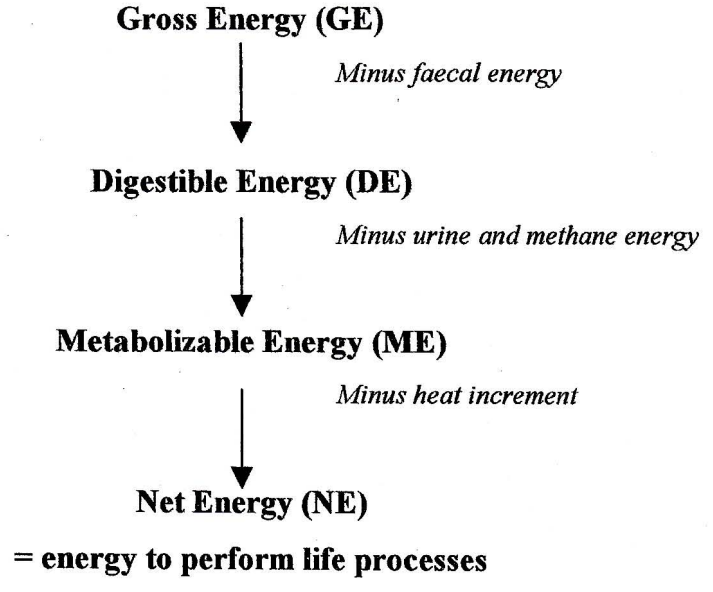

Figure 1 *The partitioning of food energy in an animal (McDonald, 1995).*

Although energy is not a nutrient on its own, it is one of the most important dietary components needed by the animal.

1.2.1 Gross Energy

Gross Energy (G.E) is a measure of the total amount of energy in the feed and is an indication of the amount of heat produced by a known weight of feed, which is burnt in an atmosphere of oxygen. Gross energy values bear little relation to what actually happens to the energy inside a horse but with some feeds, it is the only figure available. The determination is usually carried out in a bomb calorimeter. The basic unit of heat energy is the calorie, defined as the amount of heat required to raise the temperature of one gram of water by one degree Celsius, measured from 14.5 to 15.5°C. This unit is too small for use in horses, so the energy content of horse feed is usually expressed as kilocalories (Kcal) or megacalories (Mcal). Throughout this thesis joules will be the energy unit used, where one calorie is equal to 4.184 joules.

The energy-producing component of a horse feed can be divided into three classes of nutrients namely protein, fat, and carbohydrates. These three classes of nutrients typically have the following gross energy contents (Pagan, 2000):

Carbohydrates: 17.36 kJ/g
Fats: 39.35 kJ/g
Proteins: 23.64 kJ/g

Gross energy is entirely independent of the animal to which it is being fed and gives no indication of the efficiency with which the dietary energy is being utilised by the animal, therefore much effort has been put into determining the DE, ME and NE values of feeds for different animals.

Table 1 contains the gross energy content of a number of pure substances and horse feeds.

Table 1 *Gross energy values of pure substances and feeds (dry matter basis) (Pagan, 1998).*

Pure Substance	*Gross Energy (kJl/g)*	*Feed*	*Gross Energy (kJ/g)*
Glucose	15.73	Maize	18.54
Starch	17.70	Oats	19.58
Seed Fat	38.62	Soybeans	23.10
Lard	39.66	Timothy hay	18.87
Casein	24.52	Oat straw	18.54

From Table 1 we can see that GE is a poor indicator of overall feed value since the GE of corn is identical to the GE of oat straw. Gross energy does not differentiate between various carbohydrate sources, and starch and cellulose contain the same GE content (Pagan, 2003).

1.2.2 Digestible Energy

Digestible Energy (DE) is the energy that is left after the production of faeces and is representative of the energy, which is actually available to the horse. Digestible energy is available for two purposes. Firstly, some energy is lost from productive purposes as it is voided in urine and is produced as gases, both being part of the digestion process. To obtain digestible energy values, animals have to be confined in special areas and fed special feeds. These trials are very time consuming and expensive to operate so there are not many DE values available for horse feeds. Most DE values commonly used in formulating horse rations are obtained from using DE figures from other species of animals such as pigs, poultry, cattle or sheep.

1.2.3 Metabolizable Energy

Metabolizable Energy (ME) is determined by subtracting the gross energy in urine and combustible gases from the DE. The metabolizable energy value of a food is determined in a feeding trial similar to a digestibility trial, but in which urine and methane, as well as faeces are collected. ME is the part of DE, which is available for and used by body tissue maintenance, tissue replacement and physical exercise or work demand. This is the part of the energy which keeps the horse alive, keeps it fit and when too much energy is being fed in the ration can make the horse sick.

1.2.4 Net Energy

Net Energy (NE) accounts for the losses in ME as well as the energy lost during the digestion of nutrients (heat increment of a feed). The amount of heat energy generated during digestion depends on the site of absorption and the metabolic pathway the nutrient follows during digestion. NE is not a characteristic of the particular raw material, but more a characteristic of the compound diet. It is measured by feeding a particular diet and determining the energy lost in the heat increment, either by calorimetry or by comparative

slaughter technique. Of all the systems, net energy most closely reflects the energy available for production and is a very sought after value by nutritionists when formulating diets, however it is the most difficult and elusive value to determine (Batterham, 1990). NE represents the energy fraction, which is actually used by the horse for useful purposes such as maintenance and various forms of production, and is not dissipated as heat. The measurement of NE involves determining the energy that is retained in the body or other products such as the energy in mare's milk.

The effective energy system (Emmans, 1994) further extrapolates the concept of a "tax" on energy ingested that is not available for production purposes.

An energy system is essentially a set of rules relating the energy intake to that of the animal's energy requirements, be it for performance or production. The further down the "energy ladder" one can go in selecting a suitable system (e.g. DE, NE), the closer one can come to ensuring that the energy intake meets that of the animals energy requirements. As no *in vivo* energy system is in place in South Africa we will be looking at the digestible energy system, so as to hopefully provide a basis onto which further research can be conducted in this country.

1.3 Why develop an Energy system for Horses

Domestication and an increasing demand for horses to perform under circumstances that require energy intakes above those able to be provided by their more 'natural' diet of fresh forage, has resulted in the inclusion, in particular of cereal grains and their by-products as well as supplemental fat in many horse diets. Such additions may be made in the form of the raw material or processed raw material or a manufactured compound feed. The upper part of the gastrointestinal tract has a relatively small capacity and the horse has digestive and metabolic limitations to high grain diets (Kohnke, 1998). Large grain meals may overwhelm the digestive capacity of the stomach and small intestine leading to the rapid fermentation of the grain carbohydrate in the hindgut. This may result in one of a number of disorders including colic, diarrhea and laminitis (Figure 2). Providing the right amount of energy from the appropriate sources without compromising the digestive system is therefore very important especially to the performance horse.

Most horse rations in South Africa are sold according to the protein content of the feed, but it has been found that performance horses do not require a protein concentration in the ration above that for maintenance. In fact, there were some suggestions that high levels of protein could adversely influence performance, but further studies are needed (Hintz, 1985). From this it should be seen that determining the energy content of a given horse ration is important so that the concentration of the other required nutrients can be determined and a balanced ration can be formulated.

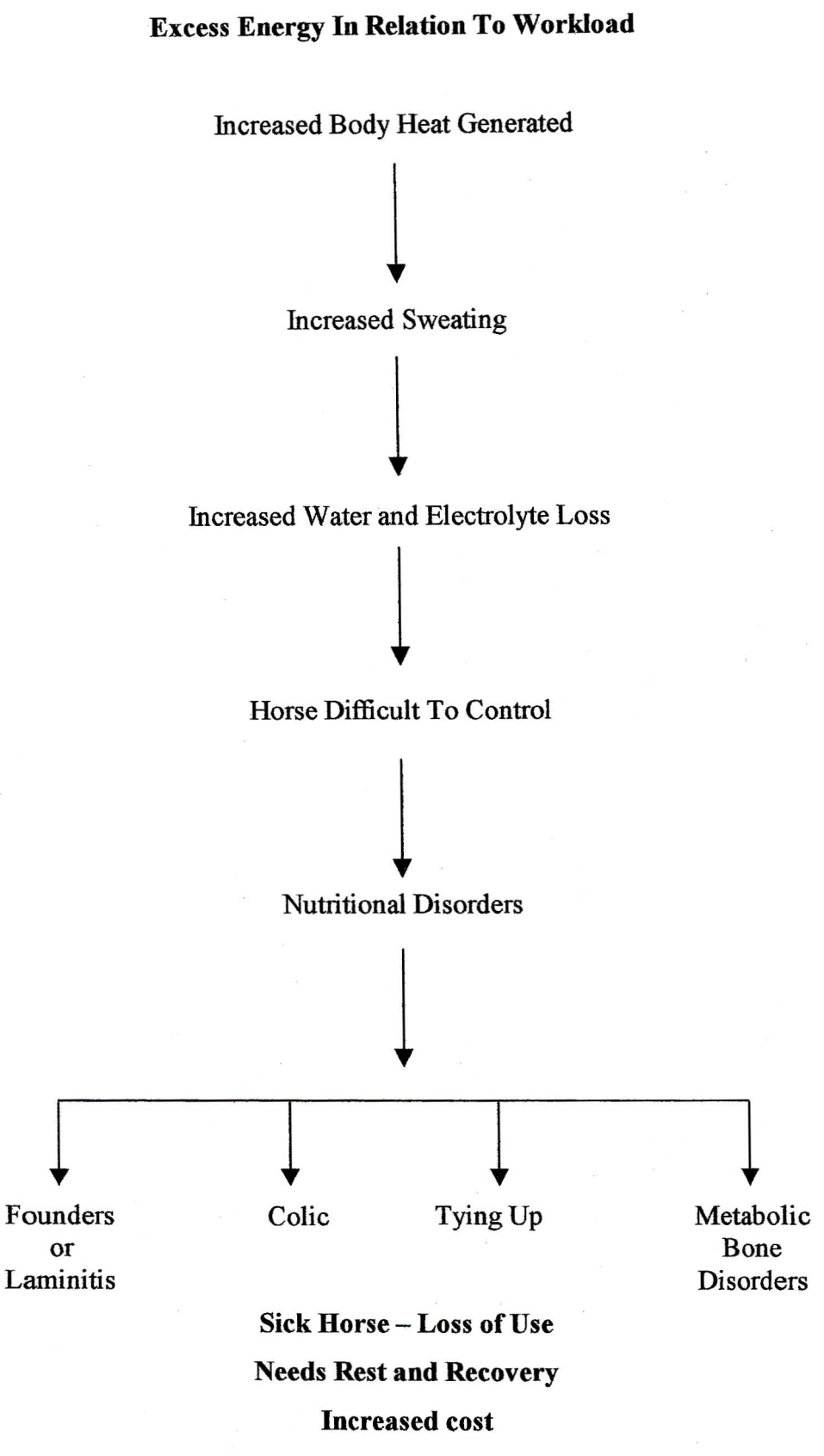

Figure 2 *Excess Energy in the Horse (Kerrigan, 1994)*

Most production animals are fed *ad libitum* but with the horse the amount of feed it will receive each day is determined by the owner. There are three questions that should be asked when determining the amount of feed the animal should receive:

- What is the animal trying to do?
- What does it need to be successful?
- What may prevent its success?

If one can answer these questions and feed according to what the animal needs to function properly under any circumstance, then we can prevent it from being placed under nutritional stress at any time. In equine nutrition, management plays a vital role in the ultimate performance of the animal. Horses have a desire to graze continuously and are therefore accustomed to taking in small and frequent meals all the time. Feeding management must ensure that the horse can still receive small and frequent meals so as to promote healthy gut function. Horses follow the controlled feeding system whereby one feed is fed to the animal and access to the feed is controlled. The owner can look at certain variables such as feed allowance and feed composition so as to affect the performance of the horse.

One must always bear in mind that the energy value of a feed is characteristic of the animal to which it is being fed and not the actual feed. Lack of sufficient energy to supply daily maintenance and exercise needs in the horse's ration will lead to a decrease in the horse's expected performance. Energy is the body's fuel and if there is not enough of it to satisfy the horse's needs, then something has to suffer. In growing horses younger then two years of age, lack of energy in the ration will cause reduced growth. In mature horses, insufficient energy in the ration will lead to poorer performance and if ration energy levels are very low then a loss in body weight could occur. Insufficient energy in the pregnant mare can cause loss of weight in the early stages of pregnancy and a reduced milk production in the last three months of pregnancy. In practical horse feeding situations, the deficiency of energy during a horse's growth period and during performance should rarely occur, provided the feeder has a knowledge of the energy needs of the horse, the energy contents of the feeds and how these all relate to the training programme.

The feeding of energy levels in excess of the horses daily needs and physiological storage ability is dangerous. Most commercial animals such as cattle, sheep, pigs and poultry, are fed energy levels in the rations well in excess of that energy required for body

maintenance. This additional available energy, i.e. that energy supplied above body maintenance requirements, is used for productive purposes, to produce more milk, wool etc. In all of these instances, feeding of excess energy, over and above maintenance needs, produces a product, which can be related to a direct commercial and financial gain to the owner of the animal. These commercial animals also have digestive and physiological systems considerably different to the horse, which allow the cow, pig or poultry to be able to effectively use quite large amounts of energy in the diet without the animal developing problems. The same situation does not exist in the horse (Kohnke, 1998). Part of the reason is the difference between the ways the various species of animals use feeds by different digestive methods which have resulted from natural selection over millions of years. Another part of the reason is due to selection, by man, of strains of these animals which can effectively utilize extra energy, over and above maintenance levels, provided in the feed (Kerrigan, 1994). When feeding excess energy, temperament and behaviour of the horse will be affected (Kohnke, 1998). This is especially true in a horse that is overfed energy and then confined to a small yard or stable where the horse is unable to exercise and "burn up" the surplus energy it does not require. In most circumstances the horse will behave exactly as required at home, in familiar surroundings, but when that horse is taken to a show or event it will probably misbehave. Instead of trying a new and harsher bit, changing other gear and changing training techniques a reduction in energy level of the ration is probably all that would be required.

1.4 Energy Sources and Efficiency of Utilization

Certain nutrients in a horse's diet provide the required energy for an individual following conversion of their chemical energy to other forms of chemical energy, mechanical energy and heat. Dietary energy is provided to the horse by four principal sources:

1- Hydrolysable carbohydrates e.g. starch
2- Cellulose, pectin, hemicellulose, etc. (i.e. non-starch polysaccharides: a component of dietary fibre)
3- Fats (normally less than 3% of the total feed intake): Most horses are able to digest and utilize fat efficiently but it is not usually recommended to feed at more than 10% of the diet and any fat supplement to the diet should be introduced gradually.
4- Proteins: This is not a nutritionally preferred option as a source of energy because it is ergogenically inefficient. Nitrogen must be removed, as excess protein is not stored, resulting in increased water requirements and potentially higher ammonia levels in the stable (Harris, 1999). This is an important reason why we need to determine the energy value of horse rations.

In general, a high proportion of the available starch ingested is degraded to glucose before absorption in the small intestine (unless the digestive capacity of the small intestine is overwhelmed). However, a proportion of the starch and a varying proportion of the dietary fibre (depending on the extent of lignification) are subjected to microbial fermentation. This occurs primarily in the large intestine, producing predominantly short chain or volatile fatty acids, some of which may be used directly as an energy fuel by the gut cells themselves while the majority is absorbed and converted to either glucose or fat (Harris, 1999). The fermentation process is ultimately less efficient than obtaining energy from carbohydrate sources directly via glucose. This helps explain why feeds with a high fermentable content provide less useable energy than those feeds with high digestible carbohydrate content. The extent to which cereal starch provide glucose or volatile fatty acids, as the end result of digestion, will depend on its pre-caecal digestibility which, in turn, will vary according to the feedstuff under consideration and the extent and nature of the processing it has been subjected to. If the excessive starch reaches the hindgut it will be

rapidly fermented resulting in high levels of lactic acid and a number of potential adverse sequellae (Harris, 1999).

1.5 Energy Requirements of the Horse

The energy requirements for horses are expressed as digestible energy (DE). If the use of TDN is preferred then 18.4 MJ of DE is assumed to be equivalent to 1 kg of TDN. Numerous factors such as individuality, body composition of the animal, environmental temperature and humidity, intensity and duration of work, weight and ability of rider, conditions of the running surfaces and degree of fatigue can influence the energy requirements of a horse (Pagan, 1998). The following amounts of energy should be considered as general guidelines.

1.5.1 Maintenance

Values for energy requirements are not based on metabolic size, as Pagan and Hintz (1986) have found no benefit in using metabolic body weight (kg $^{0.75}$) over weight (kg^{1}) in determining the energy requirements of horses ranging in size from 125 to 856 kg. The equation determined by Pagan and Hintz (1986) for horses confined to metabolism stalls is:

DE (MJ/day) = 0.975 + 0.021W * 4.184 **Equation 1**

where W is the weight of the horse in kilograms.

Maintenance requirements of a horse weighing 600kg or less can be estimated from the equation:

DE (MJ/day) = 1.4 + 0.03W * 4.184 **Equation 2**

where W is the weight of the horse in kilograms.

The digestible energy requirements for maintenance for a horse with a mature weight over 600kg can be estimated by the following equation:

DE (MJ/day) = 1.82 + 0.0383W – 0.000014W2 * 4.184 **Equation 3**

where W is the weight of the horse in kilograms (NRC, 1989)

1.5.2 Reproduction

1.5.2.1 Breeding

The body condition of mares at the time of breeding can influence the rate of conception. The dietary energy needs for reproduction therefore depend on the body condition of the mare.

1.5.2.2 Gestation

The DE requirements for foetal development are not greatly increased until the last three months of gestation when the greatest development of the foetus occurs (Platt, 1984). Estimates of the DE requirements for the ninth, tenth and eleventh month of gestation were obtained by multiplying the maintenance requirements by 1.11, 1.13 and 1.20, respectively (Platt, 1984).

1.5.2.3 Lactation

The DE requirement of a lactating mare depends on the composition and amount of milk produced. Mares of light breeds appear to produce an amount of milk equivalent to three percent of her body weight per day during early lactation (1-12 weeks) and two percent of body weight during late lactation (13-24 weeks) (Doreau *et al.*, 1980). The assumptions used by the NRC (1978) for the conversion of DE into milk is that 3313 MJ of DE per kilogram of milk is needed, and this is used today (NRC, 1989).

1.5.3 Growth

Energy for maintenance and growth rate was calculated by using the following equation:

$$\textbf{DE (MJ/day) = maintenance DE (MJ/day) + (4.81 + 1.17x - 0.023x}^{\textbf{2}}\textbf{)}$$
$$\textbf{(ADG) * 4.184} \qquad \textbf{Equation 4}$$

where ADG is the average daily gain (kg),and x is the age in months.

The digestible energy requirement per kilogram of gain (MJ/day), calculated as $4.81 + 1.17x - 0.023x^2 * 4.184$, increases with the age of the foal (Table 1) (NRC, 1989).

Table 2 *Digestible Energy Requirements for Growth of Foals*

Age	DE Requirement (MJ/kg of gain)
Weanling, 4 months	38.07
Weanling, 6 months	46.02
Yearling	64.85
Long Yearling	76.99
2 years old	82.00

1.5.4 Work

Estimations of DE requirements for work are complicated because many factors that are difficult to quantify can influence these requirements. The DE requirements have been estimated in several ways. For example, Anderson *et al.,* (1983) suggested that the amount of DE needed for work and maintenance is best described by the following quadratic equation:

$$\textbf{DE (MJ/day)} = 5.97 + 0.021W + 5.03\chi - 0.48\chi^2 * 4.184 \quad \textbf{Equation 5}$$

where W is the body weight in kilograms

$\chi = Z \times km \times 10^{-3}$

Z = weight of horse, rider, and tack in kg

The authors have shown that this equation should not be used when the work load (kg x km) is greater than 3560, which was the largest experimental value used in computing the equation. Therefore, the equation is very effective for horses performing intense work but not for horses performing endurance work (Ralston, 1988).

Pagan and Hintz (1986) suggested that the DE requirements above maintenance (kcal/kg of weight of the horse, rider, and tack/h) could be calculated by the equation:

$$\textbf{DE (MJ/kg/h)} = [\exp(3.02 + 0.0065Y) - 13.92] \times 0.06/0.57 * 4.184 \quad \textbf{Equation 6}$$

where Y is the speed (m/min).

This equation is most effective for estimating energy requirements of horses performing over long periods of time (NRC, 1989).

1.6 Evaluating a Feed

To describe or predict the performance of a horse, an effective feed evaluation system is required, which will generate information necessary to formulate diets of optimum quality. Feed evaluation systems should be simple, which is a characteristic that is often in conflict with accuracy of prediction of response over a wide range of variation of rations. Most feed evaluation systems used on a large scale are a reasonable compromise between simplicity and accuracy of prediction.

Nutritional evaluations of feedstuffs are undertaken for many different reasons:

1. To measure the extent to which one feed can replace another to support an animal function (i.e. a relative ranking of feedstuffs).
2. To relate feed characteristics to animal function
3. To allow the prediction and/or control of animal performance through nutrition (Oldham & Emmans, 1990).

Farmers throughout the world have developed intuitive and often quite elaborate methods for assessing the feeding value of available feed resources. Such knowledge became established within a community because a relationship with production could be demonstrated and used to predict and manipulate performance (Chesson, 2000). Interestingly, subsequent attempts to relate a farmer's historical understanding of nutritive value to current laboratory-based proximate analysis have often proved quite unsuccessful (Thapa *et al.*, 1997). The development of laboratory methods to characterize feedstuffs was an attempt to formalize this traditional knowledge, allowing feed evaluation to be put on the numerical basis required by the feed industry. Tables of feeds ranked by nitrogen content were available by the middle of the 19th century (Bossingault, 1843) with total digestible nutrients following (Wolf, 1874). However, it was not until the turn of the 20th century that work underpinning the present concepts of digestible and metabolizable energy and the net energy of feeds for farm animals was published (Armsby, 1903). Recently there has been a greater urgency to refine the methods used to characterize feed resources. Changes to production methods and feed formulation, the introduction of new feed resources and animals with greater genetic potential, together with falling margins have shown the need to define feeds and their ingredients more accurately in order for predictions to be made with much smaller margins of error than previously (Chesson,

2000). Existing tables of feed composition are still based on the crude nutrient fractions of the proximate analysis scheme and the cell wall fractions of the detergent fibre system (Van Soest, 1967). Additional information on fatty acid, amino acid, mineral and vitamin contents may also be included. The proximate analysis of feeds, an arbitrary and empirical series of tests, which allowed some prediction of animal performance, was in essence first described by Hanneberg and Stohmann (1864) in the 19th century. Under this scheme, five fractions; moisture, ash, ether extract, crude protein (N x 6.25) and crude fibre were determined directly and the sum of all five expressed as grams per kilogram subtracted from 100 to generate a sixth fraction, this being the nitrogen free extract (NFE). Severely criticized for its imprecision over the years, proximate analysis has been replaced by other methods in most laboratories. Crude fibre and NFE have been found to lack any consistent relationship with recognizable components of crop plants. Crude fibre contained some, but not all, of the polymers which constitute the plant cell wall, while NFE could encompass water-soluble carbohydrate, starch, organic acids and much of the pectin fraction of some cell walls. These measures have now been replaced by the direct determination of the water-soluble components and by neutral detergent fibre (NDF). Acid detergent fibre (ADF), which provides a measure of only the cellulose and lignin content of the cell wall, has found value in the description of forages because of its statistical relationship with degradability (Chesson, 2000). The limitations of these chemical analytical methods of feed description applied to predicting nutritive value are well recognized and documented.

Biological methods for estimating organic matter and energy content based on *in vitro* digestibility measurements made with rumen micro organisms (Tilley & Terry, 1963); Menke *et al.*, 1979) or cell wall-degrading enzymes (Dowman & Collins, 1982) have often proved more successful, particularly for ruminants (Aiple *et al.*, 1996). The requirements for description of feed, which allow a good prediction of responses, clearly differ between livestock classes and particularly between ruminants and non-ruminants.

Chemical analysis is the starting point for determining the nutritive value of feeds, but the value of a feedstuff does not depend entirely upon the amounts of the different nutrients it contains. The value of a feed depends upon the amounts of these nutrients that the animal can digest and use. The chemical composition alone of any feeding stuff is a very imperfect standard by which to judge its nutritive value. The main consideration is digestibility, since it is only the digestible portion of the feed that can serve to maintain the

vital functions and is of value for energy and the formation of animal products, and to ensure animal performance. However, the chemical composition and the percentage digestibility are not all that determine the value of a feed. Two feeds may be equal in composition and equally digestible, yet one may be more valuable than the other because its digested matter can be used to better advantage by the body (Schneider & Flatt, 1975).

Feed evaluation involves the use of methods to describe animal feedstuffs with respect to their ability to sustain different types and levels of animal performance. Subsequently, the acquired data are used, with appropriate animal indices, in feeding systems comprising empirical equations to determine whether a desired level of animal performance can be achieved from various diets. The ultimate goal of feed evaluation is to optimize the efficiency of feed utilization and animal performance at a cost most affordable to the animal owner. It is important to establish the potential of major feedstuffs and the need for appropriate supplements in order to overcome nutritional deficiencies and raise the level of performance (France *et al.*, 2000). Although the horse does not supply a tangible animal product of which the animal producer can receive financial return, feed evaluation is still vital so that nutritionally induced illnesses, such as colic, can be reduced, and performance in the various equine sports be improved. Horse racing, for example, does not supply a tangible product as such, but the financial returns an owner can receive on a winning racing performance make proper feed evaluation an invaluable tool.

1.6.1 The Nutrient Content of a Feed

Once the nutrient requirements of the animal have been established, a diet that provides the correct balance of nutrients can be formulated. This can only be done if correct and accurate information on the feedstuffs is available.

Concentrate feeds generally show little variation in composition. Forages, on the other hand, are extremely variable, their composition being highly dependent on their stage of growth at harvest, the plant species, the proportion of leaf to stem and the fertilizer treatment. Young grass, especially if highly fertilised, is high in protein and non-protein nitrogen compounds but low in soluble and cell wall structural carbohydrates; the cell wall is relatively un-lignified and is therefore highly degradable. At the other extreme, mature

grass is high in structural carbohydrates but the cell walls are highly lignified and of low digestibility. Mature grass is also very low in protein (France *et al.*, 2000).

The analyses of feeds generally involve determining the dry matter (DM), organic matter (OM), structural carbohydrate (fibre or non-starch polysaccharide, NSP), soluble carbohydrate, starch, ether extract (EE) and crude protein (CP) content of the feedstuff (France *et al.*, 2000).

The DM of the feed is usually determined by oven drying at 60 or 100 °C

The OM content is determined by dry ashing at 500 °C until all the carbon has been removed. The residue or ash remaining can then be used to determine the content of individual mineral elements in the feedstuffs.

The most widely used methods for analysing the structural constituents, or fibre, are the detergent extraction methods of Van Soest. These methods involve the extraction of plant biomass with neutral detergent to leave a fibrous residue of predominantly cellulose, hemi cellulose and lignin (i.e. the neutral detergent fibre or NDF of plant cell walls) or with acid detergent to leave a residue of cellulose and lignin (i.e. the acid detergent fibre or ADF of plant cell walls). The fibre content of the feedstuff may be described more accurately by NSP analysis, whereby alditol acetate derivatives of carbohydrate monomers derived from acid hydrolysis of washed, polymeric, de-starched samples are quantified by gas chromatography. With NSP analysis in addition to obtaining details of the chemical composition of the fibre, the values measured are independent of food processing and storage, and therefore the amounts present can be quantified more accurately (McDonald *et al.*, 1995).

Crude protein content is calculated from the nitrogen (N) content, determined by the Kjeldahl procedure involving acid digestion and distillation. More recently, the Dumas method, involving combustion and determination of released gaseous N, is being used. Ammonia nitrogen in fresh silage is determined on water extracts by either distillation or use of specific ion-sensitive electrodes. These methods described above measure N rather than protein; the quantity of N is therefore multiplied by 6.25 (assuming the N is derived

from protein containing 16% nitrogen) to obtain an approximate protein value (McDonald *et al.*, 1995).

Fat (ether extract) is extracted according to the Soxhlett procedure, using a Buchi 810 Soxhlett Fat extractor. Fat is extracted from the sample by the solvent petroleum ether and percentage fat is calculated on the gravimetric analysis (AOAC official method 942.05).

In recent years, near infrared reflectance spectroscopy (NIRS) has also been adopted for determining the composition of feedstuffs. In terms of accuracy, precision, speed and unit cost of analysis, the NIRS technique, provided it is calibrated correctly, is preferable to traditional laboratory methods. However, the technique ultimately relies on a set of standard samples whose composition has been determined by traditional methods (France *et al.*, 2000).

1.6.1.1 Proximate Feed (Weende) Analysis

The starting point in evaluating the usefulness of a feed to an animal is the determination of the different chemical components it contains. The best-known scheme of analysis is known as the Weende Method, generally called the Proximate Feed Analysis. This is the most commonly used system for describing feedstuffs in terms of their content of nutrients or usually its group of nutrients. The analysis fractionates the feedstuffs into six components namely:

1. Water
2. Ether Extract (EE)
3. Crude Fibre (CF)
4. Crude Protein (CP)
5. Ash
6. Nitrogen-free extract (NFE)

All of the above are determined directly, except NFE which is determined by difference (McDonald *et al.*, 1995).

1.6.1.2 Van Soest Method of Feed Evaluation

A new system of fractionation of feeds of plant origin was suggested by Van Soest as an alternative to crude fibre analysis. The procedure involves the separation of feed DM into two fractions, one of high digestibility and one of low digestibility. The first fraction, the neutral detergent fibre (NDF) can be regarded as a measure of the plant cell wall material. This fraction consists mainly of lignin, cellulose and hemicellulose and can be regarded as a measure of the plant cell wall material. The second fraction is called the acid detergent fibre (ADF), where the cell walls can be separated into soluble and insoluble fractions in the detergent. The acid-soluble fraction consists primarily of the hemicellulose in the cell wall fraction, while the residue (acid detergent fibre) consists mainly of cellulose, lignin and variable amounts of silica. ADF is now widely accepted as a measure of the fibre content of a feed, as a substitute for crude fibre (McDonald *et al.*, 1995).

1.6.1.3 In Vivo and In Vitro Methods

In addition to chemical composition, several methods have been developed to characterize feedstuffs in terms of their digestibility. These comprise *in vivo, in situ* and *in vitro* methods. *In vivo* measurements provide the standard measure of digestibility as they represent the actual animal's response to a dietary treatment. However such trials cannot be considered routine in most laboratories, and cannot be carried out for all the possible feeding situations found in practice. Therefore, a number of *in vitro* and *in situ* methods have been developed to estimate digestibility. Thus the *in vitro* and *in situ* techniques may be used to study individual processes, providing information about their nature and sensitivity to various changes (France *et al.*, 2000).

In theory, *in vivo* is the most ideal method for measuring nutrient digestibility in the animal. However, *in vivo* techniques require large amounts of feed and suffer from considerable variation due to the animal and to other factors; this variation necessitates use of sufficient experimental replication to obtain reliable results. The expense of obtaining adequate replication, when added to the costs of maintaining and sampling large numbers of animals, can make *in vivo* studies quite expensive. Animal welfare concerns are likely to contribute to further reductions in *in vivo* experimentation. This has lead to increased interest in using *in vitro* and *in situ (in sacco)* methods for estimating digestibility in the gastrointestinal tract (Broderick & Cochran, 2000). Metabolism studies are the classical means of determining apparently digestible nutrients. If one has the knowledge of

endogenous losses then we are able to calculate true digestibilities. Collection of gas and urine losses from the animal enables the estimation of metabolizable energy (ME), but this type of procedure requires calorimeters and this is difficult to conduct with horses. One such study was used to provide supportive data for the French horse net energy (NE) system (Vermorel & Martin-Rosset, 1997) but relied on assumptions in terms of the proportions of absorbed energy supplied by glucose and VFAs. Furthermore, it was assumed that DE and ME values of forages were similar when fed alone or in mixed diets. This assumption was based on the findings of Martin-Rosset and Dulphy (1987). Cuddeford *et al.* (1992) showed a non-linear increase in ration energy digestibility as alfalfa was substituted progressively with naked oats. However, this could have been a 'level of feeding effect', which might have affected the results because the rations used were adjusted on an energy basis rather than by equalising dry matter intake (DMI).

In vivo studies are the 'gold standard' against which other methods of evaluating feeds are judged. Simultaneous *in vivo* digestibility trials in horses and wethers were used by Martin-Rosset *et al.* (1984) to develop prediction equations so that *in vivo* ruminant data could be used to generate values for horses. The r^2 value for legumes was 0.71 comparable to grasses value of 0.96. Vander Noot and Trout (1971) used *in vivo* studies in steers to predict values for horses, and found that, crude fibre was the best predictor of DMD (r^2 = 0.81). However, predictions based on *in vivo* studies in different species are prone to error, and the INRA (French Net Energy System) is heavily dependent on such work (Cuddeford, 2000).

To yield useful data, *in vitro* and *in situ* techniques must somehow mimic *in vivo* digestion processes. Ideally, *in vitro* and *in situ* estimates of rate and extent of digestion should be quantitatively similar to those obtained *in vivo*. Estimates that are only correlated with *in vivo* values are also useful, indicating that important, perhaps limiting, characteristics of *in vivo* digestion had been simulated by the experimental system (Broderick & Cochran, 2000).

1.6.1.3.1 In Vivo *Systems (Digestibility Experiments)*

Digestibility refers to the proportion of the ingested feeds or nutrient not excreted in the faeces, which is assumed to be absorbed (McDonald *et al.,* 1995). The term is usually only applied to protein, carbohydrates and fat. The digestibility of ash is usually not determined as it does not contribute to the energy content of the food. Also the faeces are a pathway for mineral excretion, which would form part of the ash percentage. The digestible percentage of any substance is called the digestion coefficient of that substance. In the case of most nutrients a small proportion of the nutrient present in the faeces originated from the body, i.e. from metabolic origin. This is included in the calculation of digestibility, and the term "apparent digestibility" is used rather than the term "true digestibility" which refers to nutrients in the faeces exclusively from dietary origin. When this unrecovered fraction is expressed as a percentage of intakes, it is called the coefficient of digestion.

Digestibility coefficient = (Intake – Faecal output) / Intake * 100 **Equation 7**

Factors Affecting Digestibility of Feeds and Nutrients

1) **Animal Factors**

- Species differences: Different species of animals, due to anatomical and physiological differences in their alimentary tracts, do not digest the nutrients of various feeds with the same efficiency. The greatest differences are found between monogastrics and ruminants, although even amongst ruminants a vast difference occurs such as sheep tending to have a higher digestibility, probably due to their lower metabolic losses. Below 66% digestibility, cattle tend to have a higher digestibility than sheep, which is probably reflective of their greater capacity for fibre digestion.
- Breed or subspecies differences: Breed of animal need not be considered as a source of variation in digestibility coefficients. Although the digestion coefficients for mules are higher than those of horses, it must be remembered that the comparison involves a hybrid between two species and one of those species. Hybrid vigor has been shown to cause a more efficient digestibility.

- Variation between animals: It has been well documented (Schneider & Flatt, 1975) that individual animals may give varying digestibility coefficients for any given feedstuff, and also that any given animal may give slightly varying value's at different times. This could be due to factors such as environmental temperature, nervousness and illness. It is always recommended to use not less than three animals per feed in digestion trials, but preferably more.
- Age of animal: Older animals have been shown to exhibit a reduced digestive efficiency due to poor teeth, which makes the adequate chewing of feedstuffs difficult. In young animals, the shedding of the first incisors may influence the ability to chew. Poor digestive function due to reduced digestive enzyme activity in older animals can result in low digestion coefficients as compared to younger animals.
- Effect of exercise: Experiments with horses and cattle has shown that moderate exercise does not have a significant influence on digestibility (Schneider & Flatt, 1975). It is suggested that animals confined to crates or pens during digestibility trials be hand-walked a measured distance each day so as to prevent oedemas and impaction colic's in the case of horses.
- Health: A sick or parasite infected animal will show significantly different digestion coefficients from that of a healthy horse. It is vital that only healthy and disease and parasite free animals should be used so as to ensure accurate digestion coefficients. Diarrhoea in animals will hamper digestibility due to the faster rate of passage through the digestive tract.

2) **Feed Factors**

- Chemical composition: This is one of the most significant factors influencing digestibility. Digestibility will vary little in feeds such as Barley, showing little variation in chemical composition however, roughages are much less constant in composition therefore vary considerably in digestibility. The fibre fraction of the food has the greatest influence on digestibility, where both the amount and chemical composition of the fibre is important.

- Ration composition – associative effect: The digestibility of a feed is influenced not only by its own composition but also by the composition of the other feeds consumed with it. This associative effect provides an objection to the determination of digestibility by difference. Associative effects are usually negative and greatest when low quality roughage is supplemented with a starchy concentrate. Here the rapid fermentation of the starch drops the pH below 6, which in turn inhibits cellulolytic micro organisms, and fibre digestibility is depressed. However, the addition of a protein to low quality roughage may increase the digestibility of the roughage.
- Effect of feed processing: Changes in the physical form of a feed may influence the digestibility of the dry matter, energy, protein and any of the other organic substances in the food products. The manner of harvesting, handling, milling, treatment and storage may have an influence. The most common treatments applied to feeds include chopping, chaffing, pelleting, grinding and cooking and these can all have a marked improvement in the digestibility of a feed.

3) **Level of Feed Intake**: The digestibility of a feed decreases as level of intake increases and this is more pronounced in feeds with a high digestibility as opposed to those with a low digestibility. This food is then exposed to the action of the digestive enzymes for a shorter period of time, and there may be a reduction in its digestibility. As expected, the feed that is normally digested the slowest (cell-wall components) will show the greatest reduction in digestibility (Schneider & Flatt, 1975).

Measurement of Digestibility

a) Time collection method (classical method):

Horse digestibility trials require a collection period of about five days (Pagan, 1998). With a constant daily feed intake a state of equilibrium will be reached in which daily output of faeces will also be relatively constant (Schneider & Flatt, 1975). Faeces collected for a fixed time interval can then be related to feed intake over an equal time interval. The animals used must be docile, healthy and free from internal parasites. Females are not usually used due to the contamination of faeces with urine when using faecal collection

bags. A minimum of three animals should be used per feed but more animals are preferred. The trial is usually divided into two stages, an adaptation period and a collection period. The adaptation period usually lasts seven to fourteen days. Its purpose is to allow enough time for the microorganisms of the animals gut to adapt to a new diet as well as excrete residues from the previous diet the animal was consuming. It is not necessary to keep the animal in a metabolism crate or stall at this stage. The collection period usually lasts about five to ten days, the longer the period the more accurate the digestibility data. Here the animal must maintain a constant intake. All faeces and refusals (orts) must be collected and recorded.

b) Digestibility by Difference:

Certain feeds cannot be fed as a sole component of the diet, so to determine their digestibility they must be mixed with another basal feed which is usually roughage. The digestibility of the basal feed is known and it is assumed that the nutrients in this basal feed will have the same digestibility as it did when it constituted the entire diet. One assumes that in mixing the two feeds neither one will alter the digestibility of the other one. The level of inclusion of the test feed must be high enough to ensure that differences between the test and basal feeds are observed. The smaller the proportion of the test feed to basal feed, the greater the number of animal's required per test. A series of digestion trials can be conducted in which different levels of the test feed are added to the basal feed. The digestibilities of these mixtures can then be plotted on a graph and by extrapolation to zero basal level and the digestibility of the test feed can be calculated (Schneider & Flatt, 1975).

1.6.1.3.2 In vitro *Systems*

There is a continuing demand from the compound feed industry to have a rapid *in vitro* method capable of assessing the nutritional quality of both the raw materials, which make up the diets fed to horses and the diets themselves. *In vivo* techniques are expensive and time consuming to carry out and also requires personnel with skills.

The development of *in vitro* digestibility techniques could reduce the need for *in vivo* studies. This will become increasingly necessary due to concerns over animal welfare and ethics associated with experimentation, involving animals kept in metabolism crates and the use of surgically modified animals (*in situ).*

In vitro techniques with rumen fluid or substitutes have been routinely and extensively used for evaluation of ruminant feed. In recent years, *in vitro* methods for the evaluation of feeds for simple-stomached animals have been developed using either contents of pigs stomach and different parts of the small and large intestine (Lowgren, Graham & Aman, 1989). These methods have been shown to be well correlated with *in vivo* apparent faecal digestibilities for dry matter and energy, but no attempts have yet been made to elucidate the patterns of degradation. Since the site of absorption is of major importance for the energetic value of a feed, it would be of considerable interest to determine those feed components that are readily digestible in the small intestine and those that are degraded in the hind gut (Lowgren, Graham & Aman, 1989).

Several studies have indicated that cellulose digestion *in vitro* can be a reliable predictor of digestible energy (Hershberger *et al.,* 1959). Caecal digestion is an important part of the total digestion process in the horse. Digestion in the caecum of the horse is similar to digestion in the rumen of the steer in that complex carbohydrates are fermented to volatile fatty acids. However, rumen microflora digest freshly ingested feed whereas caecal microflora digest feed that has been partially digested in the stomach and small intestine. In addition, a major proportion of the complex carbohydrates are digested in the rumen whereas Alexander (1951) has indicated that complex carbohydrates are metabolized primarily in the caecum and large intestine of the horse. Applegate and Hershberger (1969) showed that the *in vitro* rumen fermentation technique has been successfully adapted to *in vitro* caecal fermentation studies. Acute laminitis has even be induced experimentally (*in*

vitro) in horses by the administration of carbohydrate, resulting in fermentation within the caecum and ischemia-reperfusion of the digits. The products of fermentation that trigger acute laminitis are unknown, but it is thought that compounds such as amines might play a role due to their vasoactive properties (Bailey *et al.*, 2002).

Applegate and Hershberger (1969) were probably the first to use *in vitro* digestion techniques to investigate forage digestibility in horses. Since that time a number of different approaches have been developed. These will be investigated below.

Pepsin-cellulase

Feed samples are incubated at 37°C for 48 hours in an acid-pepsin mixture either at the start of the experiment (monogastrics/hindgut fermenters), or after a 48-hour ruminal fermentation stage in the case of ruminants. This is then followed by the incubation of the samples in a cellulase buffer solution for a further 48 hours.

This method was used with 52 forages whose OMD had been determined *in vivo* by Martin-Rosset *et al.* (1996). Using multiple regressions, the following equation was derived:

$$\mathbf{OMD\ (\%) = 29.38 + X + 2.30315Y - 0.01384Y^2} \qquad \textbf{Equation 8}$$

$$\mathbf{(r^2 = 0.927)}$$

Where X = 4.12 for green forages, 0 for grass hays and –2.61 for legume hays; Y = cellulase DMD%. This relationship has been shown to be more reliable than that which depended on chemical composition. Therefore, for any feeding system, this enzymatic method deserves further study for the purpose of evaluating horse feeds.

Gas Production

Macheboeuf *et al.* (1998) used the Menke and Steingass (1988) method with caecal fluid inocula to ferment the same feeds as used previously in the pepsin-cellulase method. Two relationships were obtained, one for alfalfa hays and the other for green forages and grass hays. In the former, gas production after 24 hours was the best predictor of OMD (r^2 = 0.76) whereas in the latter case, gas production at 25 hours together with CP was the best (r^2 = 0.87). Using faeces as the source of inoculum improved the prediction of OM for alfalfa hays (r^2 = 0.96) from the rate of gas production (Macheboeuf & Jestin, 1998). The

prediction for green forages and grass hays was as good with faecal inocula as with caecal inocula using the same parameters ($r^2 = 0.86$).

Lowman *et al.* (1997) used the method of Theodorou *et al.* (1994) with faecal inocula to predict dry matter digestibility values based on the *in vivo* values of 16 diverse feeds. The best predictive equation that was obtained used gas production parameters:

$$\mathbf{DMD\ (g/kg) = 155 + 6209\ FRGP + 1.505\ GP} \qquad \textbf{Equation 9}$$

$$\mathbf{r^2 = 0.72}$$

Where FRGP is the fractional rate of gas production estimated when 50% of the gas has been produced, and GP is the total gas production. It is clear that gas production methods can provide useful data although, so far, the enzymatic methods seem more reliable (Cuddeford, 2000).

Near infrared spectrophotometry (NIRS)

NIRS is a routine laboratory procedure that is used extensively to evaluate forages for ruminants. Andrieu *et al.* (1996) applied the NIRS method to 52 forages that had been evaluated *in vivo* and obtained a prediction of OMD with an $r^2 = 0.96$. They concluded that the NIRS method was as reliable as the enzymatic method. Thus, two techniques are available for determining OMD, which is a crucial component in the calculation of UFC values (Cuddeford, 2000).

Factors Affecting the Accuracy of* in vitro *results

- Repeatability will be influenced by accuracy of weighing, fineness of samples, maintenance of anaerobic conditions and the correct pH.
- The composition of micro organisms can change rapidly in a test tube to a composition adapted to the substrate, which is different to the parent population initially collected from the animal.
- End products will accumulate which can inhibit metabolic activities.
- Extraneous reactions that do not normally take place in the animal can occur.

Therefore it is strongly advisable to include feeds of known *in vitro* digestibility as standards with each batch to ensure reliable results (Schneider & Flatt, 1975).

Advantages and Applications of an* in vitro *method

- It is simple, inexpensive and requires standard laboratory equipment
- It requires a small quantity of test feed
- It can be applied to different botanical fractions of a plant
- It is used as a rapid screening or indexing technique for a large number of forage samples
- It can be used as an initial screening of cultivars in a plant-breeding programme (Schneider & Flatt, 1975).

1.6.1.3.3 Marker Techniques

Markers are classified as internal or external. External markers are added to the feed, given to the animal in a capsule or by drenching. Internal markers are natural "inert" components of the feed, for example, lignin, alkanes, silica and acid-insoluble ash (AIA). They are used in a variety of studies relating to digestion and these include (Schneider & Flatt, 1975):

1. The estimation of total faecal output where it is inconvenient or impossible to collect total excretion.
2. A determination of dry matter and nutrient intake of the grazing animal.
3. Flow rate of digesta through the digestive tract of an animal.
4. The partitioning of digestion in various segments of the digestive tract.

External and internal markers must have the following characteristics (McDonald, 1995):

- It must be inert, i.e. be neither absorbed nor metabolised in the gastro-intestinal tract.
- It must not be of metabolic origin.
- It must have neither pharmacological action on the tract nor any toxic effect in the body.
- It must have no appreciable bulk.
- It must be mixed properly with and remain uniformly distributed in the digesta.
- It must pass through the tract at a uniform rate.
- It must have physico-chemical properties and its determination must be accurate and easy throughout the digestive tract.

Situations in which indicators prove very useful include (Schneider & Flatt, 1975):

1. when feed intake is known but total faecal collection cannot be made.

$$\text{Faecal DM Output}\,(g/day) = \frac{\text{Indicator consumed}}{\text{Indicator concentration in faeces}} \qquad \textbf{Equation 10}$$

2. neither feed intake nor faecal output is known, but an estimate of digestibility is desired

$$\text{Digestibility of nutrient}\,(\%) = 100 - \left[100 \times \left(\frac{\%\,\text{indicator in feed}}{\%\,\text{indicator in faeces}} \times \frac{\%\,\text{nutrient in faeces}}{\%\,\text{nutrient in feed}}\right)\right]$$

Equation 11

3. neither feed intake nor faecal output is known and estimates of both intake and digestibility are required

$$\text{Dry Matter Intake}\,(g/day) = \left(\frac{\text{Faecal Output}}{\%\,\text{digestibility of DM}}\right) \times 100 \qquad \textbf{Equation 12}$$

1.7 Energy Evaluation Systems for Horses

In a seminal review of the nutrition of the horse in 1955, Olsson and Ruudvere summarized the energy requirements of the horse for maintenance computed according to different authors and the systems available. At that time, systems were based on starch equivalent, Scandinavian feed units, feed units for ruminants, Russian oat feed units and total digestible nutrients (TDN). Since that time, the TDN system developed in the USA (Morrison, 1937) has given way to one based on digestible energy (NRC, 1989) which is used throughout North and South America, the UK, Australia, New Zealand, South East Asia and parts of Southern Europe. Over the last 15 years, a new French net energy (NE) system has been developed and refined from that originally proposed (INRA, 1984). This system expresses the energy value of feeds in terms of horse feed units (Unite fouragire cheval; UFC); one feed unit is the net energy content (9.42 MJ) of 1kg barley for maintenance. Another NE system currently in use in The Netherlands has feed values based on NE for milk production in ruminants (Smolders, 1990). The Scandinavian feed unit (ScFU) continues to be used in Denmark, whilst in Finland, Iceland and Norway, the fattening feed unit (FFU) is in use. Both systems are based on digestibility trial values obtained with cattle, and ruminant digestible crude protein (DCP) values are used as well. Protein rich feeds have a higher energy value calculated in the ScFU than in the FFU (Staun, 1990). However, a feed or diet containing about 110g of digestible protein per ScFU has the same calculated energy value in both units. The absence of data derived from experiments with horses in the Nordic countries precludes the use of a special feed unit for horses. Sweden previously relied on a digestible energy (DE) system and is now using metabolizable energy (ME). In the former USSR, the energy value of feedstuffs is based on the system derived from Kellner (1926) and expressed as Russian oat feed units, where one unit (1 kg) corresponds to 0.6kg starch equivalent (Cuddeford, 2000). The former USSR also uses energetic feed units (EFU), equivalent to 10.46 MJ of ME (Memedeikin, 1990), which have been derived experimentally with horses. Spain, Portugal and Greece do not use any particular system, whereas Italy has moved from using the Leroy fodder unit to adopting the French UFC system in its entirety (Miiraglia & Olivieri, 1990). More recently, it has been proposed (Austbo, 1996) that the Nordic countries should adopt the French system as well. It is therefore quiet conceivable that, in the near future, there will be only two systems of horse feeding practiced and this will be based on the DE according

to the National Research Council (NRC, 1989) devised in the USA or NE (Vermorel & Martin-Rosset, 1997) as developed by INRA in France. In the meantime the ScFU is still in use together with the Russian oat feed unit.

At present there are three main ways to describe the energy potential of a horse feed: total digestible nutrients (TDN), digestible energy (DE) and net energy (NE). Each of these has been determined in a number of ways over the years with TDN becoming less popular recently. Further confusion results from the fact that two units of energy are in common use in the horse industry: the joule (J) predominantly in Europe and the calorie in the United States (4.184 J is taken to be equivalent to 1 calorie).

The energy value of a feedstuff, as well as the total diet, for the horse will depend upon the relative amounts of hydrolysable and fermentable substrates that it contains. Determination of a feed's energy value using *in vivo* methods, especially in the horse, tends to be time consuming, labor intensive, costly and often highly impractical. Therefore, as with many other species, effort has concentrated on finding methods for assessing energy values of feeds using prediction equations. At the moment these tend to be based on the chemical composition of the feed, which may not truly reflect its functional aspects (Harris, 2001).

1.7.1 TDN

TDN is simply a figure that indicates the relative energy value of a feed to an animal. It is ordinarily expressed in pounds or kilograms or in a percent (1 lb or kg of TDN per 100 lb or kg of feed). It is arrived at by adding the following together (Schneider & Flatt, 1975):

TDN = Digestible crude protein + Digestible crude fibre + Digestible N-free extract + (Digestible crude fat x 2.25) **Equation 13**

Total digestible nutrients are not an actual total of the digestible nutrients in the feed. Firstly, it does not include the digestible mineral content. Secondly, the digestible fat is multiplied by 2.25 before being included in the TDN figure. This step is necessary to allow for the extra energy value of fats compared to that of carbohydrates and protein. As a result of this step, feeds high in fat will sometimes exceed 100% TDN.

In the determination of total digestible nutrients, the feed and faeces are separated in a manner of speaking by empirical chemical analyses into component parts. The digestibility of each component is determined and according to their respective digestion coefficients, the parts are reassembled to constitute a total digestible nutrient value expressed on a weight basis. The underlying energy aspect of the process is recognized by giving the same weight value to digestible protein and carbohydrate and multiplying the digestible ether extract by 2.25 before including it in the TDN value (Swift *et al.*, 1950).

The determination of digestible energy avoids the tortuous method involved in obtaining total digestible nutrients and possesses the greater significance accruing to a more direct procedure. DE serves the same purpose but with an increased simplicity and accuracy. As a matter of fact the determination of total digestible nutrients may be looked upon as a laborious, cumbersome and inaccurate effort to determine the digestible energy. About 70 years ago Swift (1957) suggested that digestible energy be adopted in the place of TDN. Researchers were in complete agreement as to the superiority of this experimental procedure as being not only more easily and directly determined, but also more accurate as results were now expressed as calories. Pursuing the matter further, it was recommended to the Committee on Animal Nutrition by Swift (1948) that digestible energy be adopted as a routine measure of the potential value of feedstuffs in the place of TDN.

The energy content of rations has been calculated as the percent total digestible nutrients in a number of ways:

$$\mathbf{TDN = \%DCP + \%DNFE + (\%DEE \times 2.25)} \qquad \textbf{Equation 14}$$

$$\mathbf{TDN = 78.1 - (1.01 \times ADF\ \%) + (0.823 \times CP\ \%)} \qquad \textbf{Equation 15}$$

Certain factors affect the TDN value of a feed namely:

- The percent of dry matter in a feed: Water can in no way contribute in a positive way to the TDN value of a feed. The more water present in a feed, the less there is of other nutrients therefore the lower the TDN value. For example, silage is low in TDN compared to hay mainly because of a difference in the water content.

- The digestibility of the dry matter: Unless the dry matter of a feed is digestible, it can have no TDN value. Only digestible dry matter can contribute to TDN. Feeds high in fibre are, in general, low in digestibility and relatively low in TDN. Sand is an example of dry matter that is indigestible and so would have a zero TDN value.
- The amount of mineral matter in the digestible dry matter: Mineral compounds in an animals ration may be digestible, but they contribute no energy to the animal and so have no TDN value. The more mineral matter a feed contains, the lower its TDN value will be.

- The amount of fat in the digestible dry matter: The more digestible fat a feed contains, the greater the TDN value will be.

1.7.2 DE System (NRC)

The prediction of DE in diets has been approached by attempting to define the factors that influence these values. The approach has been twofold:

1) To investigate the relationship between the digestibility of nutrients, and DE content.

2) To determine whether DE can be predicted using regression equations involving the various chemical components of the diet.

Predicting digestibility is a basic step for energy evaluation in horse feed. In horses, as in all species where microbial fermentation plays an important role in digestion, interactions between feed ingredients are even more important than those in other species (Martin-Rosset & Dulphy, 1987). In horses, suggestions for systems of energy evaluation usually refer to all feedstuffs and they are not limited to certain life stages or groups. It is quite common that the same mixed feed may be used for a leisure horse and a performance horse but in very different rations. Under these conditions it is not surprising that interactions

between feedstuffs are widely neglected in predictive equations for digestible energy. Several equations have been suggested that work quite well in some situations but can cause serious errors in others (Zeyner & Kienzle, 2002). From a total of 287 digestion trials, Zeyner and Kienzle (2002) developed the following predictive equation:

DE (MJ/kg DM) = -3.60 + (0.211 x CP) + (0.421 x AEE) + (0.015 x CF) + (0.189 x NFE) (nutrients in % DM) **Equation 16**

This equation can be applied to mixed feed with satisfactory results. The DE content of rations with added fat and a total fat content of >5% as well as rations with a high content of highly fermentable fibre tended to be somewhat underestimated (Zeyner & Kienzle, 2002).

Most commonly the energy content of horse feed is referred to by its DE or digestible energy content. Using standard digestibility balance studies, the digestible energy content of a ration can be estimated *in vivo*. This does not provide a truly accurate measurement because faecal energy includes energy originating from endogenous sources as well as undigested feed and bacteria. It is nevertheless a very useful practical guide (Harris, 1999). However, this is an expensive and time consuming way to determine DE and so a number of equations to estimate DE content of feedstuffs have been devised. Examples of such equations include:

1. Fonnesbeck (1981)

$$DE(KJ/kg) = 255 + 3660 \times TDN \times 4.184 \qquad R^2 = 0.85$$

Equation 17

2. Pagan (1988)

$$DE(KJ/kg) = 2260 + 14.17CP\% - 11.48ADF\% - 4.88HC\% + 57.2Fat\% + 24.38NSC\% - 31.77Ash\% \times 4.184$$

$$R^2 = 0.88$$

Equation 18

3. Pagan (1998)

$$DE(KJ/kg)=2118+12.18CP\%-9.37ADF\%-3.83HC\%+ 47.18Fat\%+20.35NSC\%-26.3Ash\% \times 4.184$$

$R^2 = 0.88$

Equation 19

4. Harris (2001)

$$DE(MJ/kg\,DM)=11.1+0.0034CP\%+0.0158CF\%-0.00016CF\%^2$$

Equation 20

5. Zeyner and Kienzle (2002)

$$DE(MJ/kg\,DM)=-3.60+(0.211\times CP)+(0.421\times AEE)+(0.015\times CF)+(0.189\times NFE)$$

Equation 21

6. Martin-Rosset *et al.*, (1994)

$$DE(MJ/kg\,DM)=\frac{GE\times dE}{100}$$

Where: $dE = 0.0340 + \Delta 0.9477 dOM$

$DOM = 78.33 - 0.0746CF$

Δ = -1.1 for forages

Δ = =1.1 for concentrates

Equation 22

The NRC originally used TDN to describe the energy content of feeds for horses. These units can be converted to DE since 1 kg of TDN is equivalent to 18.4 MJ of DE (NRC, 1989). This conversion factor is based on work with ruminants; the validity has been questioned particularly because of the results that were obtained in a small study with ponies (Cuddeford, 2000). Barth *et al.* (1977) estimated 19.246 MJ / kg for a hay or hay-concentrate diet, which is 1.05 times greater than the proposed factor. Now, however the

NRC tables use DE expressed as Mcal. The *raison d'etre* for the NRC (1989) using DE is that few data exist for ME or NE values of horse feeds. Although it is preferable that DE values are determined by *in vivo* experimentation, equations are becoming available for prediction of DE (Pagan, 1994). More recently, new technologies, such as *in vitro* gas production techniques have been developed and the data have been used to predict feed values (r = 0.72; Lowman *et al.*, 1997).

1.7.3 The French Net Energy System

The DE system tends to overestimate the actual mechanical energy potential of a high fibre feed compared with a highly soluble carbohydrate feed, as fibre predominantly produces VFA's which are not used as efficiently as glucose.

The NE system was introduced in France during 1984 by INRA (Institut National de la Recherche Agronomique) because the DE system was considered to overvalue high-fibre feeds. Classic experiments by Wolf *et al.* (1877) showed that more digestible energy (15%) was required to be fed to the horse when its diet consisted of 75% hay as compared to when fed a 75% cereal diet. Vermorel and Martin-Rosset (1997) showed that the DE requirements for work were 25% higher when hay was fed compared to grain. A calorimetric model was developed to derive heat production for the utilization of different diets by horses, this showed that fibre is more thermogenic and that replacement of fibre with cereal reduced the yield of heat. Thus it follows that fibrous foods will be used less efficiently by horse (Kronfield, 1997).

The French net energy system is based on two concepts:
1) Maintenance is the major component of energy expenditure in the majority of horses (50-90%) (Martin-Rosset *et al.*, 1994).
2) The net energy value of nutrients for both maintenance and work depends on the free energy (ATP) produced by oxidative catabolism (Vermorel *et al.*, 1984; Vermorel and Martin-Rosset, 1997).

This NE system uses the horse feed unit (HFU) or in French, l'unite fouragire cheval (UFC). The UFC corresponds to the net energy value (2250 kcal) of one kilogram of standard barley (87% DM) for a horse at maintenance. The UFC value of a particular feed is calculated by dividing its NE content by that of barleys net energy content.

The NE value of feeds is calculated through a stepwise procedure from:

1) The gross energy (GE) content;
2) The digestible energy (DE) as measured in horses;
3) The ratio between metabolizable energy (ME) and DE as determined in horses;
4) The efficiency of ME utilization for maintenance (k_m)

$$\mathbf{NE = ME \times k_m} \qquad \textbf{Equation 23}$$

or

$$\mathbf{NE = (DE \times ME) / (DE \times k_m)} \qquad \textbf{Equation 24}$$

The k_m is calculated from the energy cost of eating, the assumed proportions of absorbed energy supplied by the various nutrients as well as the efficiencies of nutrient energy utilization.

For concentrate feeds:

$$\mathbf{K_m = 0.85\ E\ (\mathit{Gl}) + 0.80E\ (\mathit{LCFA}) + 0.70E\ (\mathit{AA}) + (0.63\ to\ 0.68)E\ (\mathit{VFA})} \qquad \textbf{Equation 25}$$

For forages:

$$\mathbf{K_m = 0.85E\ (\mathit{Gl}) + 0.80E\ (\mathit{LCFA}) + 0.70E\ (\mathit{AA}) + (0.63\ to\ 0.68)E - 0.14\ (76.4 - \mathit{ED})\ (\mathit{VFA})} \qquad \textbf{Equation 26}$$

Where E = % of absorbed energy supplied by glucose or lactate (Gl), long chain fatty acids (LCFA), amino acids (AA) and volatile fatty acids (VFA).

ED = Energy digestibility (%) of the feed

As a result, NE content of a reference feed, such as Barley in Europe, accounts for 2.250 Mcal/kg fresh materials for a horse at maintenance.

The UFC system is an empirical model for predicting the NE value of feeds for horses. It may not give the true energy values of feeds but the values are closer than the DE system

would predict. It has been well demonstrated that methane and urinary losses as well as the utilization of ME for maintenance (or fattening) vary with diet composition in horses as with other species. In a DE system, the energy of protein rich feeds and forages is overestimated (by approximately 15% for cereal by-products, 25-30% for oil meals and 30-35% for hays), whereas those feeds rich in starch are underestimated (Table 3) (Vermorel & Martin-Rosset, 1997).

Table 3 *Energy values of feeds related to that of Barley in a DE system and in the UFC system. Relative differences between the two systems for each feed (Vermorel and Martin-Rosset, 1997).*

As fed	DE System	UFC System	DE as % NE
Maize starch	116	131	88
Maize	111	115	96
Wheat	108	109	99
Barley	100	100	100
Oats	90	85	105
Wheat Bran	88	77	114
Soyabean Meal (45% CP)	113	91	124
Lucerne Hay	70	54	130
Good quality grass hay	64	49	132
Bad quality grass hay	49	35	137

The NE value of feeds (in Mcal or UFC) can be predicted accurately from either tabulated values stated by the INRA or from laboratory analysis. The accuracy can be improved by using the digestible organic matter (DOM) content of feeds which can be estimated by the pepsin cellulose method (Martin-Rosset *et al.*, 1996) or by the near infrared spectrophotometric method (Andrieu *et al.*, 1996). The gas production technique using fecael innocula may appear promising (Lowman *et al.*, 1997), but this method has proved less reliable as indicated by recent studies (Macheboeuf *et al.*, 1998).

The nutrients available to the horse will reflect the chemical composition of the feed that it consumes and the site of digestion. Feeds with the same or similar DE value may although contribute very different absorbable nutrients. Vermorel and Martin-Rosset (1997) have tabulated the quantitive nutrients supplied from different feeds. Some values were

measured whilst others were assumed from studies with pigs and ruminants. Enzymatic digestion in the small intestine accounted for 85% of starch consumed, 5% of cell wall of dried grass and hays, 8% of fresh grass, 10% of lucerne and 15% of concentrates. Between 90% and 95% of apparently digested ether extract was long chain fatty acids (LCFA's) absorbed from the small intestine (Cuddeford, 2000). Jarrige and Tisserand (1984) estimated true digestibility of true protein pre-caecally to be about 8% for cereals and oil meals, 70% for fresh grass, 60% for dehydrated lucerne and 30-45% for hays, the last value depending on the stage of growth at harvest. Undigested feed residues that leave the ileum are subjected to microbial degradation. Based on ruminant data it was deduced that methane and heat of fermentation amounts to some 8% of the energy of fermented substrates. Furthermore, they estimated volatile fatty acid (VFA) production as follows:

VFA (g/kg DM) = OMD – OM digested in the small intestine x 0.92 **Equation 27**

The molar proportions of VFA's produced reflect the nature of the feed degraded; with high-fibre feeds resulting in high molar proportions of acetate (Cuddeford, 2000). Vermorel and Martin-Rosset (1997) were able to predict acetate (C_2) production as follows:

Acetate (%) = 0.54 CF (%DM) + 57 $r^2 = 0.8$

Equation 28

Vermorel and Martin-Rosset (1997) have shown that the k_m values for nutrients absorbed from the small intestine should be similar to those in monogastrics and for VFA's be the same as in ruminants.

Absorbed energy will reflect the sum of the gross energies of the individual nutrients that are absorbed. Individual values (KJ/g) used for glucose/lactate, acetate, propionate, butyrate, amino acids and LCFA's were respectively 15.65, 14.60, 20.76, 24.94, 23.44 and 39.76. Absorbed end products of digestion and their relative contribution in energy terms are shown in Table 4 (Cuddeford, 2000).

Table 4 *Estimated end products of digestion (g/kg of DM), the proportion of absorbed energy (AE) they contribute and derived k_m values (Vermorel & Martin-Rosset, 1997).*

End-products of digestion (g/kg DM)					
Substrate	Glucose	Amino Acids	Long Chain Fatty Acids	Volatile Fatty Acids	K_m value
Maize	628	53	32	174	0.80
AE	0.63	0.08	0.08	0.21	-
Oats	390	63	47	188	0.78
AE	0.48	0.11	0.15	0.26	-
Good Hay	74	49	12	387	0.65
AE	0.12	0.12	0.05	0.71	-
Poor Hay	40	21	5	348	0.61
AE	0.09	0.06	0.03	0.82	-

It is clear that feeds degraded in the small intestine contribute the most energy via glucose and lactate, with a resultant higher efficiency of utilization. In contrast, those feeds degraded in the large intestine provide energy via VFAs with a much lower overall efficiency of utilization. K_m is reduced further by the energy cost of eating roughages. Based on the above values of absorbed energy, k_m (%) was calculated as follows:

$$\mathbf{K_m = 0.85\ E_{GL} + 0.80\ E_{LCFA} + 0.70\ E_{AA} + (0.63\ to\ 0.68)\ E_{VFA}} \qquad \textbf{Equation 29}$$

The energy cost of eating remains to be considered. Orskov and Macleod (1990) have shown in ruminants that this is a significant factor affecting the utilization of feed energy. Experiments with ponies (Vermorel and Mormede, 1991) and horses (Vernet *et al.*, 1995) showed that the energy cost of eating per kg of feed DM was much greater than when measured in sheep (Osuji *et al.*, 1975). The physical form and nature of the diet has a large effect on energy expenditure during eating. When 300kg steers were fed long roughage, they expended 6 MJ/day compared to only 0.8 MJ/day when fed pelleted diets. In ponies there is a reciprocal relationship between OMD and energy cost of eating (Cuddeford, 2000). Vernet *et al.* (1995) measured the cost of eating hays and pelleted maize and showed that the former averaged 0.1 of ME intake compared with only 0.01 for the maize. It was

found that km values were lower than those calculated from the supply of nutrients, therefore correction factors (Δkm) were developed which depend on:

1. Digestibility of energy:

$\Delta k_m = -0.14\ (76.4 - DE\ \%)$ **Equation 30**

2. Fibre content of the DM:

$\Delta k_m = -0.20\ CF\% + 2.50$ **Equation 31**

These correction factors are applied to Equation 30 for roughages in order to calculate k_m values.

1.7.4 Comparison of TDN, DE, ME, and NE as measures of a feed's energy value for an animal

Total digestible nutrients, digestible energy, metabolizable energy, and net energy have been used for years as methods of expressing the energy value of different feeds and rations for feeding purposes. Of these, DE is probably the least precise measure of a feed's energy value to an animal, however in South Africa this will be our first step to the advancement of equine nutrition in our country. It embraces all of the energy of a feed that does not appear in the faeces but makes no allowance for other energy losses during the digestion and utilization. TDN is superior to DE in this regard since in attributing the same value to digestible protein as is attributed to digestible carbohydrates in calculating TDN, an approximate correction is effected for that part of the protein energy that is excreted in the urine. Whereas digestible carbohydrates have a DE value of approximately 17.36 kJ per gram, digestible protein has a DE value of approximately 23.64 kJ per gram. However, the two are of similar energy value to the animal and are so considered by the procedure followed in calculating TDN. ME figures, on the other hand represent a more accurate measure of the energy value of a feed to an animal, than do either DE or TDN figures. In determining the ME of a feed, allowances are made not only for the energy losses in the urine but also for those of fermentation gases. Although TDN takes into account a correction for the energy losses of urine, both TDN and DE values fail to take into account the energy losses of the fermentation gases (Perry, Cullison & Lowrey, 1987).

1.7.5 Limitations of the DE and NE Energy Systems

The net energy system has a number of potential advantages, but at the moment an English version of all the tables and factors used is not readily available. Most countries use the DE system and the DE values for horse feedstuffs are more routinely available. It is assumed that the horses needs for different functions, such as work and maintenance, are additive. Thus the daily energy or protein need is arrived at using a factorial approach, summing the needs for maintenance, growth, etc. The INRA system (INRA, 1990) assumes the efficiency with which ME is used for different functions is the same as that for maintenance (k_m). This means, for example, that the utilization of ME for maintenance is the same as that for work ($K_m = k_w$); however, the increased heat production associated with work may well invalidate this assumption.

One UFC has a NE value of 9.414 MJ and is equivalent to 1kg of 'standard barley' of 870g of DM/kg given to horses at maintenance. The reason for using maintenance as the base is that 0.50-0.90 of total energy expended by horses is for maintenance purposes in lactating and pregnant mares, respectively (Doreau *et al.*, 1988), 0.60-0.90 in growing horses (Agrabriel *et al.*, 1984) and 0.70-0.80 in working horses (Martin-Rosset *et al.*, 1994). In relation to growing animals, Vermorel and Martin-Rosset (1997) showed that only 0.20 of the total energy requirement is used for growth in light breeds, the balance being used for maintenance. The greatest differences in nutritive value between horse feeds are due to differences in OMD and are thus related to cell wall content or neutral detergent fibre (NDF). This is very relevant since requirements are expressed in terms of DE or UFC. Table 5 illustrates the significance of this.

The NE of barley is deduced from the assumption that it contains 16.13 MJ of GE/kg and has a DM of 870g/kg. The NE is determined by a step-wise procedure assuming the following relationships: DE/GE = 0.80, ME/DE = 0.931 and NE/ME = 0.785. The validity of using these conversion factors has been questioned (Harris, 1997) particularly as the NE value is assumed to be constant for whatever purpose the food energy is being used. The function of the animal, environment, nutrient content of the ration and other factors will affect the NE value of the food. For practical rations composed of 0.75 forage and 0.25 concentrate, Tisserand (1988) suggests the following relationships between energy values: DE/GE = 0.67-0.83, ME/DE = 0.83-0.91 and NE/ME = 0.63-0.80. With this magnitude of variation, it is difficult to accept the UFC values are additive (Martin-Rosset & Dulphy, 1987 No account is taken of food interactions, in other words how different feeds and the way in which they are fed affect the site of digestion, extent of digestion and the resultant nutrient uptake. It has been demonstrated (Potter *et al.*, 1992) that both the origin of starch and the manner in which it has been processed affect the site of digestion and the magnitude of pre-caecal digestion; the latter will have a major impact on whether glucose or VFA is the substrate for subsequent ATP production.

The NE system relies on the fact that maintenance requirements for energy account for the largest part of the total energy requirements, which may not be true for certain performance animals. The use of an NE system also implies amongst others that the NE requirements of a horse have been accurately described but this is not the case; validation experiments have only been conducted at or near maintenance (Vermorel & Vernet, 1991).

Certain of the equations used to predict ME and therefore the NE values of feeds, appear to have very low correlation values ($r^2 = 0.45$). However, it is possible, as more information becomes available, that this system will become more generally applicable and widely used (Harris, 1999).

Table 5 *DE (MJ/kg DM) and UFC (KJ/kg DM) values of some common feeds and as a proportion of maize (Cuddeford, 2000).*

Feed	DE	DE feed/DE maize	UFC	UFC feed/UFC maize
Maize	16.1	1.00	1.33	1.00
Barley	14.5	0.90	1.16	0.87
Oats	12.5	0.78	0.99	0.74
Beet Pulp	12.5	0.78	0.86	0.65
Lucerne	10.2	0.63	0.60	0.45
Grass hay	7.4	0.46	0.44	0.33
Barley straw	6.5	0.40	0.36	0.26

The NRC (1989) maintenance energy requirements were calculated on the basis of experiments with four animals (Pagan & Hintz, 1986) and from feeding trials, which were the basis for NRC (1978). These feeding trials also provided the foundation for the INRA system (INRA, 1990), which subsequently has been validated using calorimetry.

In summary, the DE system relies on digestibility as being the most important factor for discriminating between feeds. The NE system separates concentrates and roughages and is based on end-product usage. As well as considering the efficiency of utilization of these end-products (glucose, lactate, VFA's etc.), the NE system quantifies the impact of the energy costs of mastication, propulsion of food through the gut and heat of fermentation on k_m. For example, the k_m for barley might be 0.79 compared with only 0.60 for grass hay (Cuddeford, 2000).

Hintz and Cymbaluk (1994) noted that direct comparisons between the NRC and INRA systems couldn't be made easily or without several assumptions. Furthermore the calculations, based on either system, when expressed in feed rather than in terms of DE, UFC's, etc. produced similar values. They took the example of a 500 kg lactating mare and, in spite of using different energy units, the final rations were similar. Frape (1998)

compared the two systems for ration formulation and concluded that: (i) expected selection against poor hay by the NE system did not occur; (ii) the DE system generally assumed higher requirements for different functions which offset the higher values given to hay; and (iii) a change in assumed feed intake of working horses had a large effect on ration composition with either system.

The INRA system is flexible and can be modified and updated as new information comes available, although, at present, its complexity compared with the NRC does not appear to confer any major advantage (Cuddeford, 2000).

1.8 Comparative Digestive Physiology

The horse's digestive physiology has on many occasions been compared to that of the ruminant. Smith (1965) suggested that the flora of the caecum of the horse and the rumen of the sheep and the cow were quantitatively similar, that is, they consisted of the same types of organisms. Due to lack of information in equine nutrition, Morrison (1960) utilized digestion coefficients obtained from ruminants for fibrous components, common to both species, as a guide in compounding diets for equines. Ruminants and horses eat the same type of feed but the digestive tracts of the two species are very different in the way in which feed is broken down and absorbed into the bloodstream and used by various organs and body tissues. Ruminants are often referred to as "anterior fermenters" which simply means that the food is pre-digested by the microorganisms in the rumen before the pre-digested food enters the stomach. The horse is a "posterior fermenter" in that the micro-organisms act on the food in the large intestine after preliminary nutrient absorption has started in the horse's single stomach (Kerrigan, 1994). For compound feeds and compound feed ingredients Smolders *et al.*, (1990) has shown that horse digestibility data were on average comparable to sheep digestibility data allowing for considerable differences between feeds. He also showed that *in vitro* digestibility with rumen fluid offers a reliable estimate of OM digestibility in horses for the different groups of feeds. By conducting comparative feeding trials it can be deduced how accurate it is to use ruminant data for the formulation of horse rations.

The rapid assay method has proved to be an outstanding innovation in the evaluation of poultry and pig feeds. By using an experimental bird the metabolizable energy value can be determined by the process of tube feeding. Using this concept one might investigate the potential of finding a pilot animal, such as the miniature horse, rabbit and sheep which could be used for a rapid assay of horse food.

Slade and Hintz (1963) have found no significant differences between the pony and the horse in digestion of either alfalfa or alfalfa-grain pellets (Table 6). However, the pony tended to be more efficient in digesting both diets. The miniature horse is a "scaled down version" of a large horse with the same nutritional requirements but obviously on a much smaller scale. Whether or not miniature horses can be used as a pilot animal for large horses, will be determined by this experimental work.

Table 6 *Composition and Digestibility of Alfalfa and Alfalfa-Grain Diets (Slade & Hintz, 1963)*

Composition	**Organic matter %**	**Crude protein %**	**Ether extract %**	**Crude fibre %**	**NFE %**	**Ash %**	**Energy MJ/kg**
Alfalfa	90.1	19.7	1.8	25.2	43.443.4	9.9	18.56
Alfalfa-grain	91.6	16.7	2.0	17.8	55.1	8.4	18.29
Digestion Coefficients							
Alfalfa							
Horse	60.4	74.0	-6.4	34.7	71.5	...	56.9
Pony	62.5	76.2	-19.0	38.1	73.9	...	58.3
Rabbit	54.3[c]	73.7	23.6	16.2[c]	68.7[c]	...	51.8[b]
Guinea Pig	62.8	69.0[b]	14.7	38.2	76.0	...	59.4
Standard dev	*1.4*	*1.4*	....	*2.5*	*1.4*	...	*1.7*
Alfalfa-grain diet							
Horse	71.1	77.3	33.5	38.6	80.6	...	67.4
Pony	72.4	79.6	27.4	40.9	81.6	...	68.9
Rabbit	65.2[c]	73.2	46.0	18.1[c]	79.0	...	62.0[b]
Standard dev	*1.2*	*1.8*	....	*2.6*	*1.0*	...	*1.3*

[a] Dry matter Basis

[b] Means are significantly different ($P<0.05$)

[c] Means are significantly different ($P<0.01$)

According to Table 6, rabbits were significantly less efficient in digesting organic matter and energy than the other animals, primarily due to a much lower digestion of crude fibre. Guinea pigs were significantly more efficient in the digestion of nitrogen-free extract of alfalfa, but were significantly less efficient in the digestion of crude protein than were horses, ponies and rabbits.

Horse, ponies and rabbits digested the organic matter, NFE and energy in the mixed ration more efficiently than in alfalfa. However, there were no differences in the digestion of crude protein and crude fibre.

Guinea pigs digested the organic matter and crude fibre as efficiently as the horses and ponies, whereas the rabbits did not, being only about 48% as efficient in digesting crude fibre. Loosli *et al.* (1939) and Hintz (1961) also reported that the guinea pig was more efficient than the rabbit in the digestion of crude fibre. A recent review has shown that the crude fibre digestion coefficients obtained with rabbits were about 50 to 60% of the values obtained with horses (Hintz, 1968). On the other hand, guinea pigs were less efficient in the digestion of crude protein than were horses and ponies, whereas rabbits were similar. (Slade & Hintz, 1963). Due to the observed differences, more comparative studies are needed to be carried out so as to determine the extent to which rabbits and guinea pigs are suitable for use as pilot trial animals for evaluating horse feeds.

Three problems arise when transferring nutritional data from other species to the horse.

1. The data for other animal species is based on the measurement of an economic factor, e.g. milk yield, growth rate etc. Apart from growth rate in the horse, most of the ideals, which people are trying to attain with their horses, cannot be defined in quantitative or mathematical terms. Horse people know what they want with their animals but are unable to define, in quantitative terms, just what the requirement is.
2. The energy requirements of a horse can change continually, dependent on the current exercise programme and the eventual use of the horse. No reasonable horse trainer would ever feed the same amount of grain to a racehorse at the start of a training programme as he would at the peak of a training programme.
3. Horses are generally fed as individuals, each with its supposed specific nutritional needs, however most feed energy values are based on application to large numbers of animals all being fed basically the same, e.g. steers in a feedlot (Kerrigan, 1994).

Progress in comparative nutrition offers ample evidence that each species is not a special creation functioning in its own peculiar manner, but rather that the similarities are far more striking than the differences (Guilbert & Loosli, 1951). In Tables 8, 9 and 10 the digestive efficiencies of the horse, rabbit and ruminant are summarised. These similarities and differences will be studied in more detail in Chapter 3

The digestive system of the horse is unique in many aspects:

1. The upper part of the digestive system of the horse is much like that of the monogastric animal.
2. The hindgut contains a large fermentation organ, the caecum, which has fermentation capabilities much like that of the ruminant animal.
3. Both the rumen and the caecum contain bacteria that can break down cellulose; however, in ruminants the feed is fermented at the beginning of the digestive tract, whereas in the horse it is fermented at the end of the digestive tract.
4. There is a difference in eating rate between ruminants and horses. Whereas the ruminant, with its very large rumen, can eat feed rapidly and store it in its rumen for leisurely rumination later, the horse must eat more slowly. They must not force feed through the digestive tract too rapidly before digestion is complete as this can cause digestive upsets.
5. Forcing food too rapidly through the digestive tract of the horse causes undigested feed to enter the large intestines, with the result that starch residues are fermented too rapidly, causing excessive production of gases (Perry, Cullison & Lowrey, 1987).

The horse is classified as a non-ruminant herbivore (Table 7). The easily digested food material is first hydrolysed by the action of digestive enzymes, while the insoluble material, which is mainly cellulose, reaches the large intestine for bacterial fermentation.

***Table** 7 The major structural sections, capacity and function of the digestive system of a 500kg horse (Kohnke, 1998).*

Organ	Volume (L)	Length (m)	Passage Time	Digestive Activity
Stomach	7.5-15 L	0.25m (20-25cm diameter)	Water: 75% in 30 mins Dry Food: 25% in 30 mins and 98% in 12 hours	Some protein digestion by acid Partial feed breakdown Soaking feed mass with gastric fluid and saliva
Small Intestine	40-50L	15-22m (7-10cm diameter)	Water: 2-8 hours Food: 1-8 Hours	Major fat and protein digestion Carbohydrate 50-70% Most vitamins and minerals No fibre
Large Intestine Caecum	25-30L	0.9-1.2m (15-25cm diameter)	Water: 5 Hours Food: 6-12 hours	Fibre 50% residual carbohydrates
Large Colon	50-60L	3.0-3.7m (20-25cm diameter)	*Relative Passage Times* Fresh Grass: 24-36 hours Concentrates: 24-36 Hours Pellets: 24-36 hours Hay: 50-60 hours	Fibre Water absorption Remaining carbohydrates
Small Colon	18-19L	3.0-3.2m (7.5-10cm diameter)		Fibre Water absorption
Rectum	2-3L	0.3m (6-7cm diameter)		Faecal storage
Total	143-177L	23-31m	Total Transit Time= 78 hours	

Table 8 *Comparison of Digestive Efficiency in Horses and Rabbits*[a]

Nutrient	Diet 1		Diet 2		Diet 3	
	Horse	**Rabbit**	**Horse**	**Rabbit**	**Horse**	**Rabbit**
Dry Matter	70.0	47.4	60.4	54.3	63.4	61.8
CP	53.0	80.2	74.0	73.7	74.1	65.9
Crude Fibre	...	...	34.7	16.2	...	...
Cellulose	...	...	...	...	51.2	25.5
Starch	...	...	...	...	98.0	96.8
ADF	47.5	25.0	...	...	...	...
NDF	68.9	36.7	...	...	...	...
Energy	79.9	49.3	56.9	51.8	...	...

[a] Given as a percentage. Diet 1, whole corn plant pellets (Schurg *et al.*, 1977); Diet 2, Pelleted alfalfa meal diet (Slade & Hintz, 1969); Diet 3, complete Pelleted diet (Wolter *et al.*, 1980).

Table 9 *Comparison of Digestibility Coefficients in Guinea Pigs, Rabbits, and Equines Fed a Pelleted Alfalfa Diet*[a]

Species	**Organic Matter**	**Crude protein**	**Ether Extract**	**Crude Fibre**	**NFE**	**Energy**
Horse	60.4	74.0	-6.4	34.7	71.5	56.9
Pony	62.5	76.2	-19.0	38.1	73.9	58.3
Rabbit	54.3[b]	73.7	23.6	16.2[b]	68.7	51.8[b]
Guinea Pig	62.8	69.0[b]	14.7	38.2	76.0	59.4

[a] Adapted from Slade and Hintz (1969)

[b] Significantly lower than other values ($p < 0.05$)

Table 10 *Mean digestibility of organic matter of various feedstuffs in horses and ruminants (Loewe & Meyer, 1974)*

Diet	**Horse (%)**	**Ruminant (%)**
Straw	35	50
Hay	50	55-60
Green forage	65	70
Oat	70	70
Other cereals	80-85	80-90
Fodder beet	85	85-90

1.9 Rate of Passage through the Digestive Tract of the Horse

Rate of passage of digesta is the measure of how long individual portions of digesta are retained in the gut subject to the processes of mechanical mixing, digestion, microbial fermentation and absorption (Warner, 1981).

Rate of passage of feedstuffs through the digestive tract of the monogastric and ruminant animal has been studied in great detail using markers such as chromic oxide, carmine, carbon granules and Styrofoam. McCarthy *et al* (1974) evaluated the use of AIA as a natural marker for determining digestibility of rations in growing pigs and concluded that the AIA method was superior to chromic oxide as a marker in pig diets. Care must however be taken when faecal samples are taken off the ground so as to avoid contamination with soil, dust or bedding material.

Along with rate of fermentation, the mean retention time (MRT) of feeds within the gastrointestinal tract (GIT) is of utmost importance in determining the extent of feed digestion and efficiency of microbial synthesis in herbivores. Digestibility is the product of the retention time and the degradation characteristics of a foodstuff (Forbes, 1996). The longer a foodstuff stays in the rumen, the greater the amount of digestion possible; therefore it would be possible to be digested to the maximum extent possible.

Mathematical modelling of faecal excretion data using indigestible external markers is a non-invasive method that can be used to obtain digesta passage rate and mean retention time (MRT) in animals. These models simulate aspects of the digestive system therefore allow a greater understanding of digesta kinetics. Mathematical models can be categorised into time-independent models (Grovum & Williams, 1973; Dhanoa *et al.* 1985) and time-dependent models (Moore-Colyer *et al.* 2003). Time-independent models are deterministic in nature and assume that digesta flows irreversibly through a fixed number of sequential compartments according to first order kinetics (Lalles *et al.*, 1991). Time-dependent models are based on assumptions of probability (stochastic models) and use γ-functions (non-exponential residence time distributions) to describe the time-dependent passage of digesta through different segments of the gastrointestinal tract. Due to the flexibility of this model it has allowed it to be successfully fitted to ruminant animal faecal excretion data where the more rigid time-independent models have failed (Pond *et al.*, 1988). Digesta

passing through the caecum and large colon must pass through narrow flexures as it flows through right and left ventral, to left and right dorsal chambers. While mixing may occur quickly it is very unlikely that it will follow first order kinetics, therefore digesta passage through the equids digestive tract would be a time-dependent process (Moore-Colyer, 2000).

Length of collection period in horses as determined by rate of passage trials has been reported as 4 days by Vander Noot *et al* (1967), 5 days by Paterson (1879), 7 days by Nicholson and Friend (1965), 8 days by Lindsey *et al* (1926), and 12 days by Lathrop and Bohstedt (1938).

When indicators such as chromic oxide or coloured particles are added to hay-grain diets, about 10% of the indicator is excreted in 24 hours, 50% within36 hours and 95% within 65 hours (Alexander, 1946). The physical form of the diet can influence the rate of passage. Pelleted diets have a faster rate of passage than chopped or long hay (Hintz & Loy, 1966). Fresh grass moves more rapidly through the tract than does hay. Particle size will also influence the rate of passage in a horse (Olsson & Ruudvere, 1955) (Table 11).

Table 11 *Mean retention time (h) of digesta in horses fed various diets and measured with different markers*

Diet	Marker	Mean retention time (h)	Reference
Chopped oaten hay/concentrates	Cr-EDTA (fluid)	22	Orton *et al* (1985)
Alfalfa hay	Chromic oxide	38	Vander Noot *et al* (1967)
Alfalfa chaff/barley grain	Styrofoam particles	33	Hintz and Loy (1966)
Meadow hay	Coloured beads	36	Wolter *et al* (1974)
Chopped meadow hay	Coloured beads	25	Wolter *et al* (1974)
Pelleted meadow hay	Coloured beads	29	Wolter *et al* (1974)
Alfalfa hay/grain	Coloured beads	29	Wolter *et al* (1976)
Timothy hay	Co-EDTA (fluid)	18	Uden *et al* (1982)
Timothy hay	Cr-mordanted fibre (particles)	23	Uden *et al* (1982)

1.10 Conclusion

It is evident from the literature that the feed industry needs a conclusive assessment of the range of ingredients available for incorporation into finished feeds. The variation in composition and nutritional value that occur between batches of the same raw ingredient needs to be reduced. It is well known and expected that data on feeding stuffs produced in different locations are inadequate. Awareness of this problem is the first step towards finding a solution. A national effort to obtain new data applicable to local horse feeds may be a means of developing the equine industry in our country.

Whatever feeding system we used for horses, it cannot be effective if we do not understand the dynamic process of digestion within the horse's gut.
Measurement of degradation rates *in situ* together with the use of markers to measure the rate of digesta passage will contribute to the production of a model for digestion in the horse. The recent use of *in sacco* techniques (Hyslop & Cuddeford, 1996; Macheboeuf *et al.*, 1996; Longland *et al.*, 1997; Tomlinson, 1997) has complimented *in situ* studies and has contributed information on nutrient disappearances in different parts of the digestive tract. With these new methodologies, it is now possible to quantify the digestive process within the horse under different feeding regimes. The uses of *in vitro* techniques that rely on faecal innocula (Lowman *et al.*, 1996) provide new, non-invasive methods for further characterization of feeds.

The four main components of a rational feeding model (McDonald, 1995) are:

1. Metabolic model
2. Feed database
3. Analytical service
4. On-farm management facilities

The DE system relies on digestibility as being the most important factor in discriminating between feeds. The first three components of a rational feeding model for the horse have been discussed already, and it remains that our ration evaluation methods be evaluated in the context of on-farm management and facilities.

In simulation of the horses natural, historic, and therefore most efficient feed utilization schedule, i.e. trickle feeding, one might consider that an energy (nutrient) evaluation model would rely most heavily on those factors that are intrinsic to the provision of feed to a horse. For this reason, the DE system of digestibility relying on the feeding management in equine athletes, will reflect most on the optimal means of provision of nutrients and should therefore be explored most beneficially to the welfare and performance of the equine athlete.

Against this background of development, it is feasible to produce 'designer' diets for which it will be possible to predict the site of digestion and subsequent nutrient availability. For example, horses used in flat racing require glucose as a substrate for energy storage and release and would thus benefit from feedstuffs that are degraded pre-caecally. In contrast, horses used for endurance competitions require 'slow-release' energy which could be made available from the large intestine in the form of VFAs; appropriate mixes of raw materials would meet these diverse needs (Cuddeford, 2000).

A deficiency or over-abundance of some nutrients can also limit horse performance or production. Balancing the diet of a horse can only be done if an accurate energy value is available of the various feedstuffs. Until that time we will continue to be feeding the horse without the accuracy that is needed to ensure we are meeting the exact requirements of the animal without over or underfeeding energy.
Horse owners, veterinarians, industry and nutritionists need to continue to work together to improve the health and performance of horses.

CHAPTER 2

PART A

THE DEVELOPMENT OF A DIGESTIBILITY TRIAL PROTOCOL FOR HORSES IN SOUTH AFRICA

A Preliminary Trial to Evaluate the Accuracy of Using Miniature Horses to Replace Large Horses In Digestibility Trials

2.1 Introduction

The prediction of digestibility is the basic step in energy evaluation of an animal feed. As any large animal is more difficult to work with as well as more costly than a smaller animal, it was decided to purchase miniature horses to evaluate their similarity in digestibility to large horses so to determine whether or not they could be used as a pilot animal in equine digestibility studies. The aim of the experiment was to test the use of miniature horses in digestion stalls as a method of providing reliable digestibility results, thereby designing an equine digestion trial protocol applicable for use by feed companies in South Africa. Countries such as America and France have well developed energy evaluation systems using large equines, in operation in their countries. Lack of any local equine nutrition literature or results from experiments performed under our conditions using locally produced feeds led us to conduct this preliminary trial so as to provide data that could be used as a starting point for further investigations.

As a concentrate cannot constitute the entire diet of the horse, the digestibility of the roughage needs to be calculated by that of the 'digestibility by difference' method (Schneider & Flatt, 1975), to calculate the digestibility of the concentrate in the second collection period. During the first trial collection period, the digestibility of the roughage (*Eragrostis Curvulae*) was determined. This was followed by a second trial collection period whereby the digestibility of the concentrate was determined. Each of the digestion trials consisted of a preliminary period where the animals' digestive processes could become adapted to the specific ration they would be fed and a collection period during which faeces were collected and intake of feed dry matter was kept constant. The

preliminary period was seven days in length and the collection period was five days in length. During the second collection period a marker (Celite ®) was added to the concentrate portion so as to measure the rate of passage of the hay and concentrate through the digestive tract of the miniature horse.

Therefore the main aims of this experiment were:

- To determine the precision of miniature horses as predictors of digestible energy (DE) for large horses,
- To compare the results of this trial with literature,
- To identify the possible problem areas in digestibility experiments with horses.

This preliminary investigation is included so that a better understanding of the methodologies employed in Chapter 3 can be obtained.

2.2 Materials and Methods

2.2.1 Animals and Housing

The *in vivo* digestibility trial and rate of passage trial was carried out simultaneously at Ukulinga Research Farm at the University of Kwazulu-Natal in Pietermaritzburg. Four miniature horses, two males and two females, aged between 1.5 and 12 years old and ranging from 90 to 150 kg body weight were used (Table 12). Each horse was housed in an individual stall (1.5m x 1.7m). These stalls were arranged in two rows and the horses faced each other. These stalls were inside a roofed building. Prior to the trial these animals were on a Kikuyu pasture. The animals were in a good condition and were easy to handle both in and out of the digestion stall. All four horses had been dewormed.

Table 12 *Age and weight of experimental animals used*

Horse	Sex	Age (yrs)	Weight (kg)
Horse 1	Male	8	147.5
Horse 2	Female	8	138
Horse 3	Female	1.5	110
Horse 4	Male	1.5	93

The metabolism stalls consisted of a cement floor with three cement walls and a wire gate in the front. The floor was covered with inflexible woven rubber matting to prevent faecal contamination by urine and the cement floor, as well as to provide a comfortable surface for the animals to stand on.

The experimental food was either placed in hayrack (hay) or in black plastic feed troughs (concentrate). Each stall contained a water bucket that was changed once a day or as often as needed. Water was available to the horses *ad libitum.*

Animal comfort was ensured at all times. All horses were able to move around sufficiently in their stalls so as to prevent irritability and any possible vices developing that could affect the results of the digestion trial. While housed in these stalls, the horses were hand-walked a measured distance (on a flat surface) of 1km once a day, so as to prevent them from going off their feed, and to prevent oedema of the legs and sheath, as well as to

alleviate boredom and digestive disorders. A plastic bag was taken on all walks to collect any excreta produced. The horses were not allowed access to any form of food when exercising each day; heads were kept up at all times.

2.2.2 Dietary composition and feeding

2.2.2.1 Diet Composition

The nutrient composition of the two feeds was determined (Table 13). Homogenous samples of hay and concentrate were obtained for analysis in the laboratory.

Table 13 *The nutrient composition of the diets fed in both Trial 1 and Trial 2 (on a DM basis).*

Nutrient	Diet	
	Eragrostis Curvulae	**Concentrate (10% meal)**
Dry Matter %	93.29	86.15
Protein %	6.10	13.98
Fat %	1.01	4.0
Ash %	2.84	8.02
Crude Fibre %	38.94	10.76
Acid detergent fibre %	45.31	14.74
Neutral detergent fibre %	81.82	36.63
Calcium %	0.20	0.90
Phosphorous %	0.06	0.57
Gross Energy MJ/kg	18.49	18.31

2.2.2.2 Feeding

The horses were fed twice a day, at 8am and again at 3:30pm. They were fed at 2% of their body weight (Khonke, 1998) for a horse at maintenance during the adaptation period for both Trial A and B. In Trial B the total diet was fed in an 80:20 hay to concentrate ratio. Prior to the 8am feeding, all orts (leavings) from the previous night were collected and weighed so that a total intake from the adaptation period could be calculated. During the collection periods, 90% of the total intake during the adaptation period was fed to the horse so as to ensure that all feed was consumed. The adaptation period was seven days followed by a five-day collection period, therefore a total period of 12 days.

Trial 1A = Adaptation period 1 (All *Eragrostis Curvula* hay diet).

Trial 1A = Collection period 1 (All *Eragrostis Curvulae* hay diet).

Trial 1B = Adaptation period 2 (*Eragrostis* Hay + 10% CP Concentrate)

Trial 1B =Collection period 2 (*Eragrostis* Hay + 10% CP Concentrate + Celite®)

In the first digestion trial (Trial 1A) the horses were fed an all-hay diet (*Eragrostis Curvula*) and a digestibility study was conducted to determine the digestible energy value of the hay. The second digestion trial (Trial B) involved the horses being fed the same *Eragrostis Curvula* hay from the first trial, the 10% CP concentrate as well as the acid-insoluble ash marker (Celite®) at three percent of the total diet (Hay+concentrate) and from prior knowledge of the hay digestibility, the digestibility of the concentrate was determined by difference (Schneider & Flatt, 1975). The concentrate used in the trial was thoroughly mixed by hand so as to obtain a uniform composition.

2.2.3 Weighing of the horses

A walk-on cattle scale, accurate to 100 grams, was used to weigh the horses. This was situated very close (20 metres) to the building that housed the metabolism stalls. The horses were weighed at the beginning of each preliminary period, at the end of each preliminary period (start of collection), and at the end of each collection period. Weighing took place prior to feeding.

2.2.4 Faecal output, collection and preparation

During the collection period, faeces were collected as often as possible throughout the day so as to prevent contamination by urine and to have as many time references as possible for the rate of passage study. Time of faecal voiding and subsequent faecal weight (Table 14) was recorded for every sample. All faeces from each collection period were placed into a freezer at -18°C. At the end of each trial, a 10% sub-sample of each collection of faeces from each animal made throughout the collection period was mixed together to form one faecal sample, representative for the entire collection period for each horse. This was then stored at -15°C pending laboratory analysis.

Table 14 *Average faecal output (kg wet mass) during Trial 1A (hay) and Trial 1B (hay and concentrate) from each collection period for all four horses.*

Trial	Horse no.			
	1	2	3	4
1A(Hay)	2.20	4.02	2.67	2.32
1B (Hay and Concentrate)	2.35	3.83	3.13	2.53

2.2.5 Sample preparation

Both feed and faecal samples for digestion analysis were dried in a force-draught oven in aluminum trays at 90°C for three days, then milled through a 0.5mm screen before being analyzed in the laboratory.

2.2.6 Analytical laboratory procedure

All of the laboratory analyses were done in duplicate and repeated if results did not conform to the standard or the other result of each horse. Both feed and faeces were analyzed for dry matter, crude protein, fat, ash, crude fibre, neutral detergent fibre, acid detergent fibre, calcium, phosphorous and gross energy (AOAC, 1990).

2.2.7 Calculations

With the help of a spreadsheet (MS Office Excel, 2000), digestible nutrients were calculated. The equations to calculate the digestion coefficients, digestible nutrients and digestible energy (Equation 32, 33 & 34) were taken from McDonald *et al.*, (1998).

$$DC(\%) = \frac{\text{Amount of nutrient digested}}{\text{Amount of nutrient consumed}}$$

Equation 32

$$DN(\%\ or\ MJ/kg\ DM) = \frac{Coefficient\ of\ digestibility\ of\ that\ nutrient}{100} \times \%\ of\ that\ nutrient\ in\ the\ fee$$

Equation 33

$$DE\,(MJ/kg) = \frac{(Food\ in \times Gross\ Energy\ of\ the\ Food\ ) - (Excreta\ out \times Gross\ Energy\ of\ the\ Faece}{(Food\ in \times Gross\ Energy\ of\ the\ Food\ )}$$

Equation 34

The equations from Chapter 1 (equation 17 to 22) were used to compare the obtained experimental digestibility data to that of equations developed overseas from digestibility trials conducted using large horses.

2.2.8 Statistical methods

The Genstat (Release 6.1) statistical programme (Lawes Agricultural Trust, Rothamsted Experimental Station) was used for statistical analyses. Statistical differences between treatment means were determined from analysis of variance tables with the use of the student t-test at the 5% significance level. Correlations were performed where possible. Simple ratios were also used when establishing relationships.

2.3 Results and Discussions (Part A)

2.3.1 Body weights

All the horses endured the experimental conditions without any health problems besides a loss in a few kilograms of weight (Table 15). As there were hardly any leavings it was probably attributed to the poor quality hay and loss of muscle tone from the reduced exercise they received from being confined to a stall. Most weight loss occurred amongst the animals during Trial 1. Although *Eragrostis Curvulae* is a good quality hay, perhaps the hay had been poorly fertilized resulting in weight loss. It is more than likely however, due to the animals adjusting to the trial facilities that could have resulted in this weight loss. Horse 2 lost the most weight (11.5kg) during trial 1, probably due to the fact that it was frequently nervous, this being a trait that it always had even before entering the trial. Horse 3 lost the most weight (6.5kg) during trial 2. No apparent reasons could be given for this. From the large amount of weight lost in trial 1 it is advisable that new animals entering a digestion trial for the first time be allowed a longer adaptation period in the metabolism stalls. The animals need not necessarily be adapting to the test ration during this period, but should be allowed adequate time to accustom themselves to the new and sometimes stressful surroundings. If animals enter a trial without adequate adaptation feed intakes can be greatly affected and this could lead to subsequent weight loss.

Table 15 *Body weights (kg) of horses throughout both trials*

Horse no.	1 Start of Adaptation	1 Start of Collection	1 End of Collection	Weight loss	2 Start of Adaptation	2 Start of Collection	2 End of Collection	Weight loss
1	147.5	139	137	10.5	137.5	139	134	3.5
2	138	131.5	126.5	11.5	129.5	127.5	125	4.5
3	110	106.5	105.5	4.5	105.5	102.5	99	6.5
4	93	92.5	91.5	1.5	91	90	86.5	4.5

2.3.2 Nutrient intakes of trial animals

Fresh weight intakes of all the horses were presented in Tables 16 and 17. These animals seemed to adapt very quickly to the digestion stalls and the environment and consumed all of their diet. No concentrate was ever left, if there were any refusals, it was only ever hay.

Table 16 *Fresh weight feed intakes (2% of body weight) of horses during the adaptation periods of both trials*

Horse no.	Trial 1 (Adaptation)	Trial 2 (Adaptation)	
	Hay (kg/day)	Hay (kg/day)	Concentrate (kg/day)
1	2.10	1.99	0.61
2	2.58	2.16	0.58
3	1.82	1.82	0.47
4	1.92	1.60	0.40

Table 17 *Fresh weight feed intakes [90 %* adaptation period intake] of horses during the collection periods of both trials*

Horse no.	Trial 1 (Collection)	Trial 2 (Collection)	
	Hay (kg/day)	Hay (kg/day)	Concentrate (kg/day)
1	1.88	1.76	0.46
2	2.31	1.80	0.5
3	1.58	1.46	0.42
4	1.62	1.44	0.36

DE and DCP requirements in accordance with the NRC (1989) were compared against actual DE and DCP intakes to determine if the animal's energy and protein requirements were being met (Table 18 and 19). The DE requirements were met for all four animals when fed the *Eragrostis Curvulae* in Trial 1A and concentrate and *Eragrostis Curvulae* in Trial 1B. Therefore, it can be concluded that feeding two percent of the horses' body weight (Khonke, 1998) is an adequate estimate for DE intake when conducting digestibility trials.

However, the DCP intakes were below recommended levels in Trial 1A. This was probably due to the poor protein quality of the hay used in the trial. Requirements for DCP were adequately met in Trial 1B with the provision of a 10% CP concentrate feed.

Table 18 *Relationship between nutrient intake and nutrient requirement in Trial 1* (Eragrostis Curvulae)

Horse no.	DE intake (MJ/kg)	DE required † (MJ/kg)	DEI/DE req.	DCP Intake (g/day)	DCP required ¥ (g/day)	DCPI/DCP req.
1	20.23	17.02	1.18	76.89	88.5	0.86
2	19.70	16.19	1.22	77.85	82.8	0.94
3	15.55	13.73	1.13	48.34	66	0.73
4	17.20	12.23	1.41	56.7	55.8	1.02

† Calculated from NRC (1989): DE req. (MJ/day) = (0.021 x LW + 0.975) x 4.18

¥ Calculated from NRC (1989): DCP req. (g/day) = 0.6 x LW (liveweight)

Table 19 *Relationship* between nutrient intake and nutrient requirement in Trial 2 (Eragrostis *Curvulae and concentrate)*

Horse	DE Intake (MJ/kg)	DE required † (MJ/kg)	DEI/DE req.	DCP Intake (g/day)	DCP required ¥ (g/day)	DCPI/DCP req.
1	25.53	16.15	1.58	113.85	82.5	1.38
2	22.82	15.44	1.48	104.85	77.7	1.35
3	18.43	13.34	1.38	95.78	63.3	1.51
4	18.43	12.06	1.53	86.22	54.6	1.58

† Calculated from NRC (1989): DE req. (MJ/day) = (0.021 x LW + 0.975) x 4.18

¥ Calculated from NRC (1989): DCP req. (g/day) = 0.6 x LW (liveweight)

Although the equations used to determine DE and DCP requirements were obtained from the NRC (1989), which uses large horse data, one can accept possible inaccuracy, but this is work in progress for new trials and metabolic models for miniature horses, and these equations were used to determine only possible causes of body weight loss in this trial.

2.3.3 Analyses of digestibility results

The dry matter digestibilities were calculated for each animal and for each nutrient of the hay and concentrate. This information was then used to calculate the digestible energy of the hay (Table 20) and the digestible energy of the concentrate (Table 21) by difference.

Table 20 *Digestible Nutrients and digestion coefficients of* Eragrostis Curvulae *calculated from miniature horses in trial 1*

Nutrient	Digestible nutrient (%)				Digestion Coefficient (%)			
	Horse				Horse			
	1	2	3	4	1	2	3	4
Dry matter	59.17	46.13	53.18	56.69	63.43	49.45	57.01	60.77
Crude protein	4.09	3.37	3.06	3.50	67.09	55.17	50.20	57.43
Crude fibre	25.65	21.06	23.69	24.50	65.88	54.09	60.85	62.93
ADF	28.81	22.55	25.97	27.76	63.58	49.77	57.31	61.27
NDF	52.69	41.92	48.13	50.65	64.39	51.24	58.82	61.90
Fat	-0.42	-1.00	-0.79	-0.64	-41.58	-99.46	-77.72	-63.72
Digestible energy	11.23	8.53	9.84	10.63	60.77	46.12	53.20	57.46

Table 21 *Digestible Nutrients and digestion coefficients of concentrate calculated from miniature horses for trial 2 by digestibility by difference*

Nutrient	Digestible Nutrients (%)				Digestion Coefficient (%)			
	Horse				Horse			
	1	2	3	4	1	2	3	4
Dry matter	60.60	68.01	43.40	41.55	70.34	78.95	50.38	48.22
Crude protein	9.12	8.87	12.17	10.00	65.29	63.50	87.10	71.59
Crude fibre	4.60	6.34	-5.77	-3.92	42.82	58.91	-53.67	-36.45
ADF	1.20	7.60	-9.99	-9.57	8.17	51.55	-67.77	-64.94
NDF	18.72	26.84	-0.54	-2.22	51.12	73.29	-1.46	-6.07
Fat	1.64	3.32	3.34	2.70	41.07	83.09	83.61	67.57
Digestible energy	12.55	15.00	9.67	8.66	68.57	81.89	52.81	47.31

2.3.3.1 Investigation of aberrant digestibility results in hay

The digestion coefficients for fat in the hay showed a negative value ranging from –41.58 to –99.46 (Table 20). This is indicative of the presence of fat in the faeces. This includes fats that have escaped the action of the digestive juices, those that are not absorbable such as plant sterols, and some non-lipid ether-soluble material of feed origin. The faeces may also contain metabolic fat, which consists of ether-soluble faecal substances of body origin such as residues from digestive juices such as bile (Schneider & Flatt, 1975). In the herbivore, fat digestion is limited by a protective covering of undigested cellulose surrounding the fat, which serves as a protective barrier against fat digestion. The ether extract of the feed of the herbivore also contains more indigestible material such as the pigments found in forages such as chlorophyll. This is included in the ether extract obtained from green plants, but is not a true fat though (Schneider & Flatt, 1975). Lucas and Loosli (1944) found that with high fat rations ether extract was more digestible than with low fat rations. Extremely low and negative digestion coefficients were obtained for ether extract of rations having very low ether extract content. In Trial 1 the hay had a very low ether extract (1.01%), this could be a contributing factor to the negative digestion coefficients that were obtained with all four horses. Other factors could be the indigestible pigments of chlorophyll or metabolic fat. Gallup and Hobbs (1943) showed that the negative digestibility coefficients that occur from the conversion of carbohydrates to fat and the excretion of endogenous fat-soluble material are not unusual under ordinary circumstances and are consistent with the knowledge of lipid metabolism.

2.3.3.2 Investigation of aberrant digestibility results of the concentrate

Unlike the hay, the digestion coefficients for fat in the concentrate were positive as there were greater amounts of fat in the concentrate portion (Table 21). As there was a higher fat content in the concentrate ration it could lead to a higher digestibility of the ether extract of the ration (Lucas & Loosli, 1944). No relationship could be found between sex and digestibility of the nutrients. The two younger animals showed higher digestibilities for crude protein. They also showed negative digestion coefficients for all three of the components of the fibre fraction, crude fibre, ADF and NDF. The "digestibility by difference method" could have been the reason for this, as the assumption of this procedure is that when mixing two feeds together they do not alter the digestibility of each other (Schneider & Flatt, 1975).

This is unwarranted as it has been shown that an associative effect does in fact occur between feeds (Schneider & Flatt, 1975). Any errors arising from associative digestibility and all errors from sampling, weighing, and calculations are assigned to the feed of which the digestibility is being determined by difference (i.e. concentrate) (Table 21). The error in results will be the largest when the digestibility of a concentrate is determined this way and the proportion of roughage to concentrate is small. Those nutrients of the concentrate present in the smallest proportion, such as crude fibre and ether extract, will be affected the most (Armsby, 1917). The determination by difference of the apparent digestibility of concentrate feeds that cannot be fed alone, by adding them to a basal ration whose digestibility has been previously determined, has sometimes yielded coefficients that are negative or even greater that 100 (Schneider & Flatt, 1975). This associative effect error could be the reason negative digestion coefficients were obtained for the fibre components in Trial 2. This reasoning will remain until further digestion trials can prove otherwise, and provides further material for subsequent investigations.

2.3.4 Analysis and checking of accuracy of DE results

The DE's of hay and concentrate measured in these trials were compared with those using different equations, reported by many equine nutrition researchers, to establish how accurate this digestibility study protocol was. There were six equations used in the comparison (Equation 17, 18, 19, 20, 21 & 22). From this a conclusion was drawn as to how closely the miniature horse DE data compared with those of a large horses DE data, and as to which equation used most accurately described our experimental data (Tables 22 and 23). The DE determined from our experiments with miniature horses were compared to results using equations developed overseas to calculate DE from proximate nutrient analysis as well as digestible nutrients. From the results of DEexp: DEequ a relationship was found. It showed that the experimental DE was most similar to that of Fonnesbeck's (1981) for both the hay and concentrate with ratios of 1.05 for hay and 1.01 for concentrate. This was expected as Fonnesbeck's equation relied on digestible nutrients unlike the other equations using proximate nutrients of the food only.

Table 22 *Relationship between miniature horse experimental DE (MJ/kg) (DEexp) of* Eragrostis Curvulae *and large horse calculated DE (MJ/kg) (DEequ) of* Eragrostis Curvulae.

Diet	Experimental	Equation					
		1	2	3	4	5	6
Hay	10.05	9.56	9.27	7.39	11.74	14.29	13.02
A^1		***1.05***	1.08	1.36	0.85	0.70	0.77

A^1 = DEexp: DEequ

Table 23 *Relationship between miniature horse experimental DE (MJ/kg) (DEexp) of concentrate and large horse calculated DE (MJ/kg) (DEequ) of concentrate*

Diet	Experimental	Equation					
		1	2	3	4	5	6
Concentrate	11.47	11.35	12.82	11.72	11.29	10.52	-
A_1		***1.01***	0.89	0.98	1.02	1.09	-

A^1 = DEexp: DEequ

Equation 1: Fonnesbeck, 1981
Equation 2: Pagan, 1988
Equation 3: Pagan, 1998
Equation 4: Harris, 2001
Equation 5: Zeyner & Kienzle, 2002
Equation 6: Martin-Rosset *et al.*, 1994

A comparison of the DE experimental values versus the DE calculated values was made by using the best fitting borrowed equation and running the data through Genstat (2002). There was no significant difference ($P>0.05$) between the actual DE versus the calculated DE for both hay ($P=0.533$) and concentrate ($P=0.957$) (Table 24). The experimental data were highly correlated to the calculated data using the equations in trial 1 (hay) ($r^2 = 0.99$) and trial 2 (concentrate) ($r^2 = 0.86$). This showed that miniature horses could perhaps be used as accurate predictors of digestible energy for large horses in digestibility trials.

Table 24 *Relationship between mean of DE experimental versus DE calculated of hay and concentrate*

	Hay (MJ/kg)	Concentrate (MJ/kg)
Experimental DE	10.06	11.47
Calculated DE (Fonnesbeck, 1981)	9.56	11.35
P-Value	(0.533) n.s.	(0.957) n.s.
Correlation	0.999	0.860
s.e.d	0.746	2.10

NS = Non-significant at 5% level; s.e.d = Standard errors of difference

2.3.5 Effect of Sex and Age on DE results

2.3.5.1 Sex

There was no significant difference (P>0.05) between male and female actual DE and calculated DE values for the hay and concentrate (Table 25). From these results it can be concluded that either males or females are suitable for digestibility trials in horses. Further investigation is needed to validate the consistency of these results. Schneider and Flatt (1975) suggest using male animals in digestibility experiments because of the greater facility with which excreta can be collected without contamination.

Table 25 *The Statistical relationship between male and females when comparing actual experimental DE values*

	Hay (MJ/kg)	Concentrate (MJ/kg)
Male	10.94	10.61
Female	9.19	12.34
P-value	NS	NS
s.e.d	0.723	3.62

NS = Non-significant.; s. e. d = Standard errors of difference

2.3.5.2 Age

To determine if age had any effect on digestibility, the actual DE means of hay and concentrate between the two ages, mature two and eight year olds, were placed under statistical scrutiny (Table 26). There was no significant difference (P>0.05) between the eight and two year old horses. Armsby (1911), Blaxter (1966) and Hungerford and Foster (1921) found no influence of age on digestibility of feedstuffs with cattle. Patterson (1897)

compared the digestibilities of oats and of shelled and ground corn by young and old horses and concluded that younger horses digested whole oats better. He also obtained higher digestion coefficients of shelled corn for young horses and higher values of ground corn for older horses. Results with the miniature horses indicated that age over two years had no effect on digestibility results in miniatures (Table 26).

Table 26 *Statistical relationship between 8 years and 1.5 years when comparing actual experimental DE values*

	Hay (MJ/kg)	**Concentrate (MJ/kg)**
8 years	9.89	13.78
1.5 years	10.24	9.17
P-value	NS	NS
s.e.d	1.411	1.325

NS = Non-significant; s. e. d = Standard errors of difference

2.4 Conclusion

The use of miniatures horses in the evaluation of digestible energy for horse feeds provides substantial evidence that they are ideal pilot animal to use in digestion trials to replace large horses such as Thoroughbreds. Their size makes them ideal to use as they are not only easier to handle in the metabolism stalls as well as during weighing and exercising, but they consume less feed therefore decreasing the cost of digestion trials, a problem which often dissuades researchers from carrying out such trials on horses.

The small number of animals used in this trial and the absence of local large horse digestion data, which is important to compare the results of this trial, make it difficult for any definite conclusions to be drawn. However the results were encouraging enough to lead us to conduct a more detailed and balanced trial aimed at confirming the validity of using miniature horses as pilot trial animals and investigating the possibility of using other animals of similar digestive function.

From the results we were able to highlight problems that could occur when conducting a digestion trial with horses. These would lead us to developing methods to overcome such problems, therefore making the execution of subsequent trials a lot easier and precise thereby providing us with more accurate and reliable results upon which we can draw more definite conclusions.

PART B

RATE OF PASSAGE THROUGH THE DIGESTIVE TRACT OF A MINIATURE HORSE

A trial to determine the length of collection period to use when conducting digestibility trials with miniature horses

2.5 Introduction

Many researchers have reported rates of passage in large horses. Mean retention times (MRT) of 23 hours (Uden *et al.*, 1982), 44.2 hours (Cuddeford *et al.*, 1995), 96 hours (Vander Noot *et al.*, 1967) and 43.4 hours (Pagan *et al.*, 1998) for large horses have been reported in literature, but no information regarding rate of passage in miniature horses could be found. In any digestion trial one needs to be aware of the rate of passage of feedstuffs through the digestive tract, as this must be taken into consideration when determining the length of the collection period. If collection is terminated before all the feed residues have had time to pass through, incorrect digestibility data would be obtained. The aim of this study was to investigate the rate of passage through the digestive tract of a miniature horse so as to determine the correct length of collection period to employ when conducting digestion trials with such animals. Previous investigators have reported various lengths of collection periods as five days by Peterson (1879), seven days by Nicholson and Friend (1926) and 12 days by Lathrop and Bohstedt (1938). Haenlein *et al.* (1966) conducted rate of passage studies using chromic oxide as a marker, but no definite conclusions were drawn regarding the length of collection period to use in digestion trials. Vander Noot *et al.* (1967) found, by using chromic oxide, that a collection period of 5 days would be adequate enough time to clear the digestive tract of feed residues. Hintz and Loy (1966) using Styrofoam particles recovered most of the marker in 63 hours for both a pelleted and non-pelleted ration.

Acid insoluble ash (AIA) has been the most frequently used marker in equine studies even though it has shown problems in analysis due to it not being a discrete chemical entity (Sutton *et al.*, 1977; Cuddeford and Hughes, 1990; McMeniman *et al.*, 1990; Cuddeford *et al.*, 1992; Barbisan *et al.*, 1993). Chromic oxide is often used in ruminant studies and has been tested on horses too (Haenlein *et al.*, 1966). Orton *et al.* (1985) compared AIA to

chromic oxide and found AIA to be a more reliable estimate of digestibility coefficients as well as inexpensive as and easier to use than chromic oxide. McCarthy *et al.* (1977) found acid-insoluble ash to be superior to that of chromic oxide. Other methods have been used, these include N-alkanes, which have also been studied as markers in horses, but it has been found that horses are capable of metabolizing them to a certain extent, which could lead to inaccurate results (Ordakowski *et al.*, 2001). Ytterbium has been studied as a marker to determine rate of passage in horses by Pagan *et al.* (1998) and Moore-Colyer *et al.* (2003) and it was found to be a successful external marker. It was decided to investigate AIA in this experiment; Celite® was used as the acid-insoluble ash marker in this study. It is a form of diatomaceous earth that is non-toxic to animals.

The following investigation was conducted at the same time as the preliminary digestion trial. The same materials and methods were employed as in Part A. Therefore only those differing from that of Part A will be reported here in Part B.

2.6 Materials and Methods

2.6.1 Animals and Housing

The same four miniature horses and facilities were used as in Part A. The rubber matting on the floor was important so as to prevent contamination from the cement floor, which could affect results of the AIA analysis.

2.6.2 Dietary composition and Feeding

Celite® was administered at three percent of the total diet (hay and concentrate) for all four horses. It was administered once at the 8am feeding on the first day of the collection period. The Celite® was mixed thoroughly by hand into the concentrate portion of the diet. All of the administered marker was consumed by all four animals.

2.6.3 Faecal output, collection and preparation

Faeces were collected as often as they were voided throughout the day so as to have as many time references as possible for the rate of passage study. Faecal collection commenced on average two hours after the administration of the Celite®. This involved the collection of all defecations up to 120 hours. Time of faecal voiding was recorded for every sample. All faeces from each collection period were placed into a freezer at -18°C. A grab sample was then taken from each faecal sample at each time when made, placed in glass jars and clearly marked for date, time and animal, and then dried in a force-draught oven at 90°C immediately for 48 hours and then milled through a 0.5mm screen before being analysed using the acid-insoluble ash method in the laboratory (Schneider and Flatt, 1975).

2.6.4 Sample preparation

Both feed and faecal samples for rate of passage analysis were dried in a force-draught oven in the glass jars at 90°C for three days then milled through a 0.5mm screen, before being analyzed in the laboratory.

2.6.5 Analytical laboratory procedures

The concentrations of acid-insoluble ash in the feed and faecal samples were determined using a slightly modified method of the A101 Method from the Nutrition Research Laboratory, Adelaide University, Roseworthy, Australia (1998).

Five grams dried, ground sample was weighed into numbered crucibles and dried in a drying oven overnight. After been cooled in a desiccator for 45 minutes, each crucible was weighed (crucible + dry sample). The crucibles were then transferred to a muffle furnace where they were ashed overnight at 480°C. After allowing the crucibles to cool in a dessicator for 90 minutes each crucible was weighed (crucible + ash). Each crucible was then placed in a tall 250ml beaker. 4M HCl was slowly poured into the beaker until the sample was wetted from underneath. The beaker was then three-quarters filled with the 4M HCl. The beakers with the crucibles inside were then boiled for 15 minutes on a hotplate, ensuring that the crucibles did not boil dry. After boiling the crucibles were then placed in a Buchner vacuum flask and the HCl was removed under suction. The sample was then rinsed with 4M HCl and then distilled-deionised water. The crucibles were then transferred to the muffle furnace and ashed overnight at 480°C. After cooling once more in a dessicator for 90 minutes, each crucible was weighed and recorded (crucible + acid-insoluble ash).

2.6.6 Calculations

The following equations were used, with the help of a spreadsheet to determine the percentage acid-insoluble ash content in each faeces collected for all four animals.

$$\text{Dry Matter}\% = \frac{(\text{Crucible mass} + \text{Dry Sample mass}) - \text{Crucible mass}}{\text{Sample Mass}} \times 100 \quad \textbf{Equation 35}$$

$$\text{AIA}\% = \frac{(\text{Crucible mass} + \text{Acid Insolub le Ash mass}) - \text{Crucible mass}}{(\text{Crucible mass} + \text{Dry Sample mass}) - \text{Crucible mass}} \times 100 \quad \textbf{Equation 36}$$

2.6.7 Rate of passage data as described by the Grovum and Williams (1973) model

The Grovum and Williams model (1973) has been used to describe the rate of passage of a marker through the digestive tract of a ruminant by the equation:

$$y = A \exp^{-k_1 (t-TT)} - A \exp^{-k_2 (t-TT)} \qquad \textbf{Equation 37}$$

Where

y and A = adjusted marker concentrations in faecal dry matter

k_1 and k_2 = rate constants

k_1 represents digesta flow from the rumen

k_2 represents digesta flow from the caecum and proximal colon

t = sample time (hours)

TT = first appearance of marker in faeces

A curve peeling technique is carried out by performing a linear regression on the natural logged concentration values (values after and including the peak concentration) against time (hours), and thereby k_1 and a_1 are calculated. The gradient of the regression equation is equal to k_1 while the y intercept is a_1. The predicted values calculated from the regression are transformed to e^x and the observed marker concentration is subtracted from the corresponding predicted value (residual value). A further linear regression analysis is performed on the natural log of the residual values from the initial concentration to the peak concentration to yield k_2 which is the gradient, and a_2 which is the y intercept.

Equations 39 and 40 are used in calculating coefficients in equation 38

$$A = a_1 - (k_1 \times TT) \qquad \textbf{Equation 38}$$

where a_1 = y intercept

$$TT\,(\text{hours}) = \frac{a_2 - a_1}{k_2 - k_1} \qquad \textbf{Equation 39}$$

where TT = transit time

$$\text{MRT (hours)} = \frac{1}{k_1} + \frac{1}{k_2} + TT \qquad \textbf{Equation 40}$$

where MRT = mean retention time of marker in hours

By substituting the coefficients of k_1, k_2 and TT into Equation 38, the mean retention time, in hours, of the marker in the digestive tract is calculated.

2.6.8 Rate of passage data as described by the Castle et al (1956) model

The behaviour of Celite® as a marker through the digestive tract of the miniature horses was analysed using the Castle *et al.*, (1956) model which involved work on goats. The percentage of AIA excreted up to 120 hours was plotted against time to give excretion curves. These cumulative excretion curves were plotted for each horse (Figure 4) and 'R' was calculated by adding together the times of excretion from 5 to 95% at intervals of 10% taken from the graph and then dividing the sum by 10. This value was taken as a measure of the mean retention time, in hours, of the marker in the alimentary tract.

2.7 Results and Discussion

Two possible methods of analysing the AIA results were compared i.e. Grovum and Williams (1973) and Castle (1956). Both methods are explained below:

2.7.1 Rate of passage data as described by the Grovum and Williams (1973) model

Although this method was developed from ruminant data, it was decided to test its applicability to our horse data. Percentage marker excreted was plotted graphically over time to determine rate of passage of feedstuffs through the gastro-intestinal tract of the horses (Figure 3). All the curves followed the same general pattern of a slow initial constant excretion up to approximately 30 hours, followed by a sharp rise in marker excreted from approximately 30 to 60 hours and then as time progressed to 120 hours, the percentage excreted slowly declined. The first two horses display similar trends with higher and earlier peaks of marker excretion. They also reached initial marker concentration a lot sooner than the other two horses. This could be due to many reasons such as larger quantity of water intake, greater amount of food chewing thus a larger saliva production, or a greater amount of movement in their digestion stalls (thus promoting gut motility).

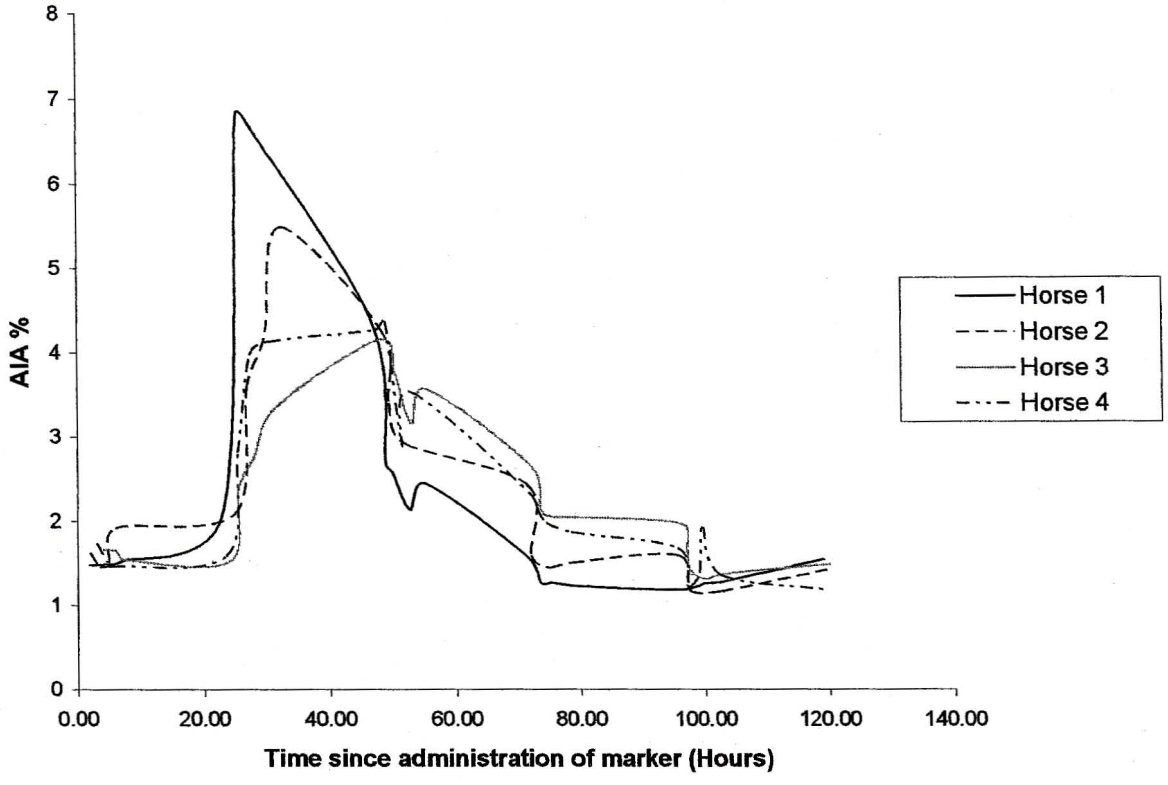

Figure 3 *Graph showing digesta flow of the AIA marker through the digestive tracts of the four miniature horses over time.*

The Grovum and Williams model was fitted to the data and coefficients as described in Equation 37, Equation 38, Equation 39 and Equation 40, were calculated (Table 27).

Table 27 *Average coefficients derived by the Grovum and Williams (1973) model of each horse on the hay and concentrate treatment and the averages thereof*

Horse no.	k_1	k_2	a_1	a_2	MRT (hrs)
1	0.0347	0.1902	2.8057	3.5364	38.78
2	0.0267	0.0682	2.5803	2.6383	52.85
3	0.0165	0.0424	2.1471	2.1263	83.39
4	0.0216	0.0494	2.3736	2.3127	64.35
Average	*0.0249*	*0.0876*	*2.4767*	*2.6534*	*59.84*

In a ruminant animal k_1 is representative of digesta flow from the rumen (Moore-Colyer *et al.*, 2003). From the data in Table 27 it can be seen that k_1 is always smaller than k_2. This could perhaps indicate that k_1 would represent the stomach (foregut) in the horse and k_2 the caecum and proximal colon (hindgut). The average mean retention time (MRT) is 59.84 hours for the four horses. According to these data, 2.97 days would prove an adequate time period to allow for a diet consisting of hay and concentrate to move completely through the digestive tract of a miniature horse. Therefore the time period used in this trial of 5 days for collection is acceptable and if needed could be shortened thereby allowing a quicker turnover of results from an *in vivo* digestibility trial using miniature horses.

2.7.2 Rate of passage data as described by the Castle et al (1956) model

The excretion curves showed the same general shape for all four horses as with the previous model. Once again, as explained in section 2.7.1, it could be seen that horse 1 and 2 showed a faster rate of passage through the digestive tract (Figure 4 and 5).

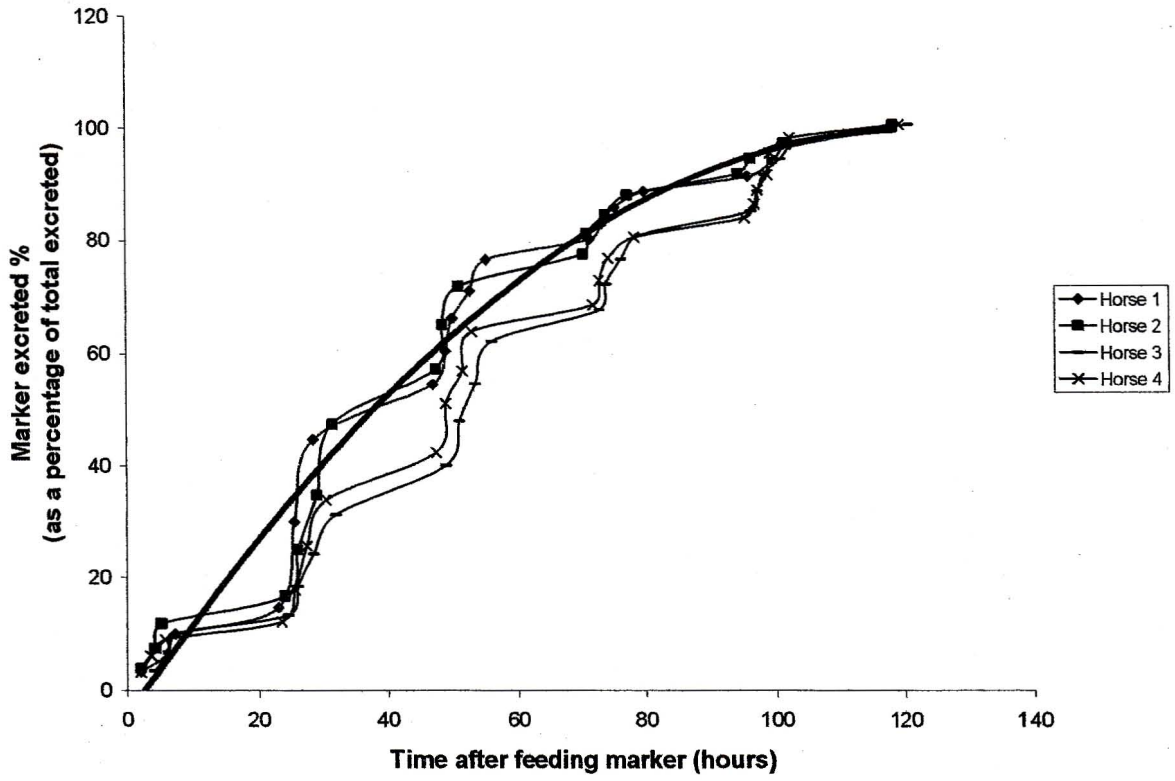

Figure 4 *Cumulative excretion curves of all four horses on a hay and concentrate diet*

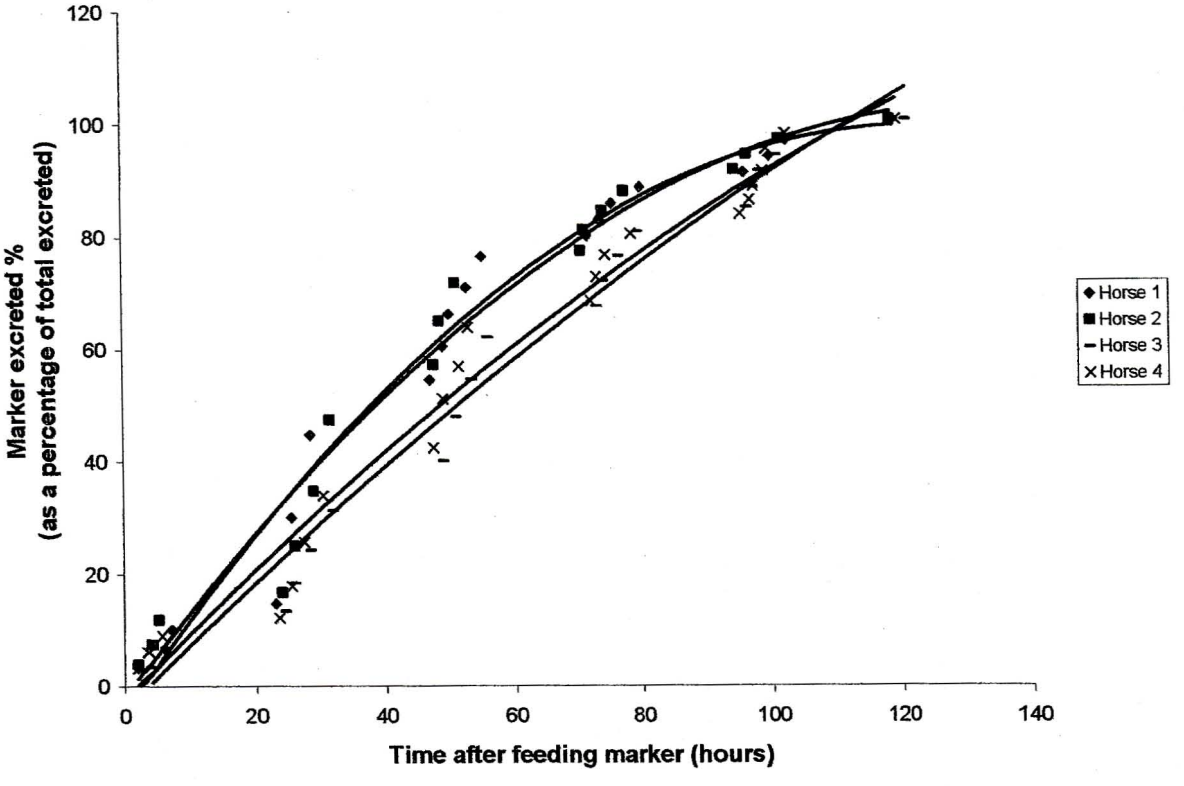

Figure 5 *Graphs showing the pattern of marker excretion for all four horses on a hay and concentrate diet. Polynomial trendlines have been fitted to show behavior of data.*

The results of the measurements from Castle (1956) for the rate of passage for each horse are shown in Table28. In all of the horses the AIA marker was first detected in the faeces between two and a half and five hours after feeding. The MRT was 45.57 hours indicating a 14.27-hour difference between the two methods employed. The excretion curves for all four horses showed the same general sigmoid shape with the curves rising sharply to about the 80% point and then gradually flattening out in the final stages of excretion. Excretion at 5% averaged 3.88 hours, while excretion at 95% averaged 87.44 hours. Although in the horses the excretion of the AIA began (5% excretion time) at approximately the same time, its final excretion (95% excretion time) showed a tendency to be characteristic of each individual animal, with wide variation in times. According to Balch (1950) the 5% excretion time is to some extent the absolute measure of passage through the omasum, abomasum and intestines in ruminants, while the 95% excretion time is indicative of the passage time through the reticulo-rumen. As a horse's digestive system is almost the reverse of a ruminant with 'fermentation vessel' at the end of the digestive tract, it could

appear that perhaps the time at 5% excretion (3.88 hrs) is indicative of time spent in the stomach (foregut) of the horse and the time at 95% excretion (87.44 hrs) is the time spent in the hindgut of the horse.

Table 28 *Mean retention times, 5% excretion and 95% of hay and concentrate for all four horses (Castle, 1956).*

Horse no.	MRT (h)	5 % excretion (h)	95% excretion (h)
1	41.15	5	78.75
2	42.28	3	89
3	55.35	4.75	116
4	43.48	2.75	66
Average	*45.57*	*3.88*	*87.44*

2.8 Conclusion

It appears from the above calculations using the two methods mentioned above, that a collection period of five days is adequate to ensure that all of the test diet used in the digestibility trial has been excreted. According to Grovum and Williams's model, which gave the longest retention times in the digestive tract, a collection period of two and a half days would prove adequate to clear the digestive tract of the test diet. From an experiment conducted using the same animals and the same hay, it was found that the MRT for a hay only diet was 56.55 hours with a 5% excretion of 2.90 hours and a 95% excretion of 107.80 (S.S Parsons, personal communication, 2004). This overall greater MRT could be attributed to the fact that roughages are maintained in the gastrointestinal tract for longer than concentrates. As the rate of passage in a hay only diet was not investigated in this trial we can use the information of 56.55 hours from the other trial to validate our recommendation that a collection period of five days is adequate in conducting digestion trials with miniature horses.

Previous equine investigators have found that time-dependent compartmental models fitted the faecal excretion data much better than time-independent models (Moore-Colyer *et al.*, 2003, Pond *et al.*, 1988, Dhanoa *et al.*, 1985). Vander Noot *et al. 1967* used the cumulative excretion method in calculating rates of passage. This, and reasons given in Section 1.9, therefore gives reason in justifying the use of the results from the Castle (1956) to draw a more definite conclusion. As the hindgut is the last region in the horse where the digesta would flow, the time of 87.44 hours would prove to be the best time to take as length of collection period. Therefore four days would be enough time to clear the digestive tract of the test diet. By increasing it to five days, inaccuracies due to the inability to make faecal collections that are truly representative of the test diets digestibility would be reduced. From the above experiment it shows that there is a definite advantage in using miniature horses as determinants of the digestible energy available in horse feed, as DE could be calculated in less time with an overall reduction in labour intensiveness and cost.

CHAPTER 3

PART A

INVESTIGATION INTO FINDING A POSSIBLE PILOT TRIAL ANIMAL FOR THE *IN VIVO* EVALUATION OF HORSE FEED IN SOUTH AFRICA

3.1 Introduction

Feed costs comprise the largest variable cost of keeping horses. The energy portion of the horse diet represents the largest and most expensive portion of the diet and has major influences on growth and performance. However, the digestible energy (DE) content of horse diets is seldom known, as DE can only be determined accurately in expensive metabolism trials. Therefore, if we could find an accurate relationship between a chemical component of the diet and DE, or an equation already used worldwide that describes DE in South Africa accurately, or even a pilot animal that we could use in metabolism studies other than a horse it would prove very valuable to feed companies and horse owners.

Although feeding standards have been recommended for horses, these were derived mainly from the extrapolation of data from ruminants. The horse is classified as a herbivore but certain anatomical characteristics of this species suggest that the nutrient requirements must be determined directly from the horse itself and not extrapolated from other species. At present there is no specific feed evaluation system for horses in South Africa, feed evaluation for horses is performed using the feed evaluation system for ruminants. The objective of this study was therefore:

- to determine how accurate or incorrect the ruminant evaluation system is for horse feed evaluation
- Investigate the possibility of using rabbits as a pilot animal.
- To investigate the rate of passage (MRT) of diets varying in crude protein content through the digestive tract of an equine

The trial reported herein was conducted so as to obtain more information on the digestion in horses, rabbits and sheep and explore the possibility of finding a pilot trial animal for

making feeding recommendations for horses. As digesta rate of passage in the herbivore is of great importance to the nutrition and feeding strategy of the animal, this was investigated in the horses as well.

3.2 Materials and Methods

The experiment was carried out at Ukulinga Research Farm at the University of KwaZulu - Natal in Pietermaritzburg from January 2004 to April 2004.

3.2.1 Experimental Design

A 5 x 5 Latin square design was used for the horses (Table 29) so that a more detailed description could be made of the individual feeds to be used later for comparison with *in vitro* results. A Latin square design effectively increases the replication of the experimental units for investigations using low animal numbers. As only four sheep were available a 4 x 4 Latin square was used (Table 30). Ten rabbits (two per diet) on a 5 x 5 Latin square design were used (Table 31). Five diets (A, B, C, D & E) were fed to the horses and rabbits but Diet D was not fed to the sheep.

Table 29 *Table showing Latin square design of diets fed to each horse during each period*

	Horse 1	**Horse 2**	**Horse 3**	**Horse 4**	**Horse 5**
Period 1	B	A	D	C	E
Period 2	E	B	A	D	C
Period 3	A	D	C	E	B
Period 4	D	C	E	B	A
Period 5	C	E	B	A	D

Table 30 *Table showing Latin square design of diets fed to each rabbit during each period.*

	Rabbit 1+6	Rabbit 2+7	Rabbit 3+8	Rabbit 4+9	Rabbit 5+10
Period 1	B	A	D	C	E
Period 2	E	B	A	D	C
Period 3	A	D	C	E	B
Period 4	D	C	E	B	A
Period 5	C	E	B	A	D

Table 31 *Table showing Latin square design of diets fed to each sheep during each period*

	Sheep 1	Sheep 2	Sheep 3	Sheep 4
Period 1	B	A	E	C
Period 2	C	B	A	E
Period 3	A	E	C	B
Period 4	E	C	B	A

Each of the trial periods consisted of a seven-day preliminary adaptation period during which the animals' digestive system could become adapted to the test diet, and a five-day collection period where all faeces were collected and feed intake was kept constant for all animals. Therefore there were 12 trial days per period.

3.2.2 Animals and Housing

3.2.2.1 Horses

Five mature miniature horses, ranging from two to twelve years old and from 90 to 150 kgs body weight were used. Two stallions, two geldings and one female were used. Each horse was housed in an individual stall as described earlier (section 2.2.1). The only modification from the first trial was that the floor of the stall was raised on bricks on the side with corrugated iron set at an angle on top and then an inflexible woven rubber matting on top of that. This was to facilitate quick drainage of the urine away from the faeces as well as allow for collection of urine. All five horses had been dewormed and were in a healthy condition.

The experimental food was placed either in a hayrack (hay) or in plastic feed troughs (concentrate) that were removed after each meal. Each stall contained a water bucket which was changed once a day or as often as needed. Water was available to the horses *ad libitum.* During the experimental period each horse was hand-walked a distance of one kilometer each day at 10am.

An external acid-insoluble ash marker, Celite®, was added to each horses concentrate portion of the diet so as to determine rate of passage. The marker was added as three percent of the total dietary allocation of the hay and concentrate together. It was administered only on day one of collection period and it was thoroughly mixed by hand into the concentrate.

3.2.2.2 Sheep

Four mature male Dorper sheep, ranging from 22 to 37 kg, were housed in metabolism crates (22cm wide x 52cm long) in the same experimental facility as the horses. A faecal collection bag was fitted to all four sheep so as to facilitate easier faecal collection. In order to prevent disturbances or anxiety, which could have affected the digestibility results of the feed, the sheep were fitted with these bags a few days before the collection period, but left open. All sheep were dewormed before the start of the experiment.

3.2.2.3 Rabbits

Ten mature male meat breed rabbits ranging in body weight from 1.8 to 2.7 kg were housed individually in wire cages (60cm long x 50cm long) with two rabbits allocated randomly per treatment. Each cage was fitted with a feed and water trough and a collection tray underneath so as to facilitate collection of faeces and urine. Water was available *ad libitum* and changed once a day.

3.2.3 Weighing of experimental animals

A walk-on cattle scale, accurate to 100 grams, was used to weigh the horses. A walk-on sheep scale was used to weigh the sheep, and a scale accurate to 0.01grams with cone, was used to weigh the rabbits. All the animals were weighed at the beginning of each preliminary period, at the end of each preliminary period (start of collection), and at the end of the collection period. Weighing took place at the same time each morning on these days before feeding.

3.2.4 Dietary composition and feeding

3.2.4.1 Diet composition

Four commercial horse diets were to be tested on all the animals (Table 32). The diets were chosen for their protein content, by which they are marketed. Diet C and D have the same crude protein percentage, but were chosen to be tested as Diet C is a grain containing diet, whereas Diet D is a "grain-free" diet. As concentrate cannot be fed to horses alone they were fed together with the hay in an 80:20 hay to concentrate ratio (Kohnke, 1998) for all the animals except the rabbits which were fed in a 60:40 hay to concentrate ratio from period three due to weight loss in periods one and two. The digestibility of the concentrate was then calculated by difference from the known digestibility of the hay.

Table 32 *The nutrient composition of the diets fed during the trial period (on a DM basis)*

Nutrient	Diet				
	A **Hay** *Eragrostis*	**B** **11% CP**	**C** **14% CP** **(grain)**	**D** **14% CP** **(grain free)**	**E** **16% CP**
Dry Matter %	88.42	83.18	84.09	86.28	85.86
Protein %	7.39	14.00	17.88	15.43	17.22
Fat %	0.88	4.57	5.71	4.51	3.97
Ash %	4.17	9.75	9.48	5.38	7.15
CF %	39.41	7.43	10.09	8.30	6.84
ADF %	44.73	12.29	13.82	9.58	8.59
NDF %	80.81	31.51	34.43	24.41	23.95
GE MJ/kg	17.49	16.90	18.04	18.29	17.28

3.2.4.2 Feeding

The daily calculated ration of all the animals was divided into two and fed twice a day, at 8:30 and again at 15:30. All animals were weighed at the beginning of each adaptation period so as to know how much to feed. The horses and sheep were fed at 2% of their body weight during each adaptation period, as were the rabbits in period one but due to weight loss this was increased to 4% of body weight from period two up until the end of period five. Any feed refusals (orts) were collected every day prior to the 8:30am feeding and recorded. During the collection periods, 90% of the intake during the adaptation period was fed to the animals so as to ensure that all feed would be consumed, thereby eliminating orts during the collection period.

3.2.5 Faecal output and collection

As a rate of passage study was conducted concurrently with that of the digestibility study, faeces from the horses were collected as often as possible. Faeces were collected when voided, weighed, recorded and placed into a freezer at -18°C pending analysis. Faeces from the sheep and rabbits were collected, weighed and recorded each morning at 8am and then placed in the freezer at –18°C pending analysis.

At the end of each collection period, a 10% sub-sample was taken from each faeces sample from each animal and mixed together to form one faecal sample, representative of the entire collection period.

Table 33 *Average faecal production (kg) for the horses, sheep and rabbits on all treatments*

Animal	Treatment				
	A	B	C	D	E
Horse	2.12	1.75	2.21	2.02	1.98
Sheep	0.42	0.46	0.39	*	0.39
Rabbit	0.03	0.04	0.03	0.03	0.03

Table 34 *Average faecal analysis for horses, sheep and rabbits on all treatments*

Animal	Treatment	DM	CP	CF	ADF	NDF	Fat	GE
Horses	A	30.13	5.57	35.60	47.34	75.46	3.22	18.53
	B	29.71	6.27	34.26	44.81	72.67	3.68	18.46
	C	29.86	6.53	33.81	45.03	73.06	3.71	18.41
	D	29.73	6.55	34.9	45.92	72.75	3.54	18.13
	E	30.61	6.11	34.81	45.53	72.59	3.40	18.32
Sheep	A	44.78	7.71	31.24	43.62	69.53	2.15	18.53
	B	44.14	8.69	30.82	42.89	68.70	2.10	18.07
	C	46.69	8.91	30.50	41.46	68.01	2.15	18.21
	E	48.13	8.75	31.67	42.61	68.52	2.06	18.40
Rabbits	A	58.22	4.63	42.93	47.28	83.52	1.51	17.58
	B	61.08	5.9	40.51	45.16	80.44	1.39	17.48
	C	69.05	6.38	40.43	46.46	79.69	1.3	17.40
	E	71.56	6.31	41.35	46.87	79.91	1.43	17.64

3.2.6 Sample preparation

All feed and faecal samples were dried in a force-draught oven at 90°C for three days, and then milled through a 0.5mm screen before being analyzed in the laboratory.

3.2.7 Analytical laboratory procedures

All laboratory analysis was done in duplicate in the Feed Analysis Laboratories of the University of KwaZulu-Natal. Both feed and faeces were analyzed for dry matter, crude protein, fat, ash, crude fibre, neutral detergent fibre, acid detergent fibre and gross energy (AOAC, 1990).

3.2.8 Calculations

All digestible nutrients, digestible coefficients and digestible energy were calculated **(Equations 32, 33 & 34)** from spreadsheets (MS Excel Windows XP, Professional, Version 2002). All apparent digestibilities were calculated from the total dry matter intakes and faecal dry matter outputs over the five-day collection periods. The apparent digestibilities of all nutrients for all animals were calculated by two methods, the digestibility by difference technique (Section 2.2.7.1), and by calculating the digestibility of the entire ration (hay and concentrate together) (Section 3.2.8.1).

3.2.8.1 Digestibility of the entire ration

Previously in section 2.2.8.1 the digestibility by difference method was discussed. Due to the unconventional digestion coefficients obtained from using this method it was decided to employ this method to evaluate critically all digestion coefficients. In this method the hay and concentrate are considered together therefore calculating one digestion coefficient for the entire ration. It has been found that in horses that forage concentrate interactions are negligible and variations in digestibility observed are due to chance variations (individual effects, period effects) as opposed to those observed in sheep (Martin-Rosset & Dulphy, 1987.

3.2.9 Statistical methods

The Genstat (7th Edition) statistical programme (Lawes Agricultural Trust, Rothamsted Experimental Station) was used for statistical analysis. Statistical differences between animals were determined from analysis of variance tables with the use of the student t-test at the 5% significance level for both collected and calculated response variables. Although a 5x5 Latin square design was used in the horses and rabbits, one of the 14% CP treatments (Diet D) was removed for statistical analysis when comparing the three animals on a 4 x 4 basis. An analysis of variance (Genstat 7th edition) compared the main effects and interaction of species and dietary treatments in a standard changeover balanced Latin square design. The horses and sheep were compared separately from the rabbits due to the large standard errors of difference that occurred when analysing all three animals together. The use of rabbits as a pilot trial animal is investigated in a separate section.

3.3 Results and Discussions

3.3.1 Feed Intake

The animals were fed at 2% (Kohnke, 1998) of their body weight in an 80:20 hay to concentrate ratio throughout the entire trial except the rabbits which were fed at 2% (80:20) for the first period but due to weight loss this percentage was increased to 4% and the hay to concentrate ratio changed from 80:20 to 60:40. Both the horse and sheep consumed most of their feed offered each day, the exception being that of the rabbits which showed large feed refusals at the beginning of the trial as well as when on the hay only treatment. Results for food and nutrient intake are given in Table 35.

There was no significant difference ($P>0.05$) between the DM intakes (g/kg BW) for the three animals. If the rabbits were to be removed from the analysis the standard error would be reduced and perhaps a significant difference could have been noted here. The rabbits consumed a higher but non-significant DM intake (g/kg BW) but showed consistently lower digestion coefficients for DM, GE, CF, ADF and NDF (Table 36). The DM intake per unit metabolic weight (g/kg $BW^{0.75}$) was significantly higher ($P<0.05$) for the miniature horses than for the sheep and rabbits.

There was a significant difference for DE intake (kJ/day) for all three species with the horses having a significantly higher ($P<0.05$) intake than the sheep and rabbits but when expressed in terms of body weight (kJ/kg BW), no significant difference could be shown between all three animals. The animals showed average body weights of 122 kg (Horse), 31 kg (sheep) and 2.16 kg (rabbits).

The DCP intake (g/day) was significantly higher for horses than for the sheep and higher in sheep than in rabbits. Diet A resulted in a significantly lower crude protein intake over Diet C and E as would be expected from a hay only diet. The DCP intake (g/kg BW) for the horses and sheep were both significantly lower than the rabbits. This could be due to the fact that the rabbits had a larger percentage of hay leavings but were consuming all of their concentrate of which they received more in comparison to the other animals (60:40 as opposed to 80:20).

Table 35 *Mean dry matter (DM), Digestible energy (DE) and digestible crude protein (DCP) intakes of horses, sheep and rabbits when given diets of varying protein percentages. Four animals in each group.*

	Diet	Type of Animal			Mean	Standard error of difference			Significance of effect		
		Horse	Sheep	Rabbit		btw species	btw diets	btw species x diet	species	diet	species x diet
DM	A	17.15	19.15	26.18	20.82						
Intake	B	17.05	19.42	32.52	23.00						
(g/kg	C	18.67	18.51	30.52	22.57	5.02	1.66	5.60	NS	NS	NS
BW)	E	17.89	19.14	22.77	19.93						
Mean		*17.69*^{a}	*19.05*^{a}	*27.99*^{a}							
DM	A	57.32	44	29.69	43.67						
Intake	B	55.48	44.61	35.60	45.23						
(g/kg	C	60.30	43.72	33.70	45.91	4.62	2.41	5.86	*	NS	NS
$BW^{0.75}$)	E	59.26	44.62	29.38	44.42						
Mean		*58.09*^{a}	*44.24*^{b}	*32.09*^{b}							
DE Intake	A	21.98	4.92	0.15	9.01						
(kJ/day)	B	21.07	5.15	0.10	8.77						
	C	24.63	5.26	0.32	10.07	0.46	0.72	1.18	*	NS	NS
	E	22.06	4.75	0.33	9.05						
Mean		*22.43*^{a}	*5.02*^{b}	*0.22*^{c}							
DCP	A	109.5	20.9	2.8	44.4^{a}						
Intake	B	129.8	27.9	2.3	53.3^{ab}						
(g/day)	C	137.8	35.4	4.1	59.1^{b}	2.59	5.32	8.39	*	*	NS
	E	149.6	29.5	4.2	61.1^{b}						
Mean		*131.7*^{a}	*28.4*^{b}	*3.3*^{c}							
DE Intake	A	0.19	0.17	0.21	0.19						
(kJ/kg	B	0.19	0.18	0.23	0.20						
BW)	C	0.23	0.18	0.19	0.20	0.01	0.03	0.05	NS	NS	NS
	E	0.21	0.18	0.18	0.18						
Mean		*0.20*^{a}	*0.18*^{a}	*0.20*^{a}							
DCP	A	0.92	0.74	1.99	1.22						
Intake	B	1.15	0.96	2.95	1.69						
(g/kg	C	1.25	1.10	3.21	1.85	0.38	0.40	0.71	*	NS	NS
BW)	E	1.23	1.10	2.89	1.74						
Mean		*1.14*^{b}	*0.97*^{bc}	*2.76*^{a}							

3.3.2 Apparent digestibility coefficients

3.3.2.1 Investigation of digestion coefficients for horses only

From Table 36 it can be see that there was a significant difference ($P<0.05$) between the calculated DE (Equation 34) of the four diets, with Diet A (hay) having a significantly lower ($P<0.05$) DE than all the other diets and Diets C and D having a significantly higher ($P<0.05$) DE than the other three diets. It was expected that Diet E, which is a race meal, would provide more available digestible energy than the other treatments yet it provided very little (11.8 *vs* 16.4) thus giving even more reason to develop an energy evaluation protocol for horse feed that could guarantee trainers a concentrate that delivers the required energy to the horse without under-providing or over-providing digestible energy. Perhaps if we could provide racehorses with concentrates that provide more digestible energy per kilogram we could reduce the amount fed to them and therefore prevent carbohydrate overload, which could ultimately lead to even more serious disorders such as colic. Larger amounts of hay could be fed so at the same time it could simulate their natural grazing as grazing trickle feeders. There was a significant difference ($P<0.05$) between the four diets in digestion coefficients of fat with Diet A (hay) having a significantly lower ($P<0.05$) fat digestibility as expected, than the other three diets, which did not differ significantly from each other. Possible reasons include those described earlier in section 2.3.3.1.

The digestion coefficients for CP differed significantly ($P<0.05$) amongst the diets with Diets A and C being significantly lower than the other three diets. This was expected from the hay diets due to its low CP content. From our results we can see that through the addition of concentrate to a horse's diet of hay only, the overall CP digestibility is increased. A possible reason for this could be due to the higher CP content of the entire ration or that the addition of the concentrate improved the digestibility of the hay protein.

In herbivores it has been shown that as the fibre content of a ration increases, the lower the nutrient digestibility value decreases (Fonnesbeck *et al.*, 1967). In this trial as the CF in the diet increased the DM and CP digestibility decreased but the DE did not respond in the same way, as Diet E which had the lowest fibre content had the second lowest DE after the hay. No other significant differences were observed amongst the other digestion coefficients in the horse.

Table 36 *Mean apparent digestibilities of dry matter (DM), gross energy (GE), crude fibre (CF), acid detergent fibre (ADF), neutral detergent fibre (NDF), fat and crude protein (CP) in horses given diets of varying protein percentages calculated for the entire ration. Five animals in each group.*

Nutrient	Diet	Apparent digestibilities and DE	Standard error of difference	LSD	Significance of effect
Calculated	A	10.85^{c}			
DE	B	12.92^{b}			*
(MJ/kg)	C	16.15^{a}	0.47	1.03	
	D	16.43^{a}			
	E	11.78^{b}			
Mean		*13.63*			
DM	A	64.19			
(%)	B	70.05			
	C	64.94	2.29	4.99	NS
	D	67.75			
	E	69.08			
Mean		*67.20*			
GE	A	62.06			
(%)	B	68.15			
	C	62.93	2.68	5.83	NS
	D	66.64			
	E	67.52			
Mean		*65.46*			
CF	A	67.64			
(%)	B	69.09			
	C	64.75	2.47	5.39	NS
	D	66.08			
	E	67.21			
Mean		*66.95*			
ADF	A	62.06			
(%)	B	65.11			
	C	59.32	2.89	6.29	NS
	D	60.88			
	E	62.49			
Mean		*61.97*			

Table 36 continued

Nutrient	Diet	Apparent digestibilities and DE	Standard error of difference	LSD	Significance of effect
NDF	A	66.56			
(%)	B	69.45			
	C	64.33	2.39	5.21	NS
	D	66.39			
	E	67.69			
Mean		*66.88*			
Fat	A	-31.4[b]			
(%)	B	31.0[a]			
	C	27.8[a]	8.92	19.44	*
	D	29.0[a]			
	E	30			
Mean		*17.3*			
CP	A	73.00[b]			
(%)	B	78.45[a]			
	C	74.16[b]	1.98	4.31	*
	D	76.46[a]			
	E	79.66[a]			
Mean		*76.35*			

3.3.2.2 Comparison of horse and sheep digestion coefficients

The coefficients of digestibility for the horses and sheep are given in Table 37 and Table 38, while the data from rabbit are presented separately in Tables 39 and 40 as many problems were encountered during the digestibility trial with these animals and comparing all three animals together gave very large standard errors. In Tables 37 and 38 the differences between the digestibility by difference method (Schneider & Flatt, 1975) and the digestibility of the complete ration method was evaluated (Schneider & Flatt, 1975). The difference in methodologies allowed us to evaluate intrinsically as to which method explains digestibility of nutrients in our experiment more accurately. Due to the large standard errors in applying the digestibility by difference technique (Table 37), the digestion coefficients for the entire ration (Table 38) were compared amongst the horses and sheep as both animals received the same proportion of hay to concentrate. The digestion coefficients obtained from the difference method may be erroneous, as they do not compare favourably with other results from literature. Schneider and Flatt (1975)

admitted that the determination of the digestibility of concentrates by the difference method introduces error as this method assumes that the digestibility of each of the feedstuffs is the same when fed together as when fed alone. There is always the possibility of associative effects between feed ingredients that needs to be taken into account. Errors arising from associative digestibility as well as errors from sampling, weighing and calculation are assigned to the feed of which the digestibility is being determined by difference, thus the range of uncertainty therefore introduced may be great (Armsby, 1885). When the proportion of concentrate to hay is low, as in our experiment, the error introduced will be the greatest. It is also the nutrients that are present in the smallest proportions, such as crude fibre and ether extract that will be affected the most (Armsby, 1917). Both these factors could have had an influence on the results obtained by the difference method in this trial. Martin-Rosset and Dulphy (1987) have shown that the proportion of the concentrate comprising the diet should preferably be at least 60% of the diet so as to get the most accurate digestibility measurements, particularly if the associated forage is of a low digestibility. Schneider and Flatt (1975) have also shown that this method can sometimes yield coefficients that are either negative or greater than 100, and although this may seem physiologically impossible they must be treated as reasonable possibilities. As from Table 37 it can be seen that DM, GE, CF, ADF and NDF all yield digestion coefficients that are greater than 100. This could be because the concentrate increased the digestibility of the hay resulting in an increase in the amount digested of one or more of the nutrients and this therefore becomes greater than the amounts present in the concentrate.

It is generally considered that ruminants have a higher digestibility of nutrients than hindgut fermenters (Hintz, 1969; Vander Noot & Gilbreath, 1970), but our results showed that the miniature horses had higher digestion coefficients than sheep for all nutrients except fat. Possible reasons for these results include:

- Horses practiced coprophagy due to boredom near the end of the 10 weeks. This could have been responsible for an increased digestibility. Faeces were however collected more often when this was found to be occurring.
- The sheep always finished their food very quickly and consumed most of the water available to them. This could have increased the rate of feed passage thorough their digestive tract and ultimately reduced digestibility.

Numerous trials have been conducted in which horses and ruminants were fed either grass or legume hay, comparing the efficiency of each to digest the various roughage components. It has been found in grass hays that dry matter was used more efficiently by sheep but CP and NFE were equally digested. Horses were also consistently unable to digest the CF and EE as efficiently as ruminants (Olsson & Ruudvere, 1955). Conversely, digestion coefficients of legume hays reported by European researchers were more favourable in horses. DM, OM, CP and NFE were digested with equal efficiency by horses and ruminants (Vander Noot & Gilbreath, 1970). The trial results follow below. Smolders *et al.*, (1990) found that the organic matter digestibility of concentrates in horses exceeded that of sheep, which agrees with the findings in this trial.

Table 37 *Mean apparent digestibilities of dry matter (DM), gross energy (GE), crude fibre (CF), acid detergent fibre (ADF), neutral detergent fibre (NDF), Fat and crude protein (CP) in horses and sheep given diets of varying protein percentages calculated by the difference method. Four animals in each group. Means with different superscripts differ significantly.*

Nutrient	Diet	Apparent digestibilities and DE			Standard error of difference			Significance of effect		
		Horse	Sheep	Mean	btw species	btw diets	btw species x diet	species	diet	species x diet
Calculated DE (MJ/kg)	A	10.76	9.22	9.99^{b}	2.47	1.64	3.18	NS	*	NS
	B	16.45	11.94	14.20^{a}						
	C	12.30	16.71	14.50^{a}						
	E	18.00	14.21	16.11^{a}						
Mean		*14.38*	*13.02*	*13.70*						
DM (%)	A	53.7	35.3	59.5^{b}	15.42	10.11	19.78	NS	*	NS
	B	96.7	67.2	31.9^{c}						
	C	71.7	89.1	80.4^{ab}						
	E	102.3	81.7	92.0^{a}						
Mean		*83.6*	*73.3*	*78.5*						
GE (%)	A	61.5	52.7	57.1^{b}	13.83	9.28	17.90	NS	*	NS
	B	97.3	70.1	83.7^{a}						
	C	71.1	92.6	81.9^{a}						
	E	102.5	82.2	92.4^{a}						
Mean		*83.1*	*74.4*	*78.8*						
CF (%)	A	68	65	66	65.2	37.4	79.6	NS	NS	*
	B	127	-26	51						
	C	34	80	57						
	E	135	7	71						
Mean		*91*	*31*	*61.0*						
ADF (%)	A	61	56	59	72.2	47.2	92.5	NS	NS	NS
	B	113	-3	55						
	C	25	96	60						
	E	153	27	90						
Mean		*88*	*44*	*66*						

Table 37 continued

Nutrient	Diet	Apparent digestibilities and DE			Standard error of difference			Significance of effect		
		Horse	Sheep	Mean	btw species	btw diets	btw species x diet	species	diet	species x diet
NDF	A	66.4	47.0	56.7	18.77	27.62	38.69	NS	NS	NS
(%)	B	107.7	33.4	70.5						
	C	50.6	19.2	34.9						
	E	106.8	54.8	80.8						
Mean		*82.9*	*38.5*	*60.7*						
Fat	A	-36.7	-8.3	-22.5[b]	2.92	11.82	14.77	*	*	NS
(%)	B	74.9	87.0	81.0[a]						
	C	72.2	95.7	84.0[a]						
	E	76.4	94.8	85.6[a]						
Mean		*46.7[b]*	*67.3[a]*	*57.0*						
CP	A	72.3	53.6	62.9[b]	3.31	4.50	6.43	*	*	*
(%)	B	89.8	66.3	78.1[a]						
	C	78.7	83.3	81.0[a]						
	E	85.6	80.2	82.9[a]						
Mean		*81.6[a]*	*70.9[b]*	*76.2*						

Table 38 *Mean apparent digestibilities of dry matter (DM), gross energy (GE), crude fibre (CF), acid detergent fibre (ADF), neutral detergent fibre (NDF), Fat and crude protein (CP) in horses and sheep given diets of varying protein percentages calculated for the entire ration (hay and concentrate). Four animals in each group. Means with different superscripts differ significantly.*

Nutrient	Diet	Apparent digestibilities and DE			Standard error of difference			Significance of effect		
		Horse	Sheep	Mean	btw species	btw diets	btw species x diet	species	diet	species x diet
Calculated DE (MJ/kg)	A	10.76	9.22	9.99^{c}	0.48	0.33	0.62	*	*	*
	B	12.93	9.72	11.32^{b}						
	C	16.21	10.66	13.44^{a}						
	E	12.29	10.20	11.24^{b}						
Mean		*13.05^{a}*	*9.95^{b}*	*11.50*						
DM (%)	A	63.70	55.34	59.52	3.26	1.91	4.01	NS	NS	NS
	B	70.16	56.10	63.13						
	C	66.53	61.84	64.19						
	E	68.89	60.50	64.69						
Mean		*67.32*	*58.45*	*62.88*						
GE (%)	A	61.54	52.72	57.13^{b}	2.85	1.94	3.71	*	*	NS
	B	68.21	55.95	62.08^{a}						
	C	64.75	60.59	62.67^{a}						
	E	70.40	58.43	64.41^{a}						
Mean		*66.22^{a}*	*56.92^{b}*	*61.57*						
CF (%)	A	67.70	64.62	66.16	3.09	1.91	3.87	NS	NS	NS
	B	69.93	60.79	65.36						
	C	66.96	65.50	66.23						
	E	70.39	62.27	66.33						
Mean		*68.75*	*63.30*	*66.02*						
ADF (%)	A	61.42	56.40	58.91	4.21	2.02	4.88	NS	NS	NS
	B	65.22	52.81	59.02						
	C	60.96	59.13	60.04						
	E	65.59	55.20	60.39						
Mean		*63.30*	*55.88*	*59.59*						

Table 38 Continued

Nutrient	Diet	Apparent digestibilities and DE			Standard error of difference			Significance of effect		
		Horse	Sheep	Mean	btw species	btw diets	btw species x diet	species	diet	species x diet
NDF	A	66.37	61.59	63.98						
(%)	B	69.72	59.23	64.47	3.26	1.93	4.03	NS	NS	NS
	C	66.00	68.83	64.91						
	E	70.57	61.14	65.85						
Mean		*68.16*	*61.45*	*64.80*						
Fat	A	-36.7	-8.3	-22.5^{b}						
(%)	B	29.2	44.2	36.7^{a}	1.80	6.43	8.08	*	*	NS
	C	32.1	54.8	43.5^{a}						
	E	33.1	45.6	39.4^{a}						
Mean		*14.4^{a}*	*34.1^{b}*	*24.3*						
CP	A	72.32	53.59	62.95^{c}						
(%)	B	77.96	57.60	67.78^{b}	1.39	1.58	2.38	*	*	*
	C	75.30	64.46	69.88^{a}						
	E	81.05	63.19	72.12^{a}						
Mean		*76.66^{a}*	*59.71^{b}*	*68.18*						

From Table 38 it can be seen that there was no significant difference ($P<0.05$) between the horses and sheep for DM, CF, ADF and NDF. The horses showed significantly higher ($P<0.05$) digestible energy values, with Diet A having a significantly lower DE value than the other diets which could be expected from a hay. Diet C showed the highest DE value amongst all diets. This was expected from Diet C as it was a grain-containing ration that is supposed to contain more fat, which could provide the animal with readily available digestible energy. Diet B showed the next highest DE value, which was unexpected as it is only a maintenance meal while Diet E which is a race meal, was expected to provide more available energy to the animal but showed the second lowest. The horse and the sheep followed very similar trends as to what ration was providing the greater amount of DE, with Diet A providing the lowest and Diet C the highest. It can also be seen from the table that the response in DE over the two levels of species is not consistent ($P<0.05$) over all levels of dietary treatments. This information alerts us to the inconsistency of energy profiling in commercial equine rations.

The horses DE values reflect discrepancies between the DE value and the energy source in the ration. This indicates a very positive area of research and investigation.

The horses showed a significantly higher ($P<0.05$) CP digestibility than the sheep. Diet A showed the significantly lowest CP digestibility with Diet C and E having the highest CP digestibility when taking the average of the horse and sheep together. As the dietary CP increased so did the apparent CP digestibility. This agrees with earlier findings in research with sheep (INRA, 1978) and horses (Martin-Rosset *et al.*, 1984). Due to the species x diet interaction, it shows that the response in CP digestibility over the two species is not consistent ($P<0.05$) over all levels of treatments. The response in DE over the two species is not consistent ($P<0.05$) over all levels of dietary treatments.

The sheep showed a significantly ($P<0.05$) higher fat digestibility than the horses. Diet A showed a significantly lower negative digestion coefficient, which could be due to the low ether extract present in the hay (Loosli, 1944). Low or occasional negative results for the apparent digestibilities of ether extract in forages have been reported before in equines (Fonnesbeck *et al.*, 1967; Darlington & Hershberger, 1968). From Table 37 and 38 it can be seen that the digestion coefficients of fat are negative for the hay only diet. Schneider and Flatt (1975) found that if the physiological effect of a feedstuff had stimulated the excretion of metabolic products such as fat or nitrogen, or it affected the digestibility of the basal ration, the feeding of this material alone or with a basal ration could produce a net negative effect, such as can be seen in this experiment. There was no significant difference ($P>0.05$) between the other three treatments fat digestion coefficients.

The horses showed a significantly higher ($P<0.05$) GE digestibility then the sheep. This corresponds to the DE results obtained. Diet A showed a significantly lower GE digestibility than the other three treatments.

3.3.2.3 Investigation into the digestion coefficients for the rabbits only

The digestion coefficients for the rabbits calculated on the entire ration is presented in Table 39. Due to large standard errors of difference reported by the digestibility by difference method in rabbits, this will not be discussed further.

Table 39 *Mean apparent digestibilities of dry matter (DM), gross energy (GE), crude fibre (CF), acid detergent fibre (ADF), neutral detergent fibre (NDF), Fat and crude protein (CP) in rabbits given diets of varying protein percentages calculated for the entire ration. Four animals in each group.*

Nutrient	Diet	Apparent digestibilities and DE	Standard error of difference	LSD	Significance of effect
Calculated DE (MJ/kg)	A	5.23	2.04	4.62	NS
	B	7.62			
	C	7.76			
	E	6.92			
Mean		*6.88*			
DM (%)	A	30.1	11.99	27.12	NS
	B	44.2			
	C	42.5			
	E	40.9			
Mean		*39.4*			
GE (%)	A	29.9	11.68	26.42	NS
	B	43.8			
	C	44.1			
	E	39.6			
Mean		*39.4*			
CF (%)	A	22.7	25.20	57.01	NS
	B	16.1			
	C	12.6			
	E	-14.7			
Mean		*9.2*			
ADF (%)	A	25.5	22.30	50.44	NS
	B	21.6			
	C	18.2			
	E	-10.4			
Mean		*13.7*			
NDF (%)	A	27.9	17.69	40.03	NS
	B	26.9			
	C	25.5			
	E	5.8			
Mean		*21.53*			

Table 39 Continued

Nutrient	Diet	Apparent digestibilities and DE	Standard error of difference	LSD	Significance of effect
Fat	A	-23.8			
(%)	B	63.8	22.83	51.65	*
	C	70.4			
	E	59.1			
	Mean	*42.2*			
CP	A	53.9			
(%)	B	66.6	8.19	18.52	NS
	C	67.9			
	E	68.1			
	Mean	*64.1*			

No significant differences in DE, DM, GE, CF, ADF, NDF and CP were found between the diets amongst the rabbits. The only significant difference was for the fat digestion coefficients, with Diet A being significantly lower ($P<0.05$) than all the other diets. This could be due to the low fat (0.88%) content of the hay diet. Extremely low and negative digestion coefficients have been found for ether extracts of rations containing low ether extract contents (Lucas & Loosli, 1944). Other possible reasons have been stated in Section 2.3.3.1.

As reported earlier by Slade and Hintz (1969) rabbits digested the CP fraction adequately but had a low fibre and energy digestibility. The rabbits had a higher CP digestibility than the sheep (Table 39) but not the horses and the fat digestibility was higher in rabbits than both horse and sheep. The high CP digestibility compared to the sheep could be due to the fact that rabbits practiced coprophagy, as this was not prevented with collars due to the smallest available collar being too big to fit the rabbits comfortably (Slade & Hintz, 1969). DE as well as the digestion coefficients of DM, GE, CF, ADF & NDF was lower in the rabbits than in the horses and sheep.

The rabbits did not fare well during the experimental period. Death, Pasteurellosis and unpalatability of the feed for the rabbits made conducting the trial a difficult procedure. The experimental feeding procedures were adjusted during the trial so as to accommodate and attempt to alleviate these problems. These included:

- Changing the total feed allocation from 2% to 4% of body weight at start of period two.
- Changing the hay to concentrate ratio from 80:20 to 60:40 in period two.
- Wetting the hay from period three.
- Treating all rabbits with 0.3ml Baytril® for three days in period four to treat Pasteurellosis.

Such an array of differences of the experimental procedures compared to the horses and sheep, as well as the differences of all major nutrients as well as their large standard errors of differences showing high individual variation, indicates that rabbits may not be suitable as pilot trial animals for equine nutrition studies.

3.3.3 Rate of passage through the digestive tract of the horse for concentrates varying in protein percentage

Numerous factors can influence the efficiency of digestion in horses. In this trial rate of passage was investigated. It is known that changes in rates of passage of foods can ultimately influence an animal's voluntary intake and the degree of food digestion as it is assumed that an increased rate of passage of food is accompanied by an increased voluntary intake of food and thus a lowered digestibility (Balch, 1950). Pearson & Merrit (1991) and Moore-Colyer *et al.* (2003) have reported MRT's ranging from 23 to 29 hours.

The method of Castle (1956) as previously described in Chapter 2 was used to analyse the results obtained in the laboratory obtained using the acid insoluble ash procedure. The cumulative excretion curves for all four diets are shown in Figures 6 and 7.

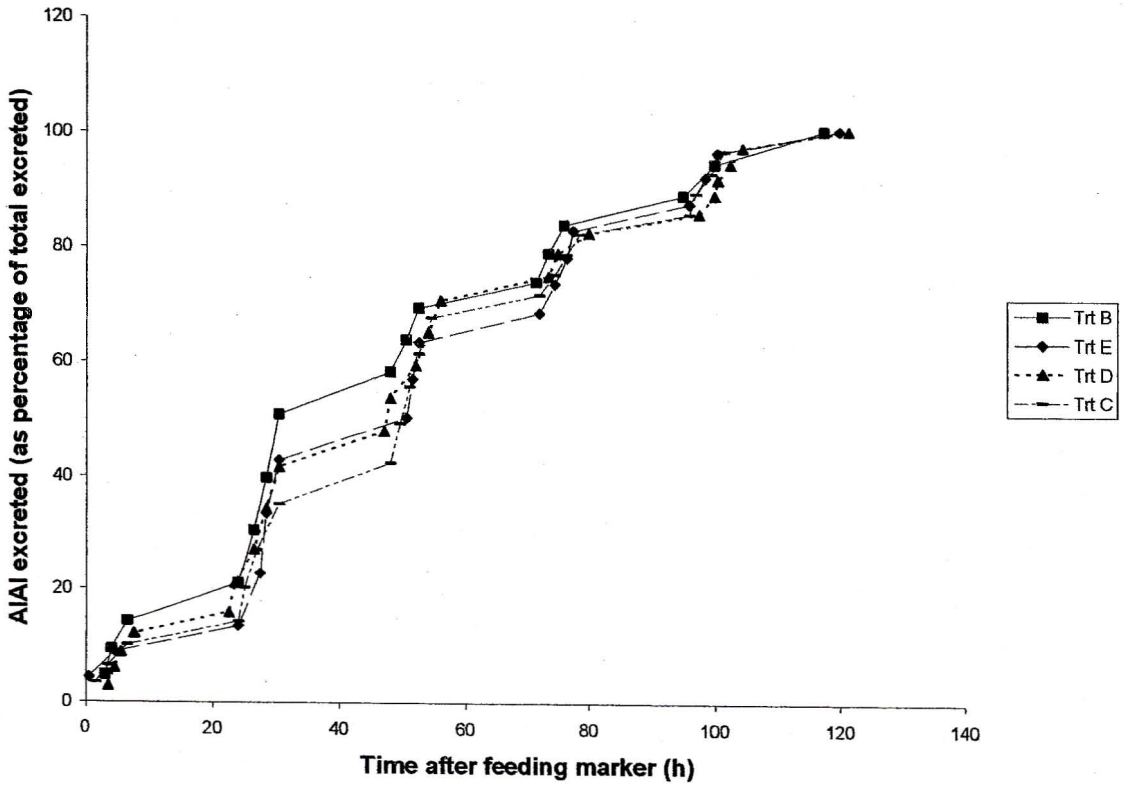

Figure 6 *Cumulative excretion curves for horse after four consecutive rates of passage studies using concentrate diets varying in protein percentage.*

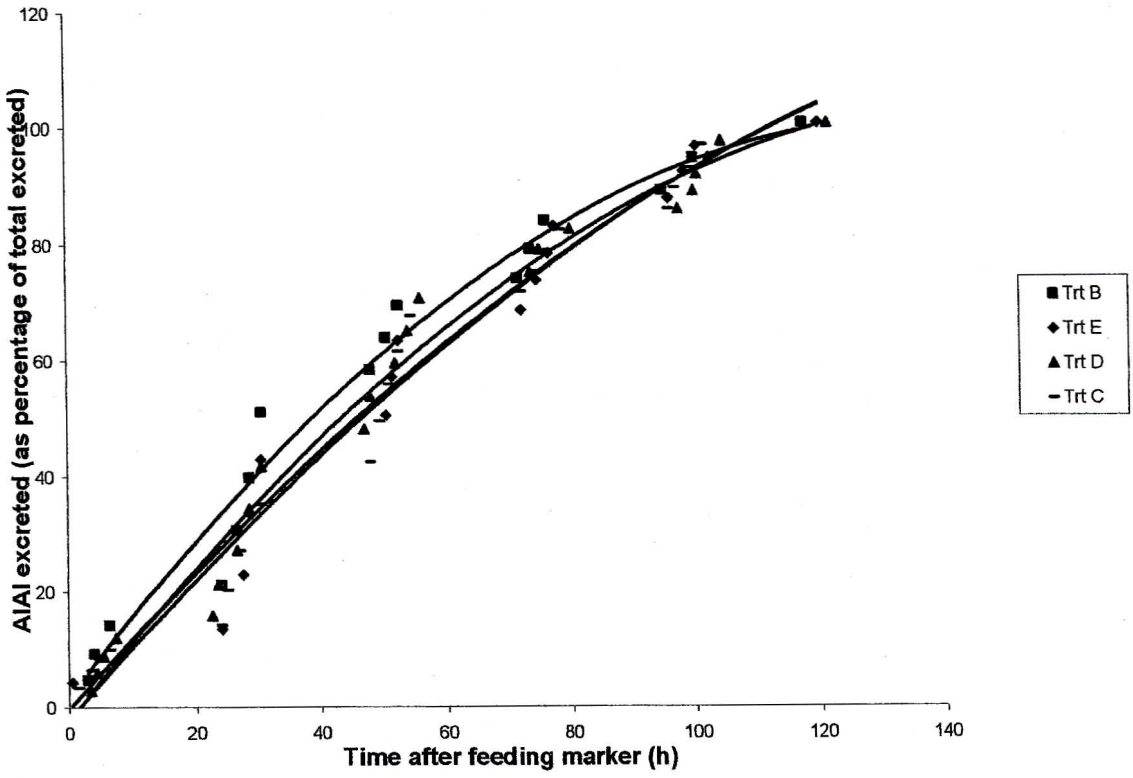

Figure 7 *Polynomial regression curves fitted to the excretion data for the horse after four consecutive rates of passage studies using concentrate diets varying in protein percentage.*

The results of the rate of passage study are presented in Table 41. Due to faulty laboratory equipment excreta from all horses could not be analyzed with the acid insoluble ash technique. Only one horse's result was available, therefore a statistical conclusion could unfortunately not be drawn in this part of the trial. Behaviour of the marker through the digestive tract of the horse on all treatments was however studied, and mean retention times were calculated from the data available.

Diet C (14% CP grain diet) showed the slowest rate of passage of 24.1 hours and was also the diet with the highest DE available to the horse. As it remained in the digestive tract the longest, it was able to be digested thoroughly and could therefore perhaps provide a greater available digestible energy for the horse. Diet C contained the highest crude fibre content of all the concentrates, which could have contributed to its longer retention in the digestive tract. Diet B (11% CP) had the fastest rate of passage of 21.08 hours and also moved through the foregut and the hindgut the quickest out of all the other diets. From the

proximate analysis Diet B had the highest moisture content and this could have been responsible for the greater rate of passage that occurred through the digestive tract.

The excretion curves for all four horses showed the same general sigmoid shape with the curves rising sharply to about the 80% point and then gradually flattening out in the final stages of excretion. All treatments showed very similar excretion patterns at the 5% excretion level ranging between 1.25 and 3 hours. This shows that the concentrate moved through the stomach very quickly and spent most of the time in the hindgut of the horse with 95% excretion occurring between 42 (Diet B) and 46 (Diet C) hours.

Table 40 *Mean retention times (MRT), 5% excretion and 95% for the four concentrate diets in hours*

Diet	MRT (h)	5 % excretion (h)	95% excretion (h)
B (11% CP)	21.08	1.25	42.00
C (14% CP (Grain))	24.10	3.00	46.00
D(14% CP (Grain free))	23.13	3.00	44.25
E (16% CP)	23.50	2.50	45.50

3.4 Conclusion

For a long time there has been lack of information pertaining to the digestive capacity of equines and this has made it necessary for nutritionists to use digestion coefficient data obtained with ruminants to formulate diets for equines. Although it has been reported earlier (Vander Noot & Gilbreath, 1970) that equines do not digest organic matter as efficiently as ruminants, the results from this experiment suggest that equines are very similar in digestive capacity to ruminants for digestibility of DM, CF, AF and NDF. There were however significant differences between the DE, fat and CP, which justifies further investigation into the adequacy of using ruminant TDN data to formulate compound feeds for horses. TDN's is equal to the combined weight of digestible crude protein and digestible carbohydrate (crude fibre and nitrogen free extractives), plus 2.25 times the weight of digestible ether extract. From the results both fat and crude protein digestibility values formed part of the value that would be used to formulate an equine diet and if these values for ruminants are not the same as the equines values then incorrect or inaccurate formulation could occur. These results indicate that the use of ruminant digestion coefficients in compounding diets for equines may give erroneous results and therefore lead to inaccurate formulations for horse. It appears from our results that sheep could perhaps be used as pilot animals when determining the fibre digestibility of a diet for horses, as there was no significant difference between horse and sheep for all fibre components (CF, ADF and NDF) of the different feeds. Digestible nutrients of horses and sheep fed roughage diets have been compared and significant differences were found (Haenlein *et al.*, 1966). It has also been reported that organic matter of low fibre feedstuffs were digested equally well by horses and cattle, but organic matter in high fibre feeds was not digested as efficiently by horses (Olsson and Ruudvere, 1955). Rabbits showed many problems during the experimental period and appear not to be ideal pilot trial animal for use in determining the digestibility of equine diets.

Although some may argue that so long as the horse can consume enough feed so as to maintain optimum body weight, condition and ensure satisfactory performance then by providing ample quantities of feed in excess of the horses requirements it will then provide for the animal, but this is costly and can lead to digestive disorders. During growth, pregnancy and lactation or when poor quality feed is being fed, feeding diets formulated to meet the horse's needs is necessary. In contrast to popular belief the horse does not select

and consume most nutrients according to its need for those nutrients (Kohnke, 1998), therefore nutritionists need to pay more attention to feeding these animals so as to ensure that their requirements are met throughout their life stages.

It appears that the Castle (1956) method could provide equine researchers with reliable rate of passage results, as our MRT's compared favourably with those of other results obtained by researchers. Although our results could not be statistically analyzed due to lack of data, it was observed that the concentrates varying in protein percentages from (10% to 16%) did not differ drastically, in mean retention times, from each other. This agrees with findings by Orton *et al.,* (1985) who found no significant difference between the rates of passage of diets (8% to 14%) through the digestive tract of horses.

As neither the sheep nor rabbit appear to be suitable pilot animals for horses, further investigation was needed to find a more suitable method in evaluating the digestible energy and digestible nutrients of equine diets. This led us to our next investigation of comparing *in vivo* results with *in vitro* results.

CHAPTER 4

AN INVESTIGATION INTO THE *IN VITRO* METHOD AS AN ACCURATE PREDICTOR OF DIGESTIBLE ENERGY IN HORSE FEEDS

4.1 Introduction

Usually feed evaluation is performed in time-consuming and costly experiments requiring animals and facilities that incur costs proportional to the accuracy required from the results obtained from the experiment.

From the previous investigation it was seen that sheep digestible energy data did not compare too favourably with those of the horse data. It was therefore decided to investigate the possibility of using the *in vitro* digestion technique as a possible method to determine the digestible energy of horse feeds. Vallance (1966) and Applegate & Hershberger (1969) have shown that the artificial rumen fermentation techniques can be utilized to study the digestibility of forages and feedstuffs by caecal microflora.

As rumen fluid was easier and more reliable to obtain than caecal fluid would be, it was decided to investigate the possibility of using rumen fluid as an inoculum for *in vitro* digestion trials and compare it to using horse faeces as an inoculum (Murray *et al.*, 2004). The results would be compared with the *in vivo* results obtained earlier.

The purpose of the present study was to:

- Compare *in vivo* digestibility results to that of *in vitro* digestibility results.
- Compare the digestibility results obtained with rumen inocula to digestibility results obtained with horse faecal innocula.
- Compare *in vitro* digestion of concentrate alone to that of concentrate in an 80:20 roughage to concentrate ratio.
- Compare the pressures (kPa) obtained from the pressure transducer investigating the effect of inocula and diets.

4.2 Materials and Methods

This trial was run at the Feed Analysis Laboratory at the University of KwaZulu-Natal. A pressure transducer (Pienaar, 1994) was used to determine pressures as well as incubate the different treatments using a modified Tilley and Terry (1963) method.

4.2.1 Experimental Design

A randomised complete blocks design was used in the analysis of 12 treatments, which were randomly allocated to 24 bottles in a modified pressure transducer machine (Figure 8). Measurements of pressure production as well as nutrient analysis before and after were obtained so as to calculate digestibilities and then analyse this in Genstat (7th Edition). Contrasts were made between concentrates incubated alone or in an 80:20 hay to concentrate ratio.

4.2.2 Animals

Rumen-fistulated, mature Jersey cows, in good condition served as donor animals for the rumen fluid required for the *in vitro* fermentation. Mature horses, in good condition served as the donor animals for the faeces required in the trial. All animals received a hay and concentrate diet.

4.2.3 Collection and 'processing' of ruminal/faecal contents

Rumen contents and horse faeces were collected by hand and squeezed through four layers of cheesecloth and placed into a thermos flask that had been previously warmed to 39 °C and flushed with carbon dioxide in the laboratory before transportation. The rumen fluid and horse faeces were then transported in separate thermos flasks back to the laboratory where 330ml of animal inoculate was mixed with 1000ml of previously prepared and warmed (39 °C) buffer solution in an Erlenmeyer flask.

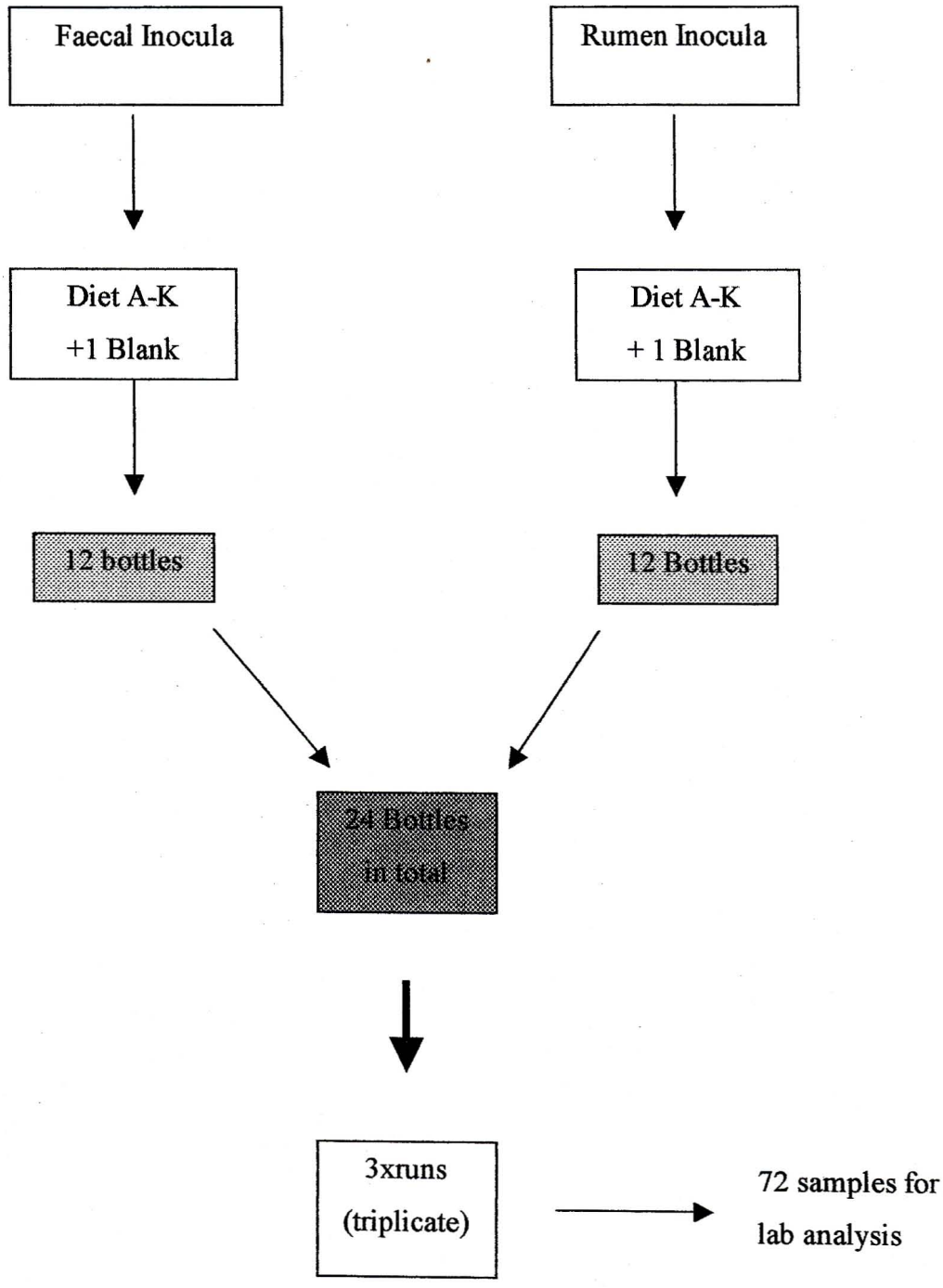

Figure 8 *Diagrammatic representation of experimental design used for in vitro trial.*

4.2.4 The analytical procedure employed in the determination of in vitro digestibility of feeds

A modified method based on the two-stage method of Tilley and Terry (1963) was used in determining the digestibility of the various feeds. Modifications included those for gas production. As fermentation occurs after digestion in the stomach in the horses and not before as in a ruminant, it was decide to swap the two procedures around therefore placing the fermentation stage as the last step and not the first.

4.2.4.1 Reagents used

1) Salivary buffer solution (McDougall, 1948)

Preparation of these buffers should be done with the following compositions:

Buffer A	g / 2000ml
Sodium bicarbonate	19.60
Disodium hydrogen phosphate anhydrous	7.40
Potassium chloride	1.14
Sodium chloride	0.94
Magnesium chloride 6-hydrate	0.26

Buffer B	g / 1000ml
Calcium chloride 2-hydrate	5.3

Immediately before use 2ml buffer B was added dropwise to 2000ml of buffer A and stirred with a magnetic hotplate stirrer for 15 minutes while carbon dioxide was bubbled through by means of a gas distributing tube. The buffer was then placed into an incubator to maintain a temperature of 39°C.

2) Rumen fluid and horse faeces inoculants

A thermos flask was heated with hot water and flushed with carbon dioxide so as to maintain anaerobic conditions. Rumen contents were removed from a fistulated heifer by hand and gently squeezed through 4 layers of cheesecloth into the thermos flask, ensuring there was at least 500ml of liquid. Once back in the laboratory, 313 ml of rumen fluid was added to 1250ml of the salivary buffer solution that was previously warmed to 39°C.

3) Acid pepsin solution

A 2000ml-graduated Erlenmeyer flask with was half filled with distilled water and 15ml of concentrated Hydrochloric acid was added and mixed well. Three grams pepsin (activity 1:10000) was added to make up to the 1500ml mark and stirring on a magnetic stirrer dissolved the pepsin.

4) Amylase

0.5ml of Amylase was added to each bottle so as to simulate any form of starch breakdown. Immediately before use, 2ml of Buffer B was added drop wise to 2000ml of Buffer A. This was mixed on a magnetic hotplate stirrer for 15 minutes while carbon dioxide was being bubbled through. This buffer was then placed into a water bath to maintain the temperature at 39°C.

4.2.4.2 First stage acid-pepsin digestion

Ten grams of each diet was placed into 250ml Pyrex/Schott bottles. Each bottle contained a magnet and was sealed with high vacuum grease (Dow Corning®). To each bottle 100ml of acid Pepsin solution was added as well as 0.5ml of Amylase. The bottles were then screwed into the lids that each contained a pressure sensor and allowed to incubate for 48 hours in the temperature controlled (39°C) pressure transducer. The stirrer was switched on for 10 seconds at the beginning of the trial. After 48 hours of incubation the bottles were removed and allowed to stand for an hour after which the supernatant was removed with a suction tube, the bottles rinsed with water and then supernatant removed once again.

4.2.4.3 Second stage rumen liquor/horse faeces digestion

The residue remaining after this acid pepsin procedure was then subjected to incubation with the respective animal inoculates. To each of the previously incubated bottles, 100ml of inoculate that had been mixed with buffer, was added to the bottles while being constantly flushed with carbon dioxide and maintained at 39 °C. These were then each screwed into lids with pressure transponders inside the temperature controlled pressure transducer so that no gas could escape. Once the door of the pressure transducer was closed, the stirrer was activated for 10 seconds. Measurements were taken every 20 minutes. After 48 hours the pressure transducer was switched off, the bottles unscrewed from their lids and then allowed to stand for an hour before following the same procedure

as before in removing the supernatant. All bottles were then placed into a force-draught oven at 100 °C for 48 hours and the residue then weighed back and sent to the laboratory for analysis. From this the weight of the residue found in the blank (which represents undigested food particles and micro-organisms present in the rumen liquor and horse faeces) was subtracted from the residue in each bottle.

4.2.5 Diets

The diets fed are shown in Table 41. Diets included a range of concentrates varying in crude protein percentage (as marketed), and a combination of that feed, as fed, with *Eragrostis Curvulae* hay in an 80:20 ratio thereby simulating a horse at maintenance.

Table 41 *Table showing the diets and their description investigated during the* in vitro *digestibility and gas production trial*

Diet	Diet description
A	*Eragrostis Curvulae*
B	10% CP Concentrate
C	11% CP Concentrate
D	14% CP (grain) Concentrate
E	14% CP (Grain-free) Concentrate
F	16% CP Concentrate
G	80 (Hay): 20 (10% CP)
H	80 (Hay): 20 (11% CP)
I	80 (Hay): 20 (14% CP)
J	80 (Hay): 20 (14% CP)
K	80 (Hay): 20 (16% CP)

A representative sample of each diet was ground through a 0.5mm sieve and then dried in a force draught oven at 60 °C for 48 hours after which they were placed into a dessicator until needed.

4.2.6 Sample Preparation

Once the residues from each run had been removed from the Schott bottles, each sample was milled through a 0.5mm screen and placed in a clearly marked bottle before being analysed in the laboratory.

4.2.7 Analytical laboratory procedures

All diets and residues remaining after the *in vitro* procedure were analysed for dry matter, crude protein, gross energy, crude fibre, ADF, NDF and fat (AOAC, 1990) in the analytical Feed Laboratory of the University of KwaZulu-Natal.

4.2.8 Calculations

All digestion coefficients and the digestible energy of all treatments were calculated (Equation 32 & 33). Pressures (kPa) were obtained for each diet every 20 minutes and these values were used to plot gas production curves that were then used to calculate the rate of gas production as well as the maximum gas production that occurred for each treatment. Results were obtained in triplicate for each treatment.

4.2.9 Statistical methods

The Genstat (7^{th} Edition) statistical programme (Lawes Agricultural Trust, Rothamsted Experimental Station) was used for statistical analysis. Statistical differences between the influences of animal inoculate, *in vivo* versus *in vitro* methodologies, feeds alone or in an 80:20 (hay to concentrate) ratio on digestibility as well as gas production were determined from analysis of variance tables.

4.3 Results and Discussions

4.3.1 Source of Inoculum

The DE and CP, GE, DM, CF, ADF and NDF digestibilities determined *in vitro* using horse faeces as a source of inoculum were not significantly ($P>0.05$) different to those values obtained *in vitro* using rumen fluid (Table 42). Murray *et al.*, (2004) reported that donor animal has little effect on the *in vitro* digestibility determinations of horse feedstuffs. There was however a significant difference ($P<0.05$) between the fat digestibility when comparing the two inocula. There was a significant difference ($P<0.05$) between the DE as well as CP, GE and DM digestibilities between the various diets regardless of source of inocula. The DE and CP, GE and DM digestibility of Diet A (hay) was shown to be the highest. A possible reason for this phenomenon is that the method employed to remove the supernatant at the end of each Tilley and Terry stage removed many of the small hay particles, which failed to settle out, therefore overestimating its digestibility. There was a trend that followed through all the nutrients showing Diets B and C to have the significantly ($P<0.05$) lowest digestibility, this could be due to there being none of the fine hay particles present in these diets therefore none of them were sucked up when the supernatant was removed. Diets B, C, D, E and F, which were all the concentrates, incubated alone without hay had the lowest DE and digestible nutrients. These low values are probably much more accurate than the other diets digestibility values due to having no small hay particles present.

4.3.2 Digestibility of concentrates incubated alone versus those incubated in an 80:20 (hay to concentrate) ratio

The digestibilities of the concentrates incubated alone were contrasted in Genstat to the same concentrates incubated in an 80 (hay) to 20 (concentrate) ratio (Table 42 & 43). There was a significant difference ($P<0.05$) for all the nutrients investigated between incubating concentrate alone or in an 80:20 hay to concentrate ratio. For Diet A to F, which are the concentrates incubated alone, the DE and digestible nutrients were consistently lower than those incubated in the 80:20 ratio. This is probably due to the method employed in this experiment to remove the supernatant as many of the small particles of hay were uncontrollably sucked up through the suction tube along with the supernatant. This would

result, as mentioned before, in an overestimation of digestibility of hay and any ration mixed with hay as has occurred here in Diets G to K.

Table 42 *Table showing the effect of the source of inocula on the* in vitro *digestibilities of the various diets.*

Nutrient	Diet	Apparent Digestibility and DE			Standard error of difference				Significance of effect			
		Horse faeces	Rumen fluid	Mean	btw inocula	btw diets	inoc*diet	contrast	btw inocula	btw diets	inoc*diet	contrast
DE	A	17.39	17.64	17.52^{a}								
(MJ/kg)	B	9.63	9.35	9.49^{d}								
	C	8.91	9.03	8.97^{d}								
	D	9.95	11.17	10.56^{cd}								
	E	11.11	10.96	11.04^{c}								
	F	11.00	11.11	11.05^{c}	0.3	0.71	0.99	1.32	NS	*	NS	*
	G	15.86	14.67	15.27^{b}								
	H	15.81	15.14	15.48^{b}								
	I	15.37	15.60	15.49b								
	J	15.18	15.42	15.30^{b}								
	K	15.83	15.55	15.69^{b}								
Mean		*13.28*	*13.24*									
CP	A	99.61	98.66	99.13^{a}								
(%)	B	78.58	75.67	77.12^{d}								
	C	71.74	72.38	72.06^{d}								
	D	79.16	85.32	82.24^{c}								
	E	82.96	82.30	82.63^{c}								
	F	81.19	83.67	82.43^{c}	1.41	3.31	4.68	3.05	NS	*	NS	*
	G	93.15	91.22	92.19^{b}								
	H	91.60	91.13	91.36^{b}								
	I	92.79	92.22	92.51^{b}								
	J	92.86	92.58	92.72^{b}								
	K	91.68	92.34	92.01^{b}								
Mean		*86.85*	*87.04*									

Table 42 Continued

Nutrient	Diet	Apparent Digestibility and DE			Standard error of difference				Significance of effect			
		Horse faeces	Rumen fluid	Mean	btw inocula	btw diets	inoc*diet	contrast	btw inocula	btw diets	inoc*diet	contras
GE	A	98.84	98.34	98.59a								
(%)	B	60.95	59.09	66.02d								
	C	56.92	57.76	57.34d								
	D	62.37	68.35	65.36c								
	E	66.62	66.54	66.58^{c}								
	F	65.85	66.51	66.18^{c}	1.75	4.10	5.79	3.74	NS	*	NS	*
	G	89.05	86.31	87.68^{b}								
	H	87.66	87.14	87.40^{b}								
	I	89.41	88.87	89.14^{b}								
	J	87.78	88.19	87.99^{b}								
	K	85.93	88.93	87.43^{b}								
Mean		*77.40*	*77.82*									
DM	A	98.90	98.40	98.65^{a}								
(%)	B	67.31	64.12	65.71^{d}								
	C	64.09	64.53	64.31^{d}								
	D	67.75	72.83	70.29^{c}								
	E	69.90	70.14	70.02^{c}								
	F	68.89	69.54	69.72^{c}	1.56	3.67	5.18	3.35	NS	*	NS	*
	G	89.93	87.55	88.74^{b}								
	H	88.63	88.27	88.45^{b}								
	I	90.00	89.62	89.81^{b}								
	J	88.71	89.02	89.86^{b}								
	K	87.01	89.83	88.27^{b}								
Mean		*80.19*	*80.32*									

Table 43 *Table showing the effect of the source of inocula on the* in vitro *digestibilities of the various diets for CF, ADF, NDF and Fat*

Nutrient (%)	Apparent digestibilities			Standard error of difference		Significance of effects	
	Horse faeces inocula	Rumen fluid inocula	Mean	Inocula	Contrast	Inocula	Contrast
CF	64.6	68.7	66.6	2.40	6.10	NS	*
ADF	63	66.9	64.9	2.71	6.63	NS	*
NDF	70.83	72.15	71.49	1.91	9.61	NS	*
Fat	72.64	76.75	74.69	1.69	4.76	*	*

4.3.3 In Vivo versus in vitro

The results comparing the *in vivo* digestibilities with that of the *in vitro* digestibilities are presented in Table 44. The two methods of determining digestibility were found to differ significantly ($P<0.05$) from each other for DE and all digestible nutrients examined. Diets were significantly different ($P<0.05$) from each other for DE and all digestible nutrients and the response in DE and all the nutrients over the three levels of feed evaluation types was not consistent ($P<0.05$) over all levels of dietary treatments. As is indicated in Table 4.4 not only were the two methods compared, but the *in vivo* method was also compared to the *in vitro* method incubated with the concentrate only as well as to the *in vitro* method that incubated the concentrate in an 80:20 ratio with hay. This was investigated closer to see the relationships.

The DE of the *in vivo* (Type 1) and both *in vitro* methods differed significantly ($P<0.05$) from each other with Type 2 (*in vitro* concentrate only) being significantly lower ($P<0.05$) than the other two types which also differed significantly ($P<0.05$) from each other. A possible reason for Type 3 (*in vitro* hay and concentrate) having the highest DE is because, as explained earlier, the method of removing supernatant between each stage of the modified Tilley and Terry method removed lots of small feed particles which could have lead to the overestimation of DE and digestibility. With it containing a small proportion of concentrate already this would have elevated the DE value even more. On investigation of the interaction between the diet and type it was observed that for Diet A and B Type 1 differed significantly from Type 2 and 3. For Diet C (14%) and Diet D (14% grain free)

Type 1 was not significantly different to Type 3 and for Diet E Type 1 was not significantly different to Type 2. Therefore from these results we can suggest that the *in vitro* method could be a reliable estimate of DE when working with diets with a higher energy and protein content.

For CP digestibility evaluation it showed that for Diets B, D and E there was no significant difference between *in vivo* and *in vitro* results obtained.

For DM digestibility it was seen that there was no significant difference between *in vivo* and *in vitro* results for Diets B, C, D and E.

GE digestibility showed no significant difference ($P>0.05$) between *in vivo* and *in vitro* results for Diets C, D and E.

For all fibre components investigated (CF, ADF and NDF) there was no relationship between *in vitro* and *in vivo* results at all and significant differences were found at all diets for all nutrients.

Fat showed no significant difference between *in vitro* and *in vivo* results for Diets B and C.

Table 44 *Table showing the difference between* in vivo *and* in vitro *digestion methods for all diets, as well as the difference between fermenting concentrate alone or in an 80:20 hay to concentrate ratio* in vitro *(1*=in vivo; *2*=in vitro*[concentrate only]; 3*=in vitro *[80hay:20concentrate]*

Nutrient	Diet	Type of digestion method				Standard error of difference			Significance of effect		
		1	2	3	Mean	Diet	Type	Diet*Type	Diet	Type	Diet*Type
DE	A	10.85^{a}	17.52^{b}	17.65^{b}	15.60						
(MJ/kg)	B	12.92^{a}	8.97^{b}	15.47^{c}	12.43						
	C	16.15^{a}	10.56^{b}	15.49^{a}	13.94	0.37	0.30	0.68	*	*	*
	D	16.43^{a}	11.04^{b}	15.33^{a}	14.14						
	E	11.78^{a}	11.06^{a}	15.60^{b}	12.87						
Mean		*13.63^{b}*	*11.83^{c}*	*15.91^{a}*							
CP	A	73^{a}	99.13^{b}	100.26^{b}	91.84						
(%)	B	78.45^{a}	72.06^{a}	91.36^{b}	80.75						
	C	74.16^{a}	82.41^{b}	92.51^{c}	83.55	1.82	1.5	3.36	*	*	*
	D	76.46^{a}	82.63^{a}	92.72^{b}	84.38						
	E	79.66^{a}	82.43^{a}	92.01^{b}	85						
Mean		*76.35^{c}*	*83.73^{b}*	*93.77^{a}*							
GE	A	62.06^{a}	98.59^{b}	104.10^{b}							
(%)	B	68.15^{a}	57.34^{b}	87.40^{c}							
	C	62.93^{a}	65.36^{a}	89.14^{b}		2.3	1.90	4.25	*	*	*
	D	66.64^{a}	66.58^{a}	87.99^{b}							
	E	67.52^{a}	66.18^{a}	87.43^{b}							
Mean		*65.46^{c}*	*70.81^{b}*	*91.21^{a}*							
DM	A	64.19^{a}	98.65^{b}	103.15^{b}	90.10						
(%)	B	70.05^{a}	64.31^{a}	88.45^{b}	74.52						
	C	64.94^{a}	70.29^{a}	89.81^{b}	75.61	2.04	1.68	3.76	*	*	*
	D	67.75^{a}	70.02^{a}	88.96^{b}	76						
	E	69.08^{a}	69.72^{a}	88.27^{b}	76.08						
Mean		*67.20^{c}*	*74.60^{b}*	*91.71^{a}*							
CF	A	67.6^{c}	105.8^{a}	89^{b}	80.9						
(%)	B	67.9^{b}	33.9^{c}	86.1^{a}	64.4						
	C	64.7^{b}	18.7^{c}	87.3^{a}	59.5	3.22	3.06	6.84	*	*	*
	D	66.1^{b}	45.7^{c}	88.7^{a}	66.6						
	E	67.2^{b}	43.6^{c}	90.7^{a}	67.2						
Mean		*66.7^{b}*	*49.6^{c}*	*88.3^{a}*							

Table 44 Continued

Nutrient	Diet	Type of digestion method				Standard error of difference			Significance of effect		
		1	2	3	Mean	Diet	Type	Diet*Type	Diet	Type	Diet*Type
ADF	A	62.1^{c}	106^{a}	88.2^{b}	77.6	3.27	3.11	6.95	*	*	*
(%)	B	65.1^{b}	36.4^{c}	84.9^{a}	63.1						
	C	59.3^{b}	14.1^{c}	87.3^{a}	55.5						
	D	60.9^{b}	36.5^{c}	88.6^{a}	61.6						
	E	62.5^{b}	40.5^{c}	90.2^{a}	63.8						
Mean		*62*^{b}	*46.7*^{c}	*87.8*^{a}							
NDF	A	66.56^{c}	105.63^{a}	89.67^{b}	80.38	2.65	2.52	5.63	*	*	*
(%)	B	69.45^{b}	44.13^{c}	86.50^{a}	67.71						
	C	64.33^{b}	34.54^{c}	87.80^{a}	62.92						
	D	66.39^{b}	50.21^{c}	89.63^{a}	67.96						
	E	67.69^{b}	54.19^{c}	90.78^{a}	69.82						
Mean		*66.88*^{b}	*57.74*^{c}	*88.88*^{a}							
Fat	A	-31.4^{b}	103.8^{a}	93.5^{a}	26.4	6.14	5.83	13.03	*	*	*
(%)	B	31^{a}	42.2^{a}	88.1^{b}	46.2						
	C	27.8^{a}	41^{a}	89.1^{b}	44.4						
	D	29^{c}	69.7^{b}	93.9^{a}	52.5						
	E	30^{c}	60.2^{b}	93.5^{a}	50.8						
Mean		*17.3*^{c}	*63.4*^{b}	*91.6*^{a}							

4.3.4 Gas production

Rates and asymptotes (maximum) of gas production were obtained by fitting an exponential curve to the gas production curves obtained for each treatment. Non-linear regression analyses were run on this data using Genstat (7th edition) to obtain these results. The results can be explained by an exponential model of the form:

$$\mathbf{Y = a + b \times (r^{x})} \qquad \textbf{Equation 41}$$

Where: a = asymptote

b = range of possible values for Y for positive values for x, such that Y = A + B if x=0 and Y = A if x is large

r = rate parameter that describes the rate of change of the curve relative to the units of x and y

a and b are linear parameters, while c is non-linear.

4.3.4.1 Rate of gas production

From Table 45 it can be seen that there was no significant difference (P>0.05) between the rate of gas production by the two different inoculums used during the *in vitro* method. The different diets did not differ significantly from each other and there was no significant interaction between inocula and the diet.

Table 45 *Table showing the rates of gas production for all diets fermented in either horse faecal inocula or rumen fluid inocula*

Diet	Rate of diff innocula			Standard error of difference			Significance of effect		
	Horse faeces	Rumen fluid	Mean	Innocula	Diet	Innoc*diet	Innocula	Diet	Innoc*diet
A	0.99713	0.99833	0.99773						
B	0.99717	0.99667	0.99692						
C	0.99823	0.99877	0.99850						
D	0.99817	0.99747	0.99782						
E	0.99765	0.99720	0.99742						
F	0.99885	0.99770	0.99827	0.0004	0.0009	0.0014	NS	NS	NS
G	0.99807	0.99703	0.99755						
H	0.99513	0.99805	0.99659						
I	0.99800	0.99840	0.99820						
J	0.99867	0.99803	0.99835						
K	0.99577	0.99767	0.99672						
Mean	*0.99753*	*0.99776*							

4.3.4.2 Maximum gas production (Asymptote)

Table 46 indicates that although there was no significant difference (P>0.05) between the maximum gas productions by the two different inoculums, there was however a significant difference (P<0.05) between the maximum gas produced by the various diets. Diet E (14% CP grain-free) had the highest maximum gas production. It has been reported that rapidly fermentable carbohydrates yield higher propionate as compared to acetate and gas is mainly only produced when a substrate is fermented to acetate or butyrate (Murray *et al.*, 2004). Therefore it was expected that Diet E would have the highest maximum gas production, as it did not contain rapidly fermentable carbohydrates, therefore it produced more acetate and butyrate as opposed to propionate, thereby having a higher gas

production. As there was no significant difference between the rate of gas production between the various diets, it is probable, looking only at maximum gas production results, that Diet E should not be a suitable choice of diet for a horse that is characteristically misbehaved and irritable as the high gas producing ability (fermentability) could contribute to the horses uncontrollability (Kohnke, 1998). If this diet is to be fed, then management factors should be controlled such as feeding the total daily allocation over smaller meals spread over the entire day

Diet F (16% CP) showed the lowest maximum gas production. This feed would be highly recommended for race horses as it is a high protein, high energy diet that does not ferment too highly in the hindgut of the horse therefore would decrease the chance of nutritional disorders developing in a horse more so than the other diets investigated. Diet's A, C and F had significantly lower maximum gas production than all the other treatments.

Table 46 *Table showing the maximum gas production (kPa) for all diets fermented in either horse faecal inocula or rumen fluid inocula*

Diet	Asymptote of diff innocula			Standard error of difference			Significance of effect		
	Horse faeces	Rumen fluid	Mean	Innocula	Diet	Innoc*diet	Innocula	Diet	Innoc*diet
A	162.2	192.1	177.1[b]						
B	245.6	179.9	212.7[a]						
C	215.5	137.7	176.6[b]						
D	168.5	199.2	183.8[a]						
E	252.6	207	229.8[a]						
F	76.8	215.2	146[b]	10.85	25.45	35.99	NS	*	*
G	236.9	201.5	219.2[a]						
H	191.4	233.7	212.6[a]						
I	218.6	219.1	218.9[a]						
J	259.4	183.9	221.6[a]						
K	195.9	236.1	216[a]						
Mean	*202.1*	*200.5*							

4.4 Conclusion

From these results we can conclude that the source of inoculum has no effect on the digestibility of a feed and when using the *in vitro* method to determine the digestible nutrients of a feed either horse faeces or rumen fluid can be used. Choice of inoculum would depend on the situation of the feed company or stud farm that required the results. If there were access to an experimental farm where fistulated cows were kept then rumen fluid would be the recommended choice due to the ease of collection. Horse defecation cannot be planned and although faecal grab samples can be taken, care should be taken so as not to damage the rectum, which could lead to septicaemia and ultimately death. However if a fistulated animal were not available then collection of fresh horse faeces would provide reliable results.

Although the *in vitro* results did not compare favourably with *in vivo* results determined previously, a possible reason would be the method of removing supernatant between each stage of the Tilley and Terry method. Further investigation is needed to find a more suitable supernatant removal method to be used in the pressure transducer that does not lose as much small hay particles as had occurred in this method. Centrifugation could be tested, however it was not used in this experiment as it was thought that transfer of the feed samples to and from the Schott bottles to the centrifuge tubes, between the two stages, would lose too much material.

There was a significant difference between incubating the concentrate alone or with hay. As mentioned before the method used to remove the supernatant between the two stages proved faulty and resulted in overestimation of digestibility for the feed in question, therefore it is recommended that the supernatant is removed by centrifugation for 15 minutes at 1,800 g (Tilley and Terry, 1963). This experiment should be rerun so as to see what effect the supernatant removal method has on the digestibility of concentrate alone versus concentrate incubated along with hay. One could also run a regression analysis using prediction equations if sequential varying (e.g. 100:0, 80:20, 70:30, 60:40 &50:50) hay to concentrate ratios were incubated *in vitro*. This would take a substantial length of time if tested *in vivo*. Therefore the *in vitro* technique remains a valuable tool of which further research would prove extremely valuable.

From the results comparing *in vivo* to *in vitro* a significant difference was found between the two methods of feed evaluation. When taking a closer look at the results it could be seen that the *in vitro* method could provide a reliable estimate for DE when feeds with a higher energy and higher protein content were being tested. This was apparent from the DE results of Diets C, D and E, which showed no significant difference between the *in vivo* and *in vitro* method of feed evaluation. It appears from the results that for most of the other nutrients, *in vitro* can be a reliable predictor of *in vivo* digestibility for diets of higher protein content.

For the gas production curves there was no significant difference in rate of gas production amongst the different diets but maximum gas production did vary. The more highly fermentable a feed is the less digestible energy it actually contains (Adesogan, 2002). This is because the fermentation process is a less efficient process than obtaining energy from a carbohydrate source directly. A greater digestible feed would however provide the animal with a greater amount of useable energy.

Once a precise diet has been formulated feeding management plays a vital role in what the horse will ultimately obtain nutritionally from its diet. For example, feeding one large meal of concentrate will result in a faster rate of passage as opposed to feeding that same amount of concentrate over three smaller meals. A faster rate of passage can result in a decreased overall digestibility (McDonald, 1985). Although this *in vitro* technique will not provide the exact results one would obtain from changing feeding strategies, it will however give a better indication of the type of change one could expect. Management decisions could be made from such results, as although exact fermentation rates or digestibilities may not be known, this is not needed for deciding on on-farm feeding management decisions. To decide on a form of feeding strategy to adopt, precise nutrient content need not be known as diets do not need to be formulated, instead the type of change to be expected needs to be determined and understood and this type of information could be obtained *in vitro*.

CHAPTER 5

GENERAL DISCUSSION, RECOMMENDATIONS AND OVERALL CONCLUSION

5.1 General Discussion

The overall aim of this thesis was to develop an energy evaluation protocol to be used in South Africa for horse feeds. Although a protocol was not as such 'developed', much investigative work was conducted that enabled any problem area to be identified so that any further research can now move forward quicker and with a greater amount of accuracy.

In Chapter Two, a preliminary trial was conducted to investigate miniature horses as possible pilot trial animals for use *in vivo* for feed evaluation. Their quiet temperament, lower feed intakes, reduced faecal output and lower overall costs makes them an ideal animal to use in digestion trials as opposed to large horses. Feeding 2% of the horses' body weight met the horses' digestible energy requirements but not the digestible crude protein requirements, this being due to the low crude protein quality in the poor quality hay that was fed. The DE results obtained from using miniature horses compared favourably to that of overseas results where large horses were used. Comparisons were based on DE equations developed overseas through large horse digestibility trial work. An equation developed by Fonnesbeck (1981) used digestible nutrients in the development of his equation and this equation showed the best fit to our digestibility results for both hay and concentrate. No significant differences were shown between DE results obtained by the male and female or the different ages. The method to calculate the digestibility of concentrates by the difference method gave questionable results, and it is believed that the assumption that the digestibility of feedstuffs are the same when fed together as when fed alone may be incorrect, and requires further testing.

Rate of passage of feedstuffs was investigated so as to determine the correct length of collection period to use when conducting digestion trials with miniature horses. Two methods were used to analyse these data namely, Grovum and Williams (1973) and Castle (1956). The method by Castle (1956) was preferred in this study due to its simplicity and ease at which results were calculated from cumulative excretion curves. The time-independent model of Grovum and Williams (1973) has also been shown to not fit

ruminant faecal excretion data very well (Pond *et al.*, 1988). Moore-Colyer (2000) has shown as well that rate of feedstuff passage through the digestive tract of equids is a time-dependent process.

A marker, Celite ®, was administered to the horses and the acid-insoluble ash method was employed to analyse the horses' faeces in the laboratory. It appears from these two methods that two and a half days is enough time to clear the digestive tract. Five days is recommended so as to ensure faeces collected are truly representative of the test diet. These two models also allow us to estimate the rates of passage through the foregut and the hindgut of the horse. This warrants further investigation as this could prove very valuable information when formulating speciality diets for horses with specific needs.

Miniature horses appeared to be the perfect *in vivo* digestibility trial animal. Using this information it was decided in Chapter Three to compare the miniature horse to other possible pilot trial animals, the sheep and rabbit. The sheep was investigated, as current equine diet formulations in South Africa are based on ruminant TDN values and the accuracy of such assumptions is unknown. Rabbits, like horses, have very similar digestive systems both being non-ruminant hindgut fermenters and would be an ideal pilot trial animal due to their size, ease of handling and reduced overall cost. Unfortunately the rabbits gave too many problems in this trial to validate their use. It is believed that the hay may have been the underlying cause of all the problems as it was quiet dusty and rabbits are very sensitive to dust. They found the hay unpalatable, therefore large hay refusals were reported and most of the rabbits lost a lot of weight. Many contracted *Pasteurellosis* and died. Feed intake was increased and proportion of hay to concentrate provided was reduced so as to try and alleviate these problems, but these did not help.

Sheep were found to be accurate predictors of digestibility for the fibre components of a horse feed, but not for other nutrients, indicating to us that there is definite room for improvement in equine diet formulation.

In Chapter Four the use of *in vitro* digestion methods as a means of predicting the digestible energy of horse feeds. As no fistulated horses were available, rumen fluid and horse faeces as sources of inoculum were compared. No significant difference was found between the two inoculums. The decision would ultimately be based on situation and animal availability as to inoculum choice.

Significant differences were found between *in vivo* and *in vitro* results. Although this perhaps shows that *in vitro* cannot be used as a predictor of digestible nutrients for horse feeds, more research is needed as the method used to remove supernatant between the two stages of the in vitro method was not reliable and removed too many fine hay particles to warrant a decision on the validity of the *in vitro* method. No significant difference was found between the rates and maximum gas production by the inoculums used. However, there was a significant difference between the maximum gas produced by the different diets. This should be investigated further as if we had the ability in this country to predict the fermentability of individual ingredients as well as the fermentability resulting from combinations of various ingredients, then we could perhaps formulate designer diets more specific to an individual horses needs and requirements.

5.2 Recommendations

The following is recommended:

- If digestibility of a concentrate is needed and the difference method is to be employed ensure that the level of concentrate inclusion is at least 60% as anything less than this could provide inaccurate results.
- As 60 % concentrate inclusion is relatively high, it may be suggested to feed the concentrate allocation over more than three times a day so as to keep carbohydrate overload to a minimum.
- Always allow adequate time for an animal to adapt to metabolism stall, especially if it has never been used before. Nervous animals can alter the true digestibility of a feed therefore animal comfort and happiness must be ensured at all times to ensure accurate digestibility results.
- Always ensure that good quality hay is provided as the basal roughage to all animals. This will prevent initial weight loss, refusals and dusty environmental conditions and prevent digestive disorders as well.
- Although it was shown that either male or female animals may be used in digestion trials, it is suggested to use mature males as it prevents urinary contamination of the faeces.
- For rate of passage studies, when using the acid-insoluble technique in the laboratory ensure that crucibles are not too old. If they are, it can slow down the procedure considerably as well as provide inaccurate results. The older the crucible gets the greater the amount of particles there are that get lodged in the porous portion of the crucible.
- If the diet whose rate of passage is being tested contains adequate AIA already, an external marker is not required. Therefore first test the diets in the lab and then decide if an external marker is needed, as this can reduce the cost of purchasing an expensive external marker.
- If using the AIA technique, ensure that the faeces do not make contact with the floor as this can affect the AIA content contained within them and provide incorrect results.
- Investigation into feeding rabbits concentrate only is needed. It is felt that the horse rations available contain enough roughage to adequately maintain the health of the

rabbit. The rabbit would be an ideal pilot trial animal for *in vivo* digestion trials for horse feeds and their ability to predict digestible energy of horse concentrates needs to be looked at in greater detail in the ideal trial conditions.

- The supernatant removal method used for the *in vitro* method between the two stages needs to be changed. Too many small hay particles were sucked up thereby affecting the overall *in vitro* digestibility results obtained. Centrifugation would be highly recommended. This method should be tested again before dismissing the possibility of *in vitro* being an accurate predictor of *in vivo* digestibility.
- A future trial recommendation would be the study of the suitability of the *in vitro* pepsin-cellulase enzyme-based prediction of digestibility. No fistulated animals are required and this method is easier to manipulate and also requires less time to complete.
- To look at results obtained *in vitro* using individual feed ingredients and then the associative effects between different feed ingredients.
- To investigate the effects on digestibility and fermentability by simulating management procedures and changing roughage to concentrate ratios *in vitro.*
- To investigate the possibility of using pigs as a pilot animal for digestibility determinations.

5.3 Overall Conclusion

Using ruminant digestion coefficients to formulate diets for horses may provide nutritionist with diets accurate enough to feed a horse adequately. The Oxford dictionary defines adequate as "passable but not outstandingly good" and this may be where our equine rations are at the moment. When considering a racehorse for example, its racing performance (phenotype) is influenced by both genetic and environmental factors. Breeders can easily manipulate the genetics today by selecting the best parents to produce offspring that hopefully go on to be our country's future champions. However, many environmental factors come into play but nutrition forms the largest part of this. Many equations are available today whereby the energy requirements of the horse can be calculated with relative accuracy. This does not help if the energy content of the diet being provided has been either overestimated or even more detrimental, underestimated. Some may argue that one can just over provide the horse with energy so that it will have at least enough to perform. This is dangerous as it can lead to severe digestive disorders such as colic that could ultimately result in death, and when a horse is worth thousands or even millions of rands, this is a chance one does not want to take. Over providing energy is not only dangerous but also very costly and can cause growth problems in the foal and foetus as well as obesity in mature horses. It is very difficult to be too precise with equine diet formulation, as each horse is truly an individual and should be fed as one. Horse nutrition in this country should be developed further by, for example, making a service available to those horse owners who want to feed their horses with a greater amount of precision such as top sport horses and during pregnancy, lactation and growth. Far too many nutritional inadequacies are subverted through volume intake. By defining gut capacities and consequences involved with substrate choice and utilization we can improve on this approach.

It is felt that although the nutrient content of the diet is very important, even more so would be the manner in which the horse is fed i.e. MANAGEMENT. Horses do not, contrary to belief, have nutritional wisdom for the digestible energy it requires (Cuddeford & Hyslop, 1996) therefore it is up to the stable manager to practice this on the horses' behalf. As the horse has evolved as a "trickle feeder", it would be only beneficial to feed it like one. Providing the daily concentrate allocation in smaller more frequent meals would simulate this type of eating behaviour and would increase digestibility and reduce nutritionally induced diseases. Most racehorses are stabled for the majority of their day except when

training and here boredom plays a huge role in their lives. This can lead to stable vices such as weaving and wind sucking which can ultimately affect their racing performance. It must be ensured that there is always a good quality, palatable hay available for each horse at all times so that stereotypies don't evolve as a function of frustrated foraging behaviour.

The feed industry in general needs rapid, robust and repeatable methods which can be used to determine the digestible nutrients available from a wide range of raw materials. NIRS may be a more widely accepted method to determine the chemical composition of raw materials but regression equations are needed to convert these values to digestible nutritive values. Due to ethical concerns an *in vitro* method must be developed so as to reduce the use of animals in digestion experiments. As horse faeces seems to be as accurate as using rumen fluid to determine digestibility, it promotes the ethical use of *in vitro* experimentation as no surgically modified animals are needed.

Although digestible energy is a poor predictor of available energy to horses as it overvalues the energy potential of high fibre foods, it is a start for us in South Africa. Development is a stepwise procedure and if the basics of feed energy evaluation are not known, then it would make further research a lot more time consuming and difficult.

Cuddeford (2000) sums it up very nicely when he said "Unfortunately, horse nutritional research is not a topical area for rigorous scientific research because there is not a simple cause and effect relationship. Coughing racehorses cannot race, but inadequately fed horses have to and, till someone takes the wider view, it is unlikely that the subject will develop apace".

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