STUDIES ON THE DEGRADATION KINETICS OF BOTANICALLY DIVERSE FIBROUS FEEDS AND THEIR APPARENT DIGESTIBILITY AND RATE OF PASSAGE WHEN FED TO PONIES

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Dedicated with love and gratitude to my mother,

Ivy H. Moore.
(1925-2000)

DECLARATION

I declare that the work in this thesis is my own, and that the thesis is my own composition.

Signed

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ABSTRACT

The horse evolved to obtain most of its dietary energy from the hindgut fermentation of fibrous feeds and so in the interests of health and welfare, the domestic horse should be fed forage-based diets. However, little information exists on the nutritive value of different forages that are potentially valuable feeds for horses. The aim of this thesis was to measure the *in vivo* apparent digestibility of a range of botanically diverse fibrous-feeds and to investigate the suitability of ruminant *in sacco* and *in vitro* techniques as routine methods for estimating, degradation kinetics and rates of passage of fibrous feeds through the gastrointestinal tract of ponies. In the first study, the *in vivo* apparent digestibility (AD) of hay (H), haylage (HY), big bale silage (BB) and clamp silage (CS) were determined in 4 ponies. Dry matter intake was significantly ($P<0.05$) greater for HY (6.3 kg/d) and BB > H > CS (2.95 kg/d). For all parameters measured AD of CS and BB were significantly ($P<0.05$) greater than for H, with HY being intermediate. The theoretical digestible energy and crude protein requirements of the ponies were met or exceeded by all diets except hay. Secondly, the *in* vivo AD, intra-caecal fermentation parameters and rates of passage of hay cubes (HC), an oat hulls:naked oats mix (OH:NO), plain sugar beet pulp (SBF), soya hulls (SH) and a hay cubes:sugar beet mix (HC:SB) were determined in 3 caecally-fistulated ponies. Total collection and mobile bag studies produced similar AD values, with SBF being greater ($P<0.05$) for all parameters measured than OH:NO and HC, with intermediate values for SB:HC and SH. All feeds maintained the intra-caecal pH and acetate molar proportions above 6.5 and 700 mmol/mol respectively. Higher levels of propionate and lactate and lower levels of butyrate were recorded for ponies fed the OH:NO compared with those on diets SBF and HC ($P<0.05$). Pre-caecal losses of total non-starch polysaccharides (TNSP) from feeds *in sacco* were highest for SBF (133g/kg) >SH > OH:NO > HC (51g/kg). In contrast, the highest crude protein (CP) losses were from OH:NO (771 g/kg) which were greater than SH = HC > SBF (296g/kg) ($P<0.05$). Of the 7 ruminant models fitted to faecal excretion data, the G3 and G4 time-dependent models of Pond *et al.* (1988) best described the passage of both chromium (Cr) and ytterbium (Yb) marked

feeds in the three diets. Mean caecal to faecal passage rates measured using Cr-mordanted feed, were *ca.* 36 h for OH:NO, which was greater than that of HC at *ca.* 26 h ($P<0.05$). Total tract mean retention time (MRT) was measured using orally administered Yb-marked feeds and averaged 43 h for OH:NO which was greater than the 30 h noted for the HC ($P<0.05$). Finally, the *in vitro* fermentation by pony faecal inoculum of hay (H) and plain sugar beet pulp (SB) in ratios of 100% H, 75:25 H:SB1, 50:50 H:SB2, 25:75 H:SB3 and 100%SB, in the presence (+N) or absence (-N) of added nitrogen was assessed by the manual pressure transducer technique of Theodorou *et al.* (1994). DM loss was significantly ($P<0.05$) greater with each addition of SB thus SB > H:SB3 > H:SB2 > HSB1 > H. Addition of N reduced the time to reach 50% of gas production (t_{50}), indicating a positive influence of N on degradation rate ($P<0.05$). Maximum DM loss (*ca.* 850 mg/g) from SB occurred within 49 h post-incubation whereas maximum degradation from the other feeds was attained at *ca.* 135 h incubation. A positive associative effect was noted on both the rate and extent of degradation of H when incubated with HSB3 (-N), indicating an increase in microbial activity associated with the addition of SB. Results from the experiments described in this thesis indicate that a range of botanically diverse fibrous feeds are suitable for incorporating into equid diets, and offer a valuable relatively energy-dense alternative to hay as the basal forage for stabled horses. Ruminant techniques for measuring *in sacco* AD and the use of markers for determining rate of passage of digesta offer rapid and reliable methods for measuring digestion of a range of fibrous feeds in ponies. *In vitro* gas production showed considerable potential as a routine method for determining DM loss and degradation rate of fibre feeds for horses

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LIST OF ABREVIATIONS

A	asymptote of as production
AbD	arabinose digestibility
Ac	acetic acid
AD	apparent digestibility
ADAS	agricultural development and advisory service
ADF	acid detergent fibre
ADFD	acid detergent fibre digestibility
ADR	acid detergent residue
AIA	acid insoluble ash
AOAC	Association of Official Analytical Chemists
ATP	adenosine triphosphate
b and c	gas production rate constants
B	gas production parameter (France *et al.*, 1993)
BB	big-bale silage
BF	bomb factor
Bu	butyric acid
^{o}C	degrees Celcius
C_1	concentration of marker in the ith sample
c2	rate parameter
Ca	calcium
CaD	calcium digestibility
Ce	cerium

CF	crude fibre
CFU	colony forming units
CH_4	methane
CO_2	carbon dioxide
CP	crude protein
CPD	crude protein digestibility
Cr	chromium
CS	clamp silage
EDTA	ethylenediaminietetraacetic acid
DCP	digestible crude protein
DCPI	digestible crude protein intake
DE	digestible energy
DEI	digestible energy intake
d.f.	degrees of freedom
DM	dry matter
DMD	dry matter digestibility
DMC	multi-compartment model of Dhanoa *et al.*,(1989)
DMI	dry matter intake
DMSO	dimethyl sulphoxide
Dy	dysprosium
ED	effective degradability
Ext D	extent of degradation
FB	faecal bags

FRGP	fractional rate of gas production
FWI	fresh weight intake
g	gramme(s)
GAX's	glucouronoarabinoxylans
GaD	galactose digestibility
GD	glucose digestibility
GE	gross energy
GED	gross energy digestibility
GIT	gastrointestinal tract
GLC	gas liquid chromatography
G1	gamma time-independent model (Pond *et al.*, 1988)
G2	gamma time-dependent model (Pond *et al.*, 1988)
G3	gamma time-dependent model (Pond *et al.*, 1988)
G4	gamma time-dependent model (Pond *et al.*, 1988)
GW	Grovum and Williams (1973) model
h	hour(s)
H	hay
H	hydrogen
HC	hay cubes
HCl	hydrochloric acid
HSB1	a 75:25 mix of hay and sugar beet pulp
HSB2	a 50:50 mix of hay and sugar beet pulp

HSB3	a 25:75 mix of hay and sugar beet pulp
HY	haylage
ICPMS	atomic emission spectroscopy
k1 and k2	passage rate constant(s)
kC	time-independent compartment
kg	kilogramme(s)
λ	lambda (rate parameter for gamma-distributed residence times
LC	lambda compartment
LED	light emitting diode
LI	large intestine
LMRT	large intestine mean retention time
LSD	least significant difference
L_T	lag time
LW	live weight
M	molar
MBT	mobile bag technique
MADF	modified acid detergent fibre
MAFF	Ministry of Agriculture Fisheries and Food
MD	mannose digestibility
ME	metabolisable energy
Mg	Magnesium
MgD	Magnesium digestibility
min	minute

mg	milligramme(s)
Mi	fraction of marker excreted in the ith defaecation
MJ	megajoule(s)
ml	millilitre(s)
MLP	maximum likelihood programme
mm	millimetre(s)
mmol	millimole(s)
mol	mol(s)
MRT	mean retention time
n	amount of substance
-N	without added nitrogen
+N	with added nitrogen
NCP	non-cellulose polysaccharide(s)
NDF	neutral detergent fibre
NDFD	neutral detergent fibre digestibility
NH_3	ammonia
NO	naked oats
NIR	near infrared reflectance spectroscopy
NRC	National research Council (1989)
NSP	non-starch polysaccharide(s)
O_2	oxygen
OH	hydroxide
OH:NO	a 67:33 mix of oat hulls:naked oats

OM	organic matter
OMD	organic matter digestibility
P	pressure
P	phosphorus
PD	phosphorus digestibility
PC	personal computer
Pr	propionic acid
PS	particle size
p.s.i.	pounds per square inch
Q and Z	gas production parameters (France *et al.*, 1993)
R^2	regression coefficient
RD	rhamnose digestibility
RG1	rhamnogalacturonan
SB	plain sugar beet pulp (experiment 3.5)
SBF	sugar beet feed (experiment 3.2 and 3.3)
SBP	un-molassed sugar beet pulp
SB:HC	a 50:50 mix of sugar beet and hay cubes
s.e	standard error
s.e.d.	standard error of difference
SH	Soya hulls
SIB	small intestine bags
Sig.	Significant
STT	small intestine transit time

t	time
ti	time elapsed since dosing to the id-point of the ith collection
T	treatment
T1	incubation time of 135 hours
T2	incubation time of 49 hours
Tb	terbium
TD	time delay
τ	time delay
t_{50}	time taken to produce 50% of the total gas production
t_{95}	time taken to produce 95% of the total gas production
TMRT	total tract mean retention time
TNSP	total non-starch polysaccharide
TT	transit time
TTT	total tract transit time
TVFA	total volatile fatty acids
μ	fractional rate of gas production
UAC	uronic acids
UACD	uronic acid digestibility
V	volume
VFA	volatile fatty acid
VFI	voluntary feed intake
WHC	water holding capacity
WSC	water soluble carbohydrates

Yb	ytterbium

Chapter 1. Introduction

> Grass is the first nourishment of all colts after they are weaned.....
> Whereas when they are fed with corn and hay, but especially with
> The first, it exposes them to unspeakable injuries.'
>
> (William Gibson 1726)

Climatic change and 55 million years of evolution altered the small dog-like creature, *Hyracotherium,* which lived in the Eocene era, into the modern-day horse, *Equus caballus* (Budiansky, 1996). The genus *Equidae* encompasses large single-hoofed Animals, which have a gastrointestinal tract designed to digest small, frequent, forage-based meals (Jackson, 1998). The *Equidae* are hindgut fermenters, with a digesta mean retention time of 36 hours (Vander Noot *et al.,* 1967). Such a digestive system is thought to be less efficient at fermenting fibrous foods than ruminants, due to the relatively limited time that the digesta is retained within the fermentation chamber (Olsson and Ruudvere, 1955; Uden and Van Soest, 1982). However, under extensive grazing systems, where low-quality high-fibre forage is abundant, this lower mean retention time can be advantageous to the horse, outweighing the lower digestive efficiency by maintaining a high passage rate of digesta and thus maximising nutrient intake. (Janis, 1976; Illius and Gordon, 1992).

Since domestication man has exploited the speed, power and endurance of the horse for agricultural, urban and military purposes, but over the last 40 years it has been primarily used as a sporting animal. Many horses are now highly trained equine athletes, whose range of sporting activities encompass long periods of relatively slow work such as endurance racing, to short bursts of high speed activity including racing and polo. These activities place great physical and metabolic demands on the animal and if it is to reach

its performance potential it must receive a balanced diet. Such a diet should take into consideration the animal's energy demands, gut capacity, digestive health and micro-nutrient requirements. However, the incidence of sub-clinical acidosis and laminitis within the racing industry alone (Cuddeford. Pers comm.) would suggest that only the first two are ever considered. Diets that maintain a healthy gut contain a minimum of 1 kg fibrous forage DM per 100 kg LW per day (NRC, 1989). On the other hand, many performance horses receive less than half this amount, as owners believe it is only possible to meet daily energy requirements if high levels of starch-based cereals are fed (Martin-Rossett and Dulphy, 1987). In the interests of efficient stable-management, these high-starch diets are generally fed in two to three meals per day, which predisposes the horse to nutrition-related metabolic disorders such as acidosis, laminitis and colic (Potter *et al.,* 1992b; Yelle, 1986). Animals with these conditions seldom perform well and frequently suffer permanent damage and accordingly are destroyed.

One strategy to help avoid such debilitating metabolic problems and to maximise digestive efficiency is to food diets that the equid gastrointestinal tract has evolved to digest. Such diets are based on fibre. However, the most common conserved fibrous-food offered to stabled horses in the UK is hay, which is generally of such low nutritional value (Frape, 1986), that supplementation with energy-dense cereals is the only way in which the animal's energy demands can be met. Recent advances in food processing for human consumption have produced high-fibre food by-products, such as citrus pulp, sugar beet pulp and Soya hulls, all of which have potential as animal foods. These foods are highly digestible by pigs (Longland and Low, 1995) and may be suitable high fibre energy-dense alternatives to hay in diets for horses. Diets containing these botanically diverse fibrous foods will increase the proportion of energy derived from the forage fraction of the diet and should allow the quantity of cereals fed to be reduced. Thus energy-dense fibre foods could help to reduce the incidence of metabolic disorders and thereby decrease the subsequent 'loss' of many performance horses.

Although many investigations have evaluated the *in vivo* digestibility of different hays in horses (Pearson and Merritt, 1991; Cymbaluk, 1990; Croizer *et al.,* 1997), few have examined the suitability for horses of fibrous foods of a diverse botanical origin. Additionally, the type of fibre these foods contain needs to be determined by detailed chemical analysis, as fibre type can influence both the speed and extent at which it is digested *in vivo* and may also affect the digestibility of other dietary ingredients. The site of digestion within the gastrointestinal tract is also important, as the yield of adenosine tri-phosphate (ATP) gained from absorbed glucose, obtained from starch digested in the small intestine, is significantly greater than that gained from absorbed volatile fatty acids (VFAs) resulting from the fermentation of food within the hindgut. Thus, the prediction of the nutritive value of a certain food within the digestive tract depends partly on knowledge of the composition of that food (Southgate *et al.,* 1978), and also the manner with which the food is processed within the gut.

Plant biomass, which forms the bulk of diets for horses, are composed of carbohydrates, proteins, lipids, nucleic acids, organic acids, vitamins and minerals. The relative quantity of each of these constituents depends on the species and the stage of maturity of the plant, although the carbohydrates generally constitute the largest proportion. Plant carbohydrates can be divided into three groups, the free sugars, glucose and fructose, the storage carbohydrates, fructans, starch and sucrose (Longland, 2000) and the structural carbohydrates, cellulose, hemicellulose and pectins, which are found in the plant cell walls. The free, soluble carbohydrates are readily digested in the small intestine of the horse, as are sucrose and starch, whilst the plant cell walls and fructans are not, but have to undergo microbial fermentation to yield VFA, which are then absorbed and metabolised to yield ATP. Chemical analysis of foodstuffs generally involves determining dry matter, organic matter, crude protein and fibre content and a detailed discussion of the methods used to determine fibre content can be found in Chapter 2 of this thesis.

In addition to knowledge of the chemical composition of a food, it is also important to know the digestibility of the constituents present, since the value of a foodstuff to an animal depends on the quantity of nutrients that are digested, absorbed and metabolised (McDonald, *et al.,* 1996). Traditionally, the total collection digestibility trial has been used to determine the digestibility of different foods in equids (Darlington and Hershberger, 1968; Cuddeford *et al.*, 1995; Aiken *et al.,* 1989). But, such a method is labour intensive, costly, requires large amounts of food and is limited to determining end-point digestibility. Several other *in vivo* Techniques for estimating digestibility, such as *in sacco* and indigestible food marker methods have been widely used in ruminants and pigs (see section 2.4). These Techniques offer the opportunity to measure degradation rate as well as increasing the number of foods tested at any one time. Since microbial degradation of foodstuffs is a time-dependent process, information on the rate of passage of foods is also of considerable value. Moreover, mathematical modelling of digesta passage through the gastrointestinal tract, coupled with information on degradation rates can be used to predict the amount of energy derived from a food and should thus improve the accuracy of ration formulation for horses.

Despite recent advances in equine nutrition research, there is still a paucity of information on the digestion and utilisation of forage by horses (Faurie *et al.,* 1992). Accordingly, this thesis attempts to investigate the nutritive value of a range of botanically diverse fibrous foods for ponies, through adapting, for use in horses, a variety of *in vivo*, *in sacco* and *in vitro* Techniques, which were originally developed for use in pigs and ruminants, coupled with detailed chemical analysis of the fibrous fraction of the foods.

The experiments undertaken were as follows:

1. **Intake and apparent digestibility of four types of conserved forage by ponies.**

   A 4X4 Latin square *in vivo* total collection digestibility trial was conducted, using 4 Welsh-cross pony geldings and four forage foods, hay, haylage big bale silage and

clamp silage. Ponies were individually housed in 10X12 ft boxes and fed 1.65 kg dry matter (DM) per 100 kg LW per day. DM intake and AD values for DM, organic matter (OM), acid detergent fibre (ADF), neutral detergent fibre (NDF), crude protein (CP), starch, gross energy (GE), calcium (Ca), phosphorus (P), magnesium (Mg), individual and total non-starch polysaccharides (NSP) were recorded for each pony. The results were then analysed for differences between foods.

2. Intra-caecal fermentation parameters and *in vivo* apparent digestibility of four botanically diverse fibre-based diets in ponies.

A 3X3 Latin square digestibility trial was conducted using three caecally fistulated Welsh-cross pony geldings. The foods tested were hay cubes (HC), sugar beet pulp (SBF), and an oat hulls:naked oat (OH:NO) food mix. Ponies were individually housed and fed *ca.* 4 kg DM per day. DM intake and AD of DM, OM, ADF, NDF, CP, starch, GE and NSP were recorded for each pony. In addition caecal chyme samples were taken 5 hours after the morning food each day, and hourly on day four and analysed for pH, VFA and lactic acid content. On completion of this experiment the same ponies were used in a single period trial to determine AD and intra-caecal fermentation parameters, when a diet of 50:50 sugar beet:hay cubes was fed.

3. Digesta passage rate and mean retention times of four botanically diverse fibre-based diets in ponies.

During the above 3X3 AD trial, rates of digesta passage through the hindgut and total tract were also examined using indigestible external markers. Chromium (Cr) mordanted food was pulse-dosed into the caecum of each pony and an oral pulse dose of ytterbium (Yb) marked food was offered just before the morning meal. Faecal samples were collected on a sliding scale of frequency for *ca.*100 hours post-dosing and analysed for Cr and Yb concentrations. Caecal samples were also collected from 9am to 5pm post-dosing in order to determine caecal outflow rates. Faecal and caecal

data were then subjected to mathematical modelling to determine digesta mean retention time and to determine whether time-independent and time-dependent ruminant compartmental models could be used to describe the marker faecal excretion patterns of ponies.

4. Degradation of four botanically diverse fibrous foodstuffs in the small intestine and total tract of ponies, as measured by the mobile bag Technique.

The mobile bag Technique was used to measure the AD and degradation kinetics of hay cubes, sugar beet, soya hulls and an oat hull : naked oats mix in three caecally fistulated Welsh-cross pony geldings. 6 X 1 cm bags were administered via a naso-gastric tube to each animal. Bags were retrieved at the ileo-caecal junction *via* a magnet placed inside the caecal cannula while those travelling through the total tract of the ponies were collected with the faeces. Bag residues were bulked for each pony for each food, and analysed for DM, OM, ADF, NDF, CP, and NSP contents. Degradation curves were then fitted to DM disappearance data, and effective degradability for 4 different residence times were calculated for each food.

5. *In vitro* degradation of mature grass hay and plain sugar beet pulp using the manual pressure transducer Technique.

The final experiment was an *in vitro* gas production study using the manual pressure transducer Technique of Theodorou *et al.* (1994). Five food mixtures, 100% hay, 75:25 hay:sugar beet, 50:50 hay:sugar beet, 25:75 hay:sugar beet and 100% sugar beet, were incubated with pony faecal inoculum in the presence or absence of added nitrogen, with residues harvested at two time periods . The resulting gas profiles and DM loss associated with each food mixture, incubation time and nitrogen treatment were compared using the model of France *et al.,* (1993). The potential of this Technique for the routine analysis of the nutrient value of fibre-food for horses was discussed.

These experiments were undertaken to both a) increase knowledge on the nutritive value of fibrous foods for ponies, with a view to providing recommendations for levels of inclusion of a range of potentially valuable fibrous foods in horse rations; and b) to investigate the suitability of different *in vivo* and *in vitro* Techniques, originally developed for determining the nutritive value of foods for ruminants and pigs, for use in equine nutrition studies.

Chapter 2. Review of literature

For those whose pleasure it is to rear horses
It is of utmost importance to provide a painstaking
Over-seer and plenty of fodder.

(Columella, 50 AD)

2.1 Equid gastrointestinal tract anatomy

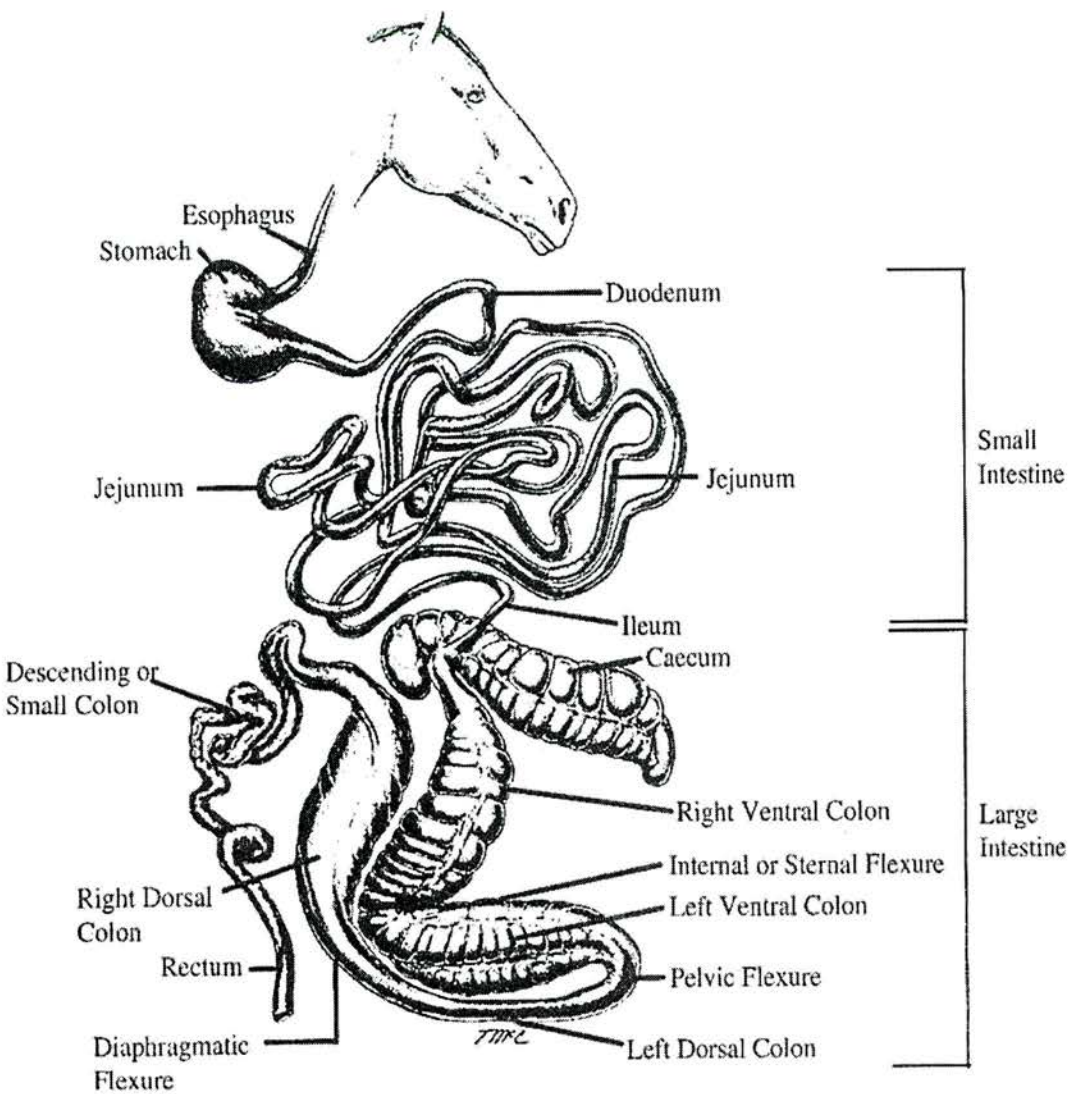
2.1.1. Introduction

Although the importance of feeding high levels of dietary fibre to horses has long been recognised, modern-day performance horses receive a narrow range of conserved fibrous foods which are based on either grass hay (the basal forage in the UK) or alfalfa hays which are popular in the USA (Pagan, 1988). Many other high-fibre foods exist such as ensiled forages, sugar beet pulp, citrus pulp and Soya hulls, which could potentially play a much greater role in equine diets. Such foods tend to be more energy-dense than grass hay and if incorporated into rations for equids, the daily energy requirements could be met on diets containing more fibre and fewer concentrates. However, before this can be achieved the suitability of these foods for horses which are hindgut fermenters, needs to be thoroughly investigated. Such studies would require a detailed examination of the type of fibre in the food, coupled with finding a reliable method to evaluate the nutritive value of that food within the horse. This review discusses equid gastrointestinal tract anatomy with particular reference to hindgut physiology in order to increase the understanding of the process of fibre degradation in the horse. Additionally, the structure of plant fibre is examined and how this may influence *in vivo* digestibility. Finally, the most commonly used *in vivo* and *in vitro* methods for determining the nutritive value and rate of digesta passage of foods in ruminants are discussed in relation to their potential application in horses, as such methods form the basis of the experiments reported in this thesis.

2.1.2. Digestive anatomy of the horse

The *Equidae* are monogastric hindgut fermenters, with the entire gut comprising only 15% of the body weight of the adult horse (Frandson, 1981). A diagrammatic representation of the digestive tract is shown in Figure 2.1 and shows the first organ of the gastrointestinal tract, the stomach to be relatively small, corresponding to *ca.* 8 litres in a 500 kg horse. Digesta leaving the stomach passes through the pyloric sphincter and enters the first region, the duodenum, of the 20 m long small intestine. The large intestine, which alone comprises 69% of total gastrointestinal tract volume, is proportionally the largest hindgut of any domestic animal (Frandson, 1981). Twenty four premolar and molar teeth chew the food gathered into the mouth, and this initial chewing is vital to ensure sufficient reduction in particle size, as food does not undergo the same degree of breakdown in the equid stomach as occurs during the rumination process in foregut fermenters (Frape, 1986). Saliva production only occurs when the horse chews. During chewing, saliva is continually secreted from the parotid, sublingual and sub maxillary glands (Alexander, 1972), therefore the quantity of saliva secreted is directly proportional to the amount of mastication. Saliva both lubricates the bolus to assist passage down the oesophagus and acts as a buffer to the hydrochloric acid produced in the stomach.

Figure 2.1 Diagram of the digestive tract of the horse.



From Lewis (1995)

On passing through the cardiac sphincter, food enters the a-glandular oesophageal region of the stomach. Here the bacterial species *Lactobacilli streptococcus* and *Bellonella gazogenes* (Kern *et al.*, 1973 & 1974), initiate lactic acid fermentation, and this fermentation continues into the fundic region of the stomach (Eldsen *et al.*, 1946). The fundic and pyloric regions are lined with gastric mucosa and produce pepsinogen, hydrochloric acid (HCl) and mucus (Argenzio, 1993). Although the production of volatile fatty acids (VFA) and lactic acid from gastric fermentation of foods is probably insignificant in terms of overall energy balance in the animal, the fermentation process does provide a source of vitamin B12, and the necessary ingredient, the 'intrinsic factor', which is required for B12 absorption further down the tract (Argenzio, 1990). The presence of HCl and pepsinogen is important for the initiation of protein breakdown, which occurs in the pyloric region of the stomach (Frape, 1986). Strictly limited nutrient absorption occurs from the stomach, although Argenzio, Southworth and Stevens (1974b) reported some absorption of VFA through the pyloric mucosa into the blood. No protein absorption occurs from the stomach and most of the lactic acid produced is either absorbed in the small intestine, or passes through to the large intestine (Alexander, 1972).

The arrival of high levels of polypeptides and acidic chyme into the small intestine triggers the release of intestinal secretions, initiating the enzymatic part of the digestive process. The exocrine glands associated with the small intestine are the pancreas, Brunners glands and Crypts of Lieberkühn, which supply a cocktail of enzymes that digest soluble carbohydrates, proteins and fats to glucose, amino acids and glycerol plus fatty acids respectively. The soluble carbohydrates and starch that are digested in the small intestine are absorbed in the jejunum and ileum, mostly as glucose although some fructose is also absorbed. Radicke *et al.* (1991) and Potter *et al.* (1992b) have suggested that ponies have a greater ability to digest sucrose and maltose than starch, because amyolytic activity in the equine small intestine is thought to be limited. Therefore, to ensure that complete enzymatic digestion of starch occurs in the small intestine, it has

been recommended that an upper limit of 0.4% of LW as starch should be fed in one meal (Potter *et al.,* 1992b). The extent of protein digestion in the small intestine is three times that which occurs in the stomach (Frandson, 1981), and potentially 500 fold that which occurs in the large intestine (Kern *et al.*, 1974), thus the small intestine is the primary site of protein digestion in the horse (Hintz and Cymbaluk, 1994). Almost all of the digestible fat is absorbed from the small intestine as either triglycerides or glycerol and fatty acids. Horses can digest diets that contain 15-20% of dietary DM as fat, with no apparent detrimental effects on digestive function (Hintz, 1990; Potter *et al.,* 1992a; Swinney *et al.,* 1995).

The large intestine of the horse consists of the sacculated caecum and large colon, the small colon and the rectum. The caecum, the first chamber of the large intestine, is a comma-shaped, 30-litre blind sac through which digesta moves cranial towards the diaphragm. The base of the caecum, which is located in the dorsal right flank, opens into the sacculated large colon, which is compartmentalised by sternal and pelvic flexures into 4 regions, the right and left ventral colon and left and right dorsal colon (Argenzio, 1993). The transverse colon, small colon and rectum follow on from the right dorsal colon and are considerably narrower than the large colon, but can be readily dilated for the storage of faeces (Frandson, 1981; Hintz, 1990). The chyme passing from the small intestine into the caecum through the ileo-caecal junction is described as 'fibrous food residues, undigested food starch and protein, micro-organisms, intestinal secretions and cell debris' (Frape, 1986). This chyme undergoes extensive microbial degradation, which continues throughout the large colon. The high rate of digesta passage through the small intestine, which can result in food arriving in the caecum 45 minutes after a meal (Frape, 1986), is greatly reduced in the large intestine. The mean retention time (MRT) of digesta in the large intestine is *ca.* 30 hours (Vander Noot *et al.,* 1967), and allows the cellulolytic bacteria and protozoa time to ferment the structural carbohydrates to VFA, carbon dioxide and methane. The three major VFA's, acetate, propionate and butyrate are normally produced in a 75:15:10 ratio when horses are fed

all forage diets, but as the proportion of grain in the ration increases, eg 3:1 forage to grain diet, values of 73:21:6 have been recorded (Glinsky *et al.*, 1976). Caecal pH is normally maintained around 6.8 to 7 (Suhartanto *et al.*, 1993; Jackson, 1998), although declines in pH have been reported by Willard *et al.* (1977) and Goodson *et al.* (1988) when starch-based diets are fed. The microbial population also produces water-soluble vitamins (the B complex) and vitamin K. Absorption from the large intestine is aided by the enlarged surface area, which allows *ca.* 95% of the fluid entering the large intestine to be reabsorbed. The large volume of highly fluid digesta, coupled with an anatomy which turns large sacs through narrow flexures, pre-disposes this region of the gastrointestinal tract to twists and impactions which can result in fatal episodes of colic (Beyer, 1998). It is therefore of paramount importance that the diet fed maintains large intestine function as well as providing essential nutrients for maintenance and production. Such diets generally contain > 50% fibre (NRC, 1989).

2.2. Dietary Fibre

2.2.1. Introduction

Hintz (1983) suggested that diets for horses should contain at least 0.12 kg of crude fibre / 100 kg LW, whereas Wolter (1993) recommended a minimum of 20% NDF or 12% ADF on an as-fed basis, of the daily ration (Hintz, 1994). Although these values do give some guideline for dietary fibre inclusion levels, they do not consider the solubility of different fibre sources, which inevitably affect digestive function. Thus, a more detailed understanding of fibre composition is required if maximum nutritive value and optimum digestive function is to be achieved when feeding horses.

2.2.2. The definition of dietary fibre

The term dietary fibre was originally defined by Trowell *et al.* (1976) 'as the remnants of plant cell walls that are resistant to digestive enzymes'. Since the introduction of this term there has been much debate as to which plant constituents should be included in the definition of dietary fibre. Currently, both a physiological and a chemical definition are commonly used and reflect the controversy surrounding this fraction of foodstuffs (Åman and Graham, 1990). The physiological definition, describes fibre as the plant cell wall components that are resistant to digestion by endogenous mammalian enzymes (Van Soest, Robertson and Lewis, 1991; Åman, 1987a; Åman, 1987b; Low, 1987; Southgate, Hudson and Englyst, 1978), whereas the chemical definition proclaims dietary fibre to be the sum of the non-starch polysaccharides (NSP) plus lignin (Noblet and Henry, 1993; Graham and Åman, 1991). The physiological definition allows for the inclusion of cell wall proteins, cutins, waxes, resistant starch and unavailable oligosaccharides (Åman and Graham, 1990), that are excluded by the chemical definition, which only recognises the polysaccharides and lignin of cell walls as dietary fibre (Theander and Åman, 1979; Southgate *et al.*, 1978). The term 'fibre' therefore, does not refer to a single botanical or chemical entity, but rather a fraction of the plant, largely consisting of cell wall material. However, for the purposes of this thesis, dietary fibre will be defined as non-polysaccharide (NSP) + lignin.

2.2.3. Biosynthesis, structure and composition of plant cell walls

Plant cell wall polysaccharides are composed of only 10 of the numerous monosaccharides that exist in nature and consist of the hexoses glucose, galactose and mannose, the pentoses xylose and arabinose, the 6-deoxyhexoses rhamnose and fucose and the hexuronic acids galacturonic, glucuronic acid and 4-O-methyl-glucuronic acid (Butler and Bailey, 1973; Åman and Graham, 1990). These monosaccharides can form many different complexes and are the result of formation of either pyran or furan rings which are connected by glycosidic linkages at carbon 3, 4 or 5 of the available hydroxyl

groups in either an α or β configuration (McDougall *et al.*, 1996). Southgate and Englyst (1985) and McDougall *et al.* (1996) categorised the cell wall carbohydrates into two major classes, the first being cellulose and the second encompassing the non-cellulose polysaccharides (NCP). The NCP category is further divided into hemicellulose composed of neutral sugars and pectins. Fahey (1994) simply divides cell wall polysaccharides into insoluble fibres composed of cellulose and hemicellulose, with soluble fibres comprising of pectin, mucilages and gums. Åman and Graham (1990) arrange the groupings of pentoses, hexoses, de-oxyhexoses and uronic acids into the five following categories:

1. Glucans - cellulose, callose, mixed linked glucans and xyloglucans.
2. Rhamnogalacturonan - plus associated arabinans and arabinogalactans.
3. Mannans - glucomannans and galactoglucomannans.
4. Xylans
5. Glucuronomannans.

However, none of these groupings clearly identifies the individual monomers with the three familiar components in cell walls ie. cellulose, hemicellulose and pectin. Longland and Low (1995) do this, producing a generalised picture of where the different cell wall monomers are largely located, although they emphasise that the particular plant part and species under investigation will dictate exactly which monomers comprise each fraction. Their grouping is as follows:

Cellulose is composed of β1-4 linked glucose units in a complex crystalline structure that constitutes the structural framework of plant cell walls.

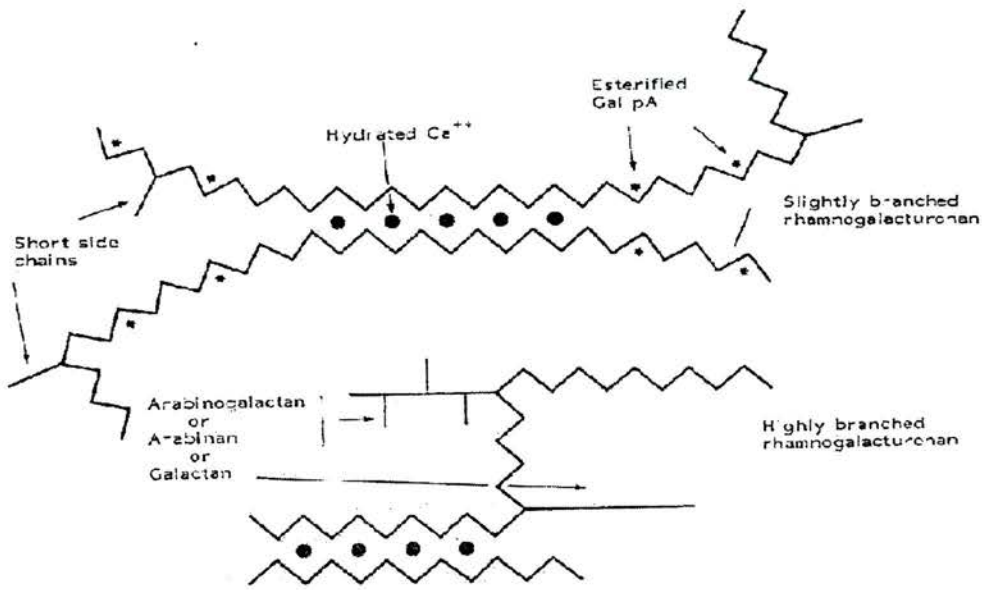
Hemicellulose is mainly composed of xylose, mannose and galactose with arabinose and mannose as minor components, whereas the pectins contain polygalacturonic acid, rhamnogalacturonan, arabinan, galactan and arabinogalactan.

Plant cell walls can vary in both chemical and physical properties, both inter- and intra-species and at different stages of growth (Graham, 1987). The development of the plant cell wall structure conveniently divides the wall into three regions, the middle lamella, a primary cell wall and a secondary cell wall. These form and develop at different stages

of growth and at different rates according to the plant species, although in all plants the middle lamella is the first stage in cell wall formation.

Growth in all living organisms is a process of hyperplasia (cell division) and hypertrophy (cell extension) and is a carefully controlled sequence, which in plants is initiated by favourable environmental conditions such as moisture, heat and light. During mitosis rapidly dividing cells in the meristematic regions each become bisected by a cell plate, which, once fully developed join onto the walls of the parent cells and allow each cell to continue with its own development (Birch and Parker, 1983). The cell plate itself then becomes the semi-fluid middle lamella, which is rich in pectic substances and functions as inter-cellular cement. The middle lamella contains a high proportion of the acidic polysaccharides, D- galacturonic acid and rhamnogalacturonan (RG1) (Fahey *et al.*, 1987; Butler and Bailey, 1973). In addition to these, pectins from different plant sources contain variable amounts of the neutral polysaccharides arabinan, arabinogalactan and galactan, which are attached to one of the acidic polysaccharides as large side chains (Smith, 1999; Reid, 1997). Some pectins, known as homogalacturonans, have a polysaccharide backbone which consists mainly of α 1-4 linked D-galacturonic acid residues with varying numbers of the carboxyl groups being methyl esters (Reid, 1997). Other pectins are not in this formation and are linked to adjacent galacturonic acid chains through Ca^{2+} bridges (Åman, 1987a; Butler and Bailey, 1973; Smith, 1999). In pectins with a low degree of esterification, the formation of gels is achieved by the straight parts of the chains lying parallel with a Ca^{2+} ion, linking a pair of residues together, while the rest forms a network which holds water within the structure (Birch and Parker, 1983) (see Figure 2.2.1).

Figure 2.2.1. Diagrammatic representation of some structural features of Rhamnogalacturonan chains.



The high water holding capacity of the pectic-rich material means that foods such as dried sugar beet pulp must be pre-soaked before being fed to horses to avoid gut distension and choke-related digestive disorders (McCarthy, 1998). Pectins are almost completely digested by pigs (Longland *et al.,* 1993; Longland *et al.,* 1994) so are a good source of dietary calcium (Ca). In monocotyledons the pectic layer accounts for only 3-6% of the cell wall DM, whereas in dicotyledons this layer can be as high as 10-20% (Chesson, 1985), thus dicotyledons are generally a better source of dietary Ca than monocotyledons.

On either side of the cell plate, deposition of pectic polysaccharides and arabinogalactans occurs forming the basis of primary cell walls (Southgate and Englyst, 1985). The primary cell wall is the thinnest component of the cell wall, being only 0.1-1μm in cross section and is formed interior to the middle lamella around the protoplast;

thus it encloses cells, which have not yet differentiated (Fahey *et al*, 1987; Reid, 1997). These cell walls undergo rapid hypertrophy, increasing in size by 10 to 20 fold by laying down a well-organised pattern of pectins, structural proteins (Showalter, 1993), hemicellulose and cellulose before growth ceases. The cellulose is composed of β 1-4 linked D-glucose units, which are twisted 180° to the next unit. The molecular weight of cellulose varies from different plant sources and ranges from 50,000 to 2,500,000, which is equivalent to 300 to 15,000 glucose residues (Lehninger, 1977; McDougall *et al*, 1996). X-ray diffraction analysis indicates that cellulose molecules, accurately referred to as 4-O-β-D-glucopyranosyl- D- glucopyranose cellobiose, are held together by strong H bonds, which are grouped together into crystalline microfibrils and it is this network which forms the structural framework of plant cell walls (Butler and Bailey, 1973; Nordvist, 1987). The cellulose microfibrils are coiled transversely around the cell and are cross linked by the hemicellulose matrix composed of either xyloglucans or glucouronoarabinoxylans (GAX's), depending on whether the primary cell wall is a type I or type II (Smith, 1999). The type I primary cell wall which is rich in pectins and hemicellulose, is found in dicotyledons and the majority of monocotyledons with the exception of the *Graminaea* (cereals and grasses), in which β-linked glucans, arabinoxylans and glucouronoarabinoxylans are more important (Åman and Graham, 1990; McDougall, 1996). The xyloglucan, which comprises 20% of cell wall DM, is highly ordered and binds tightly to the exposed faces of the microfibril glucan chains through H bonds. This cellulose - xyloglucan framework is surrounded by a pectin matrix, which although not linking to the microfibril framework, does create a network of its own. This results in the formation of a gel-like substance as interactions are formed between homogalacturonan and positively charged Ca^{2+} ions, creating junction zones. RGI is also distributed throughout this gel and the frequency of the junctions and the type of side chains on the RGI influences the structure of the gel and determines its strength. The contribution of the pectic matrix to cell strength is independent to that of the cellulose-xyloglucan network (Reid, 1997; Smith, 1999).

Type II primary cell walls are found in the *Graminaea* and contain very little pectin and xyloglucan. Instead the cellulose microfibrils are linked by glucouronoarabinoxylans (GAX's) which bind to the microfibrils and themselves through H bonds. GAX's comprise *ca* 20% of both primary and secondary cell walls and are linear polymers of β 1-4 D xylose residues with simple glucosyluronic acid residues at the O_2 position and arabinosyl residues at O_3 position of the xylosyl backbone (Smith, 1999). Binding in type II primary cell walls is not as strong as the xyloglucan - cellulose link in type I, because the side chains of arabinosyl - α glucosyluronate restricts the formation of H bonds and thus limits the degree of branching and cross linking in the wall.

The final stage of development in plant cell growth is the formation of secondary cell walls, which are formed inside the primary cell wall and outside the plasma membrane. Accompanying this development is the process of lignification, which is the laying down of phenolic compounds starting in the corners of the primary cell wall and extending outwards to the middle lamella and inwards into the developing secondary cell wall. Depending on the plant species and the stage of growth, secondary walls may account for up to 90% of cell wall DM (Åman and Graham, 1990). The composition and ultra structure of secondary cell walls varies according to cell type, although cellulose and xylans are often important constituents. Cellulose is the major component, with lignin and hemicelluloses comprising the matrix. The cellulose microfibrils are laid down in a highly ordered pattern, forming three distinct layers (Reid 1997). In the inner layer (S3) the fibrils are transversely arranged, in the next layer (S2) they are in a longitudinal orientation while in the thinner outer layer (S1) the fibrils again lie in a transverse pattern (Birch and Parker, 1983). The hemicellulose is mainly composed of 4-O-methyl-D-glucuronoxylan and lesser amounts of glucomannan.

Lignification, which is the formation of covalent linkages between lignin and the hemicelluloses, forms aromatic polymers of coniferyl, sinapyl and α p-coumaryl alcohols. P -coumaric acid and ferulic acid which are known to occur as esters in cell walls, bind to OH groups of the polysaccharides, while lignin may be bound using ether

bonds (Smith, 1999). Binding of the lignin complex is highly variable and it is believed that twenty or so different bonds exist between lignin molecules and between lignin and the polysaccharide matrix (Ford, 1986). The whole molecule is hydrophobic and lacks hydrolysable bonds, thereby making the structure extremely strong and resistant to microbial breakdown.

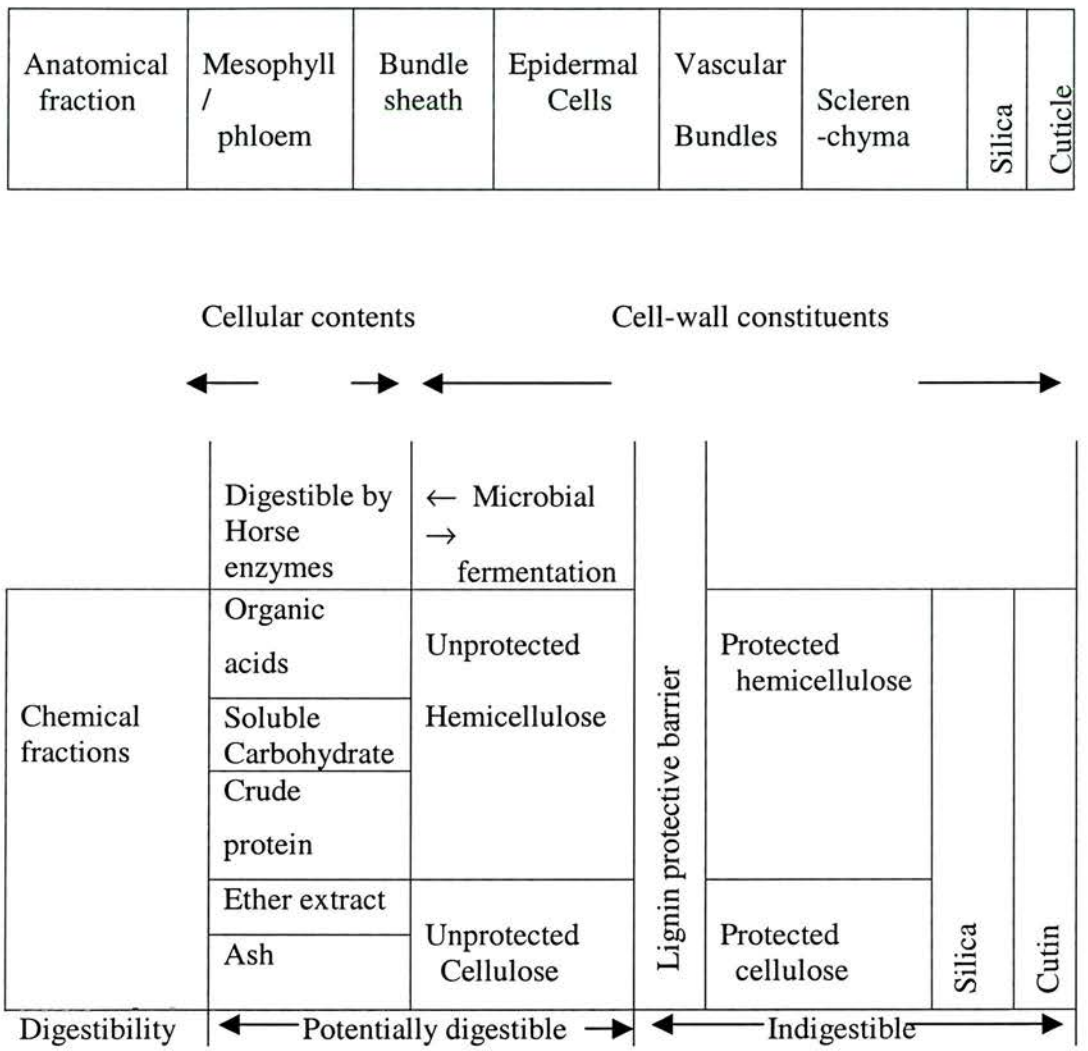
The most common proteins found in primary cell walls are extensin and a glycine rich compound in a β pleated formation found in the plasma membrane at the cell wall interface. Extensin is a glycoprotein (*ca.* 50% protein) and comprises approximately 5% of the primary cell wall in vegetables but only 0.5% in cereals (McDougall, 1996). Extensin contains *ca* 40% hydroxyproline and large amounts of serine and lysine in a rod - like formation (Lehninger, 1977; McDougall, 1996). The rod is stabilised by the presence of tetrasaccharides of arabinose and galactose, which wrap around the hydroxyproline (Butler and Bailey, 1973). Extensin appears to play a role in wall stability and extension, but how it attaches to the cell wall is poorly understood (Smith, 1999). Tyrosine residues are implicated in phenolic cross-linking of extensin and may account for the remarkable insolubility of this protein in cell walls (McDougall, 1996).

Water is a constituent of all living cells and is an important component of plant cell walls. The water content decreases as the plant cell matures, with the matrix polysaccharide - water complex (arabinoxylan) being replaced with a hydrophobic polysaccharide - lignin complex (Southgate and Englyst, 1985). In equitable environmental conditions polysaccharides contain about 8 to 10% water molecules as water of hydration. These molecules H - bond at sites not involved in inter - molecular bonding between polysaccharides. If a particularly soluble polysaccharide such as pectin is placed in water, the water penetrates the amorphous matrix and binds with available sites, thereby reducing inter - polysaccharide associations and partly breaking up the polymer, this could partially account for the high digestibility noted in monogastrics of foods such as soaked sugar beet pulp. This association with water does not happen with cellulose, as H - bonds between micro fibrils maintain the structure

(Birch and Parker, 1983), thus the bonds holding the cellulose molecule together can only be hydrolysed by microbially-secreted cellulase, which limits the extent of digestion of this structural polysaccharide within the hindgut of the monogastric.

The above account of plant cell wall development and composition illustrates the highly diverse nature of cell wall material found within foods commonly fed to horses. As cell walls contain only 10 different monosaccharides most of the diversity can be attributed to the proportions of each monomer present and the type of bonds formed between them. Thus the hydrophilic bonds found in pectins render them more degradable by microbial fermentation than the highly complex, β1-4 bonds found in cellulose. Moreover, the lignification that accompanies secondary cell wall formation renders some potentially degradable polysaccharides eg. hemicellulose and cellulose, unavailable to hindgut microbial fermentation as indicated in Figure 2.2.2. Thus, knowledge of plant cell wall development can help to explain why the glucose (comprising the cellulose) found in primary cell walls is more degradable within the equid large intestine than the glucose associated with secondary cell walls (Moore-Colyer *et al.,* 1997). Furthermore, an understanding of the relationship between the different sugar monomers comprising the three regions of the cell wall will improve interpretation of chemical analysis and thus enable a more accurate prediction of nutrient value from food composition data. However, to maximise the information obtained from food analysis the type of chemical analysis performed should be chosen with care so that an accurate profile of all the constituents of the food is achieved.

Figure 2.2.2. A conceptual model of the relation between plant anatomy and chemical fractions indicating areas of potential digestibility



(adapted from Minson, 1990)

2.3. Laboratory Analysis of Dietary Fibre.

2.3.1. Introduction

As fibrous foods often comprise the bulk of the daily dry matter intake (DMI) of horses and other large herbivores, it is particularly important to be able to quantify this fraction of the diet accurately. The first attempt to do this was by Einhoff in 1806 who used sequential applications of acids and alkalis to remove all but the reportedly indigestible fraction of the food (Cummings and Englyst, 1986). Henneberg and Stohman subsequently developed this process at the Weend Research Station, into the crude fibre method.

2.3.2. Crude Fibre

Crude fibre analysis is an empirical method which produces a residue with low nitrogen and ash content and a high C : H ratio (Cummings and Englyst 1986). However, the method is far from ideal, as frequently large amounts of hemicellulose are lost during the acid / alkali treatment and the recovery of cellulose and lignin is highly variable (Theander and Åman, 1979). The shortcomings of this method led Van Soest in 1963 to develop a new method for measuring the fibre content of foods and this he called the detergent system of analysis (Barton, 1991).

2.3.3. Acid Detergent Fibre and Neutral Detergent Fibre

Detergent analysis of dietary fibre in animal foods is still widely used and involves quantifying the residue remaining after boiling the food sample with either neutral detergent solution which yields a value for neutral detergent fibre (NDF) or boiling with an acid detergent solution, with the resultant residue being termed acid detergent fibre (ADF) (Beauchemin, 1996). Theoretically the NDF residue contains hemicellulose, cellulose and lignin, whereas ADF contains just cellulose and lignin (Morrison, 1980).

Values for hemicellulose can therefore be obtained after the subtraction of the ADF value from the corresponding NDF value and the cellulose content can be calculated after subtraction of the lignin content of the acid detergent residue (ADR); lignin is commonly measured as either acid detergent lignin hydrolysis, or by permanganate lignin (Cummings and Englyst, 1986).

This system is a rapid method for determining the fibre content of food and faeces and as the recovery of cell wall polysaccharides is relatively complete, compared to the crude fibre method, the detergent system for evaluating the nutritive value of fibrous foods is more reliable than analysis of crude fibre (Butler and Bailey, 1973; Prosky *et al.*, 1984). However, the system has several major flaws; the empirical nature of the measurement means that the end result is obtained by calculation of what is left after repeated boiling with detergent solutions, rather than what the sample contains *per se;* additionally, the ADF fraction may not just contain cellulose and lignin, but may incorporate matrix polysaccharides as well as structural polysaccharides (Morrison, 1980) and important constituents of the cell wall complex, such as pectic polysaccharides may be solubilised by neutral detergent (Theander and Åman, 1979; Johansson *et al.*, 1982; Southgate *et al.*, 1978).

Akin *et al.* (1975) found that ADF and NDF analysis produced variable recoveries of cell wall constituents in both cool and warm season grasses, with some potentially digestible components remaining in the acid detergent residue (ADR) of the warm season grasses. This contamination was also noted by Morrison (1980), who found significant quantities of hemicellulosic sugars in the ADR of 26 botanically diverse samples. The incomplete extraction of these polysaccharides was attributed to the covalent bonding known to occur between lignin and hemicellulose in secondary cell walls, which produces a compound highly resistant to chemical hydrolysis. As a result of these studies, Morrison (1980) suggests that when using gravimetric procedures to determine cell wall constituents, cellulose analysis, determined by the method of Crampton and Maynard (1938), should replace ADF as it contains less hemicellulose

contaminants due to the removal of lignin and some of the hemicellulose / lignin complexes, than the residue left after extraction in acid detergent solution. While this substitution may indeed improve the accuracy with which cell wall constituents are calculated, it still leaves room for error as both the NDF and the cellulose fractions are derived values and variable levels of hemicellulose contamination are still present. Additionally, replacing one type of gravimetric analysis with another does not overcome the problem of the loss of pectins from the residue. The loss of this fraction of the cell wall is not usually significant in members of the *Graminea* as these plants contain little pectin (Longland and Low, 1995). Collings and Yokoyama (1979) found, the detergent system over estimated the cell wall content of grasses, compared with the values obtained by more the detailed gas liquid chromatography (GLC) analysis. However, the opposite occurred with legumes where detergent analysis yielded a total cell wall value of 36.3% as opposed to the 43.51% obtained by GLC.

Despite these shortcomings, commercial laboratories still use ADF as the primary analysis for determining the cell wall content of animal foods. Indeed Canadian dairy farmers have been encouraged to have their forage analysed for ADF and NDF on the basis that it will improve the predictability of dry matter intake (DMI), assist in the estimation of digestible energy (DE) content and ensure adequate fibre is supplied in the diet (Beauchemin, 1996). Van Soest *et al.* (1991) found a significant positive correlation between NDF content and rumination time and thus concluded that NDF was a better indicator of intake and gastrointestinal fill, than any other measurement of fibre. Some researchers have developed DMI predictive equations for dairy cattle based on the NDF content of these diets eg.

$$\text{DMI (\%LW)} = 1.2 / \text{NDF (\%of DM)} \qquad \text{(Mertens, 1987)}$$

Although the above work suggests that a clear relationship exists between NDF content and DMI and DE values, other workers have not found such a relationship (Mathison, 1990). For example, Beauchemin (1996) compared the DMI of legumes and grasses

with similar NDF contents and found that the NDF from these two classes of forages were digested at different rates and had different rumen-fill characteristics, which inevitably influenced DMI. These results illustrate the limitations of gravimetric analyses as tools for predicting either the DMD or DMI of fibre-based forages by animals.

While the detergent system of analysis is considerably more accurate than that of crude fibre it still only produces values for cellulose, hemicellulose and lignin, and does not yield any information on the amounts or proportions of individual cell wall constituents. Moreover, NDF and ADF analysis cannot reveal the subtle differences in polysaccharide composition, which exist between different plants that show similar NDF values. Indeed Chesson (1985) found that NDF content alone was insufficient to explain the noted differences in digestibility between stem and leaves of the same plant that had similar NDF contents. In order to explain this some researchers felt that a more precise measurement of cell wall composition was required.

Although an evaluation of cell wall composition was detailed by Southgate (1981), it was the methods of Theander and Åman in Sweden and Englyst and Cummings in the UK who, working independently, developed methods which resulted in plant cell wall polysaccharides being described in terms of their individual monomeric sugar constituents (Englyst, 1978; Theander and Åman, 1979). This work laid the foundation for the non-starch polysaccharide (NSP) analysis of Englyst and Cummings (1984) and of Theander and Åman (1979), which are becoming more widely used.

2.3.4. Non-Starch polysaccharide (NSP) analysis

NSP analysis (detailed in appendix 1) involves three discrete steps. The first involves de-starching the sample and is particularly crucial when determining the fibre content of cereals (Englyst *et al.*, 1989). Initially de-starching procedures used only enzymes and boiling water, which proved to be inadequate for removal of retrograded or 'resistant

starch'. This starch was then erroneously measured as part of the cellulose fraction, thereby falsely increasing the NSP value. This problem has been overcome by boiling the sample with 100% dimethyl sulphoxide (DMSO) for 1 hour prior to overnight incubation with the enzymes α-amylase and pullanase to ensure complete starch removal (Englyst and Cummings 1984 and 1988).

In the second step, the cell wall polysaccharides are hydrolysed to their monomers, by boiling the de-starched sample with 12 M $H_2 SO_4$, with the lignin fraction remaining as an insoluble residue.

In the third and final step, alditol acetate derivatives of the monomers are prepared prior to their quantification by gas-liquid chromatography (GLC). Total NSP is the sum of the monomeric sugars. The acidic sugars, the uronic acids are determined using the spectrophotometric method of Scott (1979) (see appendix 1).

The relative accuracy with which fibre is determined by NSP and gravimetric procedures has been the subject of a number of studies, and in all reports improved accuracy was achieved using NSP analysis (Theander and Åman, 1982; Englyst *et al.,* 1989; Collings and Yokoyama, 1979; Longland and Low, 1995). Vervaeke *et al.*, (1991) compared CF, NDF, ADF and NSP analysis for use in determining the digestibility of fibrous foods in pigs. They reported under and over-estimation of fibre degradation in the hindgut and ileum respectively, when using the gravimetric methods compared with the chemical analysis of NSP. The reason for the discrepancies between the gravimetric and chemical analyses were discussed by the authors, and include factors such as the high inherent pectic losses, hemicellulose contamination of the ADF fraction or the formation of Maillard products. The chemical analysis of NSP does not suffer from these shortcomings and is unaffected by food processing, thus the formation of Maillard products or other artefacts do not interfere with the accuracy of the analysis (Van Soest and Mason, 1991). This coupled with the fact that evaluation of NSP content provides detailed information on food composition, which in turn may indicate the

relative ease with which the individual monomers are degraded, suggests that this procedure has considerable potential for improving the knowledge and predictability of fibre digestion in equids.

2.3.5. Near-infrared reflectance spectroscopy.

Although the analysis of NSP is considerably more accurate than the gravimetric Techniques for determining nutrient composition, it is a labour-intensive, expensive and potentially hazardous procedure. Additionally, as with all the wet chemistry methods, NSP analysis destroys the sample and is subject to the inevitable variations which occur where a high degree of manual sample manipulation is involved (Murray, 1986).

Since 1984, a modern automatic Technique, near infrared reflectance spectroscopy (NIRS), has been used by ADAS to predict CP, modified acid detergent fibre (MADF) and water-soluble carbohydrate (WSC) contents in grass silage. This procedure is used in preference to the wet chemistry Techniques, as it is fast, repeatable and less labour intensive than wet chemistry, and is widely recognised to have considerable potential as a rapid tool for evaluating forage quality. However, although NIRS is widely used by food companies for forage evaluation (Jones *et al.,* 1987), the Technique is not 100% reliable, as the problem of finding suitably robust calibration data sets, which will allow the chemical composition of a variety of forages to be calculated, has not yet been resolved. NIRS is thus only as accurate as the wet chemistry methods used to produce the calibration data set (Murray, 1986). However, once a calibration data set is compiled NIRS should provide a routine method for evaluating the chemical composition of horse foods.

2.4. Fibre digestion in equids.

2.4.1. The importance of fibre in equine diets.

Wolter (1993) details three major roles of dietary fibre in horse rations. The first is to supply the horse with an economical source of energy (from the fermentable fraction of the fibre) albeit at a lower digestive and metabolic yield than foods composed of cereals; the second is to prolong mastication which aids the psychological well-being of the animal, stimulates saliva production and regulates gastrointestinal tract motility; and the third is to supply ballast (from the indigestible fraction) which is essential for maintaining digesta transit through the gut. Other researchers concur (Tisserand, 1992; Willard *et al.,* 1977; Saastamoinen, Manninen and Rantanen, 1992; Pagan, 1997) particularly emphasising the importance of dietary fibre for maintaining gut health in horses. However, despite the fact that the popular press (Hayes, 1987; Frape, 1986) strongly recommend feeding at least 1% of body weight per day as forage DM, the role of dietary fibre in the diet of stabled horses is frequently under-rated, with many owners, preferring to food energy-dense cereal-based diets in order to meet the animal's energy requirements (Martin-Rosset and Dulphy, 1987). High grain diets frequently overshadow the contribution of forage to the energy balance of the animal (Pagan, 1998), and blithely ignore the fact that the *equidea* evolved to live on a diet of herbage alone (Janis, 1976). The quantity of ATP produced from the aerobic catabolism of the major VFA's acetate, propionate and butyrate is 12, 17 and 25 moles of ATP per mol of substrate respectively. Although this is not as efficient as the aerobic metabolism of glucose, which yields 38 moles of ATP per mol of glucose (McDonald *et al.,* 1996), Glinisky (1976) found that on a high grain : forage diet (ratio of 2:1), VFA production in the caecum alone accounted for approximately 30% of the digestible energy intake thus, VFA production is of major importance to the energy balance of the animal.

The amount of energy obtained from forage is governed by the extent of its degradation by the micro-flora within the gastrointestinal tract of the horse. The extent of

degradation is in turn determined by; a) plant species, leaf to stem ratio, and physiological maturity; b) composition, numbers and activity of the gut micro-flora and c) residence time of digesta within the fermentation chamber. (Demment and Van Soest, 1983).

2.4.2. Factors affecting the in vivo degradation of dietary fibre

2.4.2.1. Plant species and maturity

Forages fed to horses are traditionally derived from two classes of plants, grasses and legumes. At similar stages of growth legumes contain more lignin, protein, cell contents and Ca then grasses (Figure 2.4.1) therefore the energy content of legumes tends to be higher than grasses (Pagan, 1998). Whereas grasses have a higher amount of hemicellulose and thus a higher NDF content than legumes (see Table 2.4.1). If a comparison of the digestibility of the fibre portion is made between these two plant types, and fibre content is determined by NDF analysis, the digestibility of grass-fibre is higher than legume-fibre (Pagan, 1998). However, as indicated in section 2.3.3, NDF analysis results in loss of cell wall pectins, which can account for 10-20% of cell wall DM in dicotyledons (Chesson, (1985). Thus, comparing the digestibility of two plant types based on detergent analysis can lead to inaccurate conclusions on the nutrient value of different forages for horses.

Figure 2.4.1. A comparison of the fibre content of early bloom alfalfa and orchard grass hay. (source: Pagan, 1998)

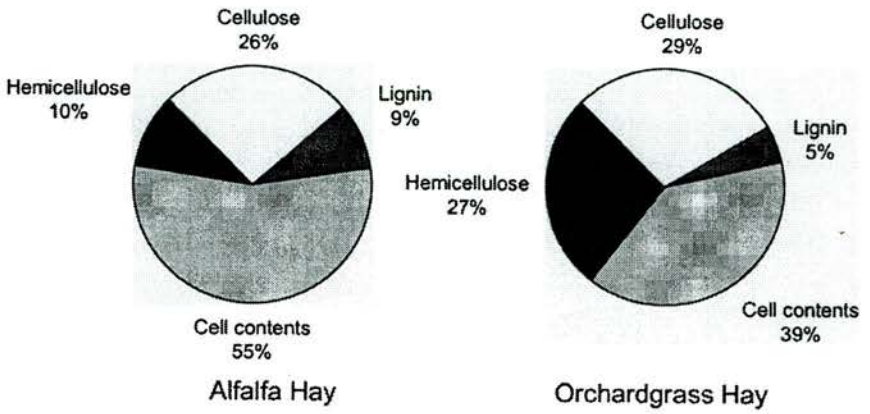


Table 2.4.1. ADF, NDF and CP content of legume and grass hays at two different stages of maturity, values expressed on a DM basis.

Species and Stage of maturity	Physical description	CP %	ADF %	NDF %
Legume pre-bloom	40-50 % leaf	> 19	31	< 40
Legume mid-bloom	25-40 % leaf	13-16	36-41	47-51
Grass hay pre-head	50 or more leaf	> 18	< 33	< 55
Grass hay at head	30 % or more leaf	8-12	39-41	61-65

(source: Pagan, 1998)

Similar caution should be exercised when choosing analytical methods for evaluating the effect of increasing plant maturity on the *in vivo* apparent digestibility of plant fibre.

Increasing physiological aging in legumes results in a greater proportion of stem to leaf and although the leaves, which have no structural role in legumes, remain highly digestible, the stems become less digestible due to increasing lignin deposition. When obtaining a legume sample for forage evaluation, particular care is required to ensure that the sample obtained is representative of the leaf to stem ratio of the forage. Grasses also decrease in digestibility as they mature, but in contrast to legumes, grass leaves do have a structural role, therefore both leaf and stem become lignified with age. The crude protein content (CP) of both legumes and grasses decreases with increasing maturity, although the decrease in CP content in grass hay is greater than the drop noted with increasing maturity in legume hays (see Table 2.4.1).

A number of studies have been published that report *in vivo* apparent digestibility coefficients for a range of grass hays in horses and ponies (Crozier *et al.*, 1997; Corino *et al.*, 1995; McLean *et al.*, 1995; Pearson and Merritt, 1991; Smoulders *et al.*, 1990; Vandernoot and Gilbreath, 1970; Darlington and Hershberger, 1968). The average *in vivo* apparent digestibility (AD) for Timothy hay, calculated from the above experiments, was 0.53 although the range was from 0.31 (McLean *et al.*, 1995) up to 0.66 (Darlington and Hershberger, (1968). Much of the variation in digestibility can be attributed to the stage of plant maturity at the time of harvest. Olsson and Rudvere (1955) noted that as the fibre content of the food increased the horses digested progressively less of the OM, CF and cellulose present, and Cuddeford *et al.* (1992) noted a similar decline in DMD when the NDF content of a food increased, indicating that there is an inverse relationship between apparent digestibility and fibre content. The composition of NDF can also have a marked affect on the degradation rates of fibre-based foods (Reid *et al.,* 1988). Stefánsdóttir, (1996) investigated the apparent digestibility (AD) of two non-forage, high-fibre foods, sugar beet pulp (SB) and Soya hulls (SH). The foods were incubated in the caecae of four ponies in forward and reverse sequences and the results recorded indicated that although the foods had similar NDF contents they were degraded at markedly different rates. After 20 hours incubation the NDF content of the SB had been reduced by 80% whereas only 55% of

the SH NDF had been degraded in this time. Clearly the type of fibre comprising the NDF fraction of these foods determined their degradation rate. Separation of cell wall polysaccharides into their individual sugar monomers by NSP analysis can help to explain the differences in fibre composition, which are not clarified by NDF analysis (Nordkvist and Åman, 1986). Such a clarification could help to explain the difference in degradation between foods and thus allow the energy content of equid diets to be manipulated by selecting foods on monomer content. Varvaeke *et al.* (1991) related the monosaccharide content of 6 cereal plus fibre diets to their digestibility in pigs. Of the monosaccharides present in significant quantities, xylose and glucose were the least degradable at 53 and 60% respectively, while the arabinose and uronic acids were the most degradable at 75 and 82% respectively. Additionally, diet degradability increased with increasing inclusion of sugar beet pulp, and this was attributed to the higher content of arabinose and uronic acids in the sugar beet food. Quantifying individual sugar monomers can also be used to study monomer proportions and how the ratios of these alter with increasing plant maturity. Albrecht, Wedin and Buxton (1987) examined monomer proportions in alfalfa and found that the proportions of glucose and xylose increased with increasing plant maturity, whereas the proportions of arabinose and galactose decreased. The altering ratios were accompanied by a decrease in the digestibility by ruminants. When comparing the digestibility of alfalfa leaves with stems, the higher digestibility of the leaves was attributed to the lower proportion of glucose, half the amount of xylose and twice the amount of arabinose detected in the leaves compared with the stems. These studies indicate the value of obtaining a detailed profile of plant cell wall constituents, which can provide some insight into the nutritional significance of the variation in fibre composition (Albrecht *et al.,* 1987).

Gibbs *et al.* (1988 and 1996) used fistulated ponies to measure the protein digestibility of hay and reported that the large intestine was the major site for CP degradation in hay. The protein held within the fibre matrix is fermented by the microbes to NH_3, which may be subsequently processed in the liver to urea, recycled or excreted via the urine (Potter *et al.*, 1992a). A high proportion of protein passing through the ileo-caecal junction is

excreted with the faeces as intact microbial protein and is therefore lost to the system (Hintz and Cymbaluk, 1994). The absorbed N appears to travel to the portal blood but according to the N^{15} labelled studies of Slade (1971), this N is poorly absorbed by the horse, however it is likely that any of the N liberated as a result of fermentation will be utilised by the microbial population. A proportion of the protein content of some high quality fibrous foods such as alfalfa, are digested in the small intestine. Gibbs *et al.* (1988) reported a proportional increase of 19.1% in pre-caecal CP digestibility when a Coastal Bermuda hay containing 110g/kg DM CP was replaced with an 180 g/kg DM CP alfalfa hay.

These studies clearly indicate the importance of feeding high quality forage to horses. Leafy well-conserved forage will provide a high proportion of readily digestible cell contents ensuring a degree of small intestine protein digestibility, while a lower ADF content will maximise fermentation of structural carbohydrates and liberate CP for the regeneration and maintenance of a healthy microbial population. Manipulation of poor quality forages, such as treating straw with ammonia, can increase the DMD and NDFD of the forage, but it does not appear to increase the protein digestibility (Hansen, Webb and Webb, 1992). These workers reported that six quarter horse mares digested 62% of the CP in wheat straw, but only 57.6% of the CP contained in ammonia treated straw. The lower digestibility on the ammonia straw was attributed to a greater faecal N output suggesting that no additional N was absorbed through the large intestine wall. Moreover, the authors also reported a higher total tract CP digestibility when horses were fed alfalfa hay than from either of the straw forages, thus confirming the superiority of alfalfa forage for maximising CP digestion (CPD) in horses. The superior CPD of alfalfa is undoubtedly due to the higher cell contents found in legumes compared with that found in the *graminea* at a similar stage of growth. Such findings clearly demonstrate the importance of feeding horses leafy fibre-foods, harvested at the vegetative stage of growth, if nutrient digestibility is to be maximised from this food source.

2.4.2.2. Hindgut microflora

Julliand (1992) has reviewed the limited number of published studies on the microbiology of the equid hindgut. The major micro-organisms present are bacteria, protozoa and fungi, which exist in the LI in a similar ecosystem to that of the rumen.

The bacteria comprise the largest proportion of the microbial population in the hindgut, with the highest concentration being found in the caecum (Mackie and Wilkins, 1988). Caecal bacteria numbers vary according to the diet and experimental conditions. The highest concentrations of 2.6 X 10^9 colony forming units (CFU) per ml of caecal digesta, recorded by Mackie and Wilkins (1988), from slaughtered grass-fed Arab horses, down to 1.2 X 10^8 CFU/ml from fistulated ponies fed 15% CP pellets (de Vaux, 1992). The major groups of bacteria present in the large intestine of the horse are detailed by Julliand (1992).

Mackie and Wilkins (1988) characterised the bacteria in the caecum and colon of one horse and found a diverse population with glycolytic, amylolytic, proteolytic, hemicellulolytic, cellulolytic and lactate utilising activities. Kern *et al.* (1974) found the cellulolytic bacteria to be particularly active in the caecum, despite the population of this group of bacteria accounting for only 1% of the total caecal flora. Hemicellulolytic activity was also high in the caecum with 60 to 70% of caecal bacteria demonstrating hemicellulolytic activity (Mackie and Wilkins, 1988).

There is little information in the literature on the concentration of proteolytic bacteria in the caecum. However, ranges of 19.7 (Kern *et al.*, 1974) to 41 % (Baruc *et al.*, 1983) of the caecal micro-flora have been reported to have proteolytic activity. *In vitro* work suggests that caecal fluid has a considerable capacity for N degradation, with 34% of caecal bacteria able to degrade gelatin and 27% able to degrade casein. However, urea-degrading activity *in vitro* was strictly limited (Baruc *et al.*, 1983).

The protozoa characterised in the equine caecal and colonic chyme, are ciliate oligotrichs; the two typical genera are *Cycloposthium spp.* and *Blepharocorys spp.* (Bonhomme, 1986a). Both of these genera have β-galactosidase and lipolytic activities, although the *Cycloposthium spp* have limited cellulolytic activity. Once attached to a plant fragment the protozoa ingest the plant tissue thus demonstrating that they have the capability to ferment both non-cellulosic and cellulose polysaccharides. The symbiotic relationship between protozoa and bacteria result in the production of two pectin degrading enzymes, depolymerase and esterase, while *Cycloposthium spp.* and *Blepharocorys spp.* produce polygalacturonase. Enzymes involved in hemicellulose degradation are also produced by the protozoa, with xylan endo-1,3-β-xylosidase activity being significantly higher than the β-mannosidase activity (Julliand, 1992).

The concentration of fungi at less than 10^3/ml of equine caecal chyme, is considerably less than those of either the bacteria or protozoa. Orpin (1981) and Julliand (1992) isolated fungi from equine caecal digesta and found members of the anaerobic *Phycomycetes*, with particular isolates showing similarities to *P.communis* and *Caecomyces communis* species often found in ruminants. *In vitro* these fungi readily degraded the pectins, hemicellulose and cellulose in milled grass particles, suggesting that despite their low concentration they have a role in fibre degradation (Orpin, 1981).

Moore and Dehorty (1993) conducted an experiment to determine the relative importance of protozoa and bacteria in the DM degradation of forage and concentrate diets in the caecum and colon of the horse. They reported little affect on degradation after defaunation (removal of protozoa) and no affect on cellulose degradation, suggesting that the bacterial population in the hindgut of the horse is more important than protozoa in degrading dietary DM. However, it must be remembered that the results reported above are from one experiment only where dietary and environmental conditions could have influenced the balance of the microbial population in the LI. Additionally, there are numerous important functions carried out by the microbial population, such as O_2 scavenging, pH buffering, absorption of methane and vitamin

synthesis which cannot be assessed by examining DM degradation alone. Since the number of reported studies on the microbial population in the equid hind-gut are very few, it would be premature to draw any firm conclusions as to the relative importance of protozoa or fungi to hind-gut function.

Mackie and Wilkins (1988), Koller *et al.* (1978), Kern *et al.* (1974) and Alexander *et al.* (1952) have examined the activity of the microbial population within the caecum and colon of the horse, to determine if differences exist between the two regions of the hindgut and whether this activity is inherently different to that found within the rumen. Kern *et al.* (1974) found that cellulolytic bacterial numbers in ponies were six times higher per gram of digesta in the caecal chyme than in the colonic chyme, indicating that microbial activity may be greater in the caecum than in the colon. However, Alexander (1951) and Applegate and Hershberger (1969) showed that only part of the degradation of fibre by the horse occurs in the caecum and that a high proportion of digesta passes from the caecum to the ventral colon before fibre degradation is completed, indicating that colonic fermentation plays an important role in fibre degradation in the horse. Despite lower microbial numbers in the hindgut in comparison with the rumen, the activity of the hindgut population is sufficient to produce degradation rates equal to the ruminant when a high quality fibre diet such as alfalfa is fed (Smoulders *et al.*, 1990; Cymbaluk, 1990). Additionally, acid digestion of cell wall material in the stomach and small intestine will initiate the breakdown of inter-cellular bonds, which will subsequently enhance the speed and efficiency of degradation of the more readily degradable hemicellulose fraction of the fibre within the hindgut (Keys *et al.,* 1969 and 1970).

In general published data to date seems to suggest that the hindgut micro-flora are less effective at fibre degradation than the population found in the rumen (Kern *et al.*, 1974; Koller *et al.*, 1978; Van Soest *et al.*, 1978). However, this does not appear to adversely affect the ability of horses in extensive grazing conditions to meet their energy requirements, as their high rate of digesta passage, results in more food passing through

the digestive tract in a given time, which in turn maintains sufficient nutrient intake. In contrast, horses which have high-energy demands, limited access to forage, or with limited time to eat will require a more nutrient dense diet than rough grazing can provide.

The maintenance of a healthy microbial population depends on an adequate supply of energy and nitrogen to the large intestine (Demment and Van Soest, 1983). As foregut fermenters, the rumen microbial population has access to all the protein, amino acids and non-protein nitrogen (NPN) supplied by the diet. By contrast, horses digest available food protein enzymatically in the small intestine, thus digesta entering the large intestine may be low in protein. Gibbs *et al.* (1996) found that 60% of the CP in oats was readily digested pre-caecally, and although this potentially left 40% for microbial degradation in the large intestine, the CP held within the cell walls would only be liberated if the fibre matrix was susceptible to breakdown by the hindgut micro-flora. Harbers, McNally and Smith (1981) noted a decrease in CP fermentation as the ADF content of grass hay increased. Thus, a deficiency of CP supply to the hindgut could arise if low quality fibre is the sole nutrient source fed to equids. Cuddeford *et al.* (1995) and Suhartanto *et al.* (1993) found donkeys to be more efficient at degrading dietary fibre than ponies. This superior degradation could be partly due to a higher MRT exhibited by donkeys. However, Izraely *et al.* (1989) observed than donkeys have a more efficient mechanism for recycling N to the hindgut (achieved by a decreased urea filtration). This mechanism could also play a significant part in increasing fibre degradation by allowing donkeys to provide a short term N flow to the micro-flora in the hind-gut thereby maintaining microbial activity on high-fibre, low-protein diets.

2.4.2.3. Digesta mean retention time.

The degradation of dietary fibre by horses has been observed by Cymbaluk (1990), Smoulders *et al.* (1990), Vandernoot and Gilbreath (1970) and Hintz (1969) to be lower than corresponding measurements in ruminants. This lower degradation is frequently

attributed to the well-documented shorter total tract mean retention time (TMRT) of approximately 48 hours, in horses (Alexander, 1946; Haenlein *et al.*, 1966b; Vander Noot *et al.*, 1967) compared with the 70 to 90 hour TMRT noted in ruminants (Balch and Campling, 1965). This high rate of passage therefore reduces degradation by reducing the time the digesta is in contact with the fermenting micro-flora. Horses have evolved this faster rate of passage in order to survive on poor quality herbage (Janis, 1976). This system allows horses to increase intake to maintain absorption per unit time of the readily available nutrients, by compromising slightly on cellulose digestion, whilst maintaining the overall levels of nutrient absorption (Janis, 1976). A higher rate of passage coupled with lower cellulose degradation will inevitably result in a higher faecal output. This in turn may partly account for the smaller microbial population in the hindgut of the horse, measured by Kern *et al.* (1974), compared with that observed in ruminants. MRT is regarded by Pearson and Merritt (1991) and Cuddeford *et al.* (1995), to be a critical factor in achieving maximum fibre degradation. They attributed the superior DM digestibility of an alfalfa : straw diet, attained by donkeys in comparison with ponies, to be due to the higher MRT recorded for the donkeys. In this diet a limited nitrogen supply could not be cited as a possible reason for decreased microbial activity, thus in this case MRT would seem to be the over-riding factor determining DMD of this diet.

Fibre digestibility in equids is clearly influenced by the type and maturity of the plant material offered and the activity of the hindgut micro-flora. All these factors inter-relate and can be further influenced by other dietary constituents, frequency of feeding, body condition and exercise. In order to maximise digestive efficiency a clearer understanding of how individual fibre foods influence microbial degradation is required. Only then can diets containing several different ingredients be manipulated to maximise their nutritive value for horses performing a wide range of activities.

2.5. Methods to Determine *In vivo* Digestibility in Animals.

2.5.1. Introduction

In order to determine the contribution of any forage to overall nutrient balance in the horse, it is necessary to conduct *in vivo* digestibility trials. This involves detailed knowledge of food composition coupled with precise measurements of food intake and faecal output so that the digestibilities of a range of parameters can be calculated. This can be achieved using several different experimental Techniques.

2.5.2. Total collection digestibility trials.

The most basic form of food evaluation *in vivo* is the total collection digestibility trial (Cochran and Galyean, 1994). This type of experiment involves measuring and sampling all the food given and the faeces produced during a pre-determined collection period, which follows 2-4 weeks of adaptation to the chosen diet. The samples are then analysed for the parameters under study and the digestibility is calculated using the following formula:

$$\text{Digestibility} = \frac{\text{Nutrient consumed - nutrient in the faeces}}{\text{Nutrient consumed}}$$

(Source: Church and Pond 1988)

Digestibility can be expressed as a coefficient, or a %. The digestibility derived from the above formula is termed apparent digestibility (AD) and is the standard against which the accuracy of indirect methods, such as *in vitro* experiments, are measured (Minson, 1990). AD underestimates true digestibility (TD) as the faeces contain a high proportion of endogenous secretions, microbial cell debris and gut endothelium, which increases the weight of the faeces above that derived from undigested food alone. True digestibility of a food is difficult to determine in practice due to the similarities of many

of the faecal constituents derived form endogenous and plant fractions, thus for convenience, AD values are commonly used in *in vivo* experiments (Mc Donald *et al.*, 1996). Mason (1969) found the metabolic faecal output (MFO) of herbivores to be largely composed of microbial biomass. Uden and Van Soest (1982) agree, adding that the MFO expressed per g DM consumed, is similar across heifers, goats, sheep, rabbits and horses, but noted that the form of the output differed between foregut and hindgut fermenters. In ruminants the microbes passing from the rumen into the small intestine are subjected to enzymatic digestion, whereas in the horse the microbes passing from the colon are voided with the faeces (Moore *et al.,* 1978). This has implications with regard to N balance in these Animals, the ruminant has access to microbial N whereas the horse does not.

Cochran and Galyean (1994) thoroughly reviewed experimental protocols for farm animal *in vivo* trials and made several useful recommendations with regard to animal numbers, housing, duration of periods, collection procedures, data analysis and the use of markers for determining digestibility. *In vivo* trials can run for many weeks, involve a large number of animals and are labour intensive as they usually involve weighing all the food offered and rejected, and all the faeces produced over a 5-10 day balance period. Sutton *et al.* (1977) using four geldings, found a 4-day collection period, which he based on a preliminary rate of passage and AD trial, to be adequate to determine the AD of a roughage : concentrate diet, however, it is more common to use 5 to 7 day collection periods for determining nutrient balance (Uden and Van Soest, 1982; Parkins, Snow and Adams, 1982), as it yields more data and reduces error associated with varying daily intake and output.

When evaluating a new food the preferred method is to give the food as the sole diet, which gives a value of AD for that food without any confounding associative effects from another dietary ingredient. Although Martin-Rosset and Dulphy (1987), Hintz, Argenzio and Schryver (1971) and Cuddeford, *et al.* (1992) have fed horses a range of forage : concentrate diets and found no associative effects on digestibility between the concentrate and forage fractions of the diets, this is unlikely to be the case when feeding

two forages. When offering concentrate : forage diets the two foods are digested in different segments of the digestive tract, the small and LI respectively, whereas with an all forage diet, the two foods are both digested in the LI. Thus, the nutrient content and digestibility of one forage could affect the digestibility of the other. Concentrate foods cannot, due to health reasons, be fed to horses as sole diets and so must be mixed with another foodstuff, which is generally a fibre-based forage. In order to determine the AD of the concentrate (test food) the following calculation must be performed.

$$AD = \frac{\text{AD of test diet - (AD of basal diet X fraction of basal diet in test food)}}{\text{Fraction of test food in test diet}}$$

(Source: Church and Pond 1988)

Determination of the AD of a food with a basal diet can be performed at one level, 50:50, or on an ascending proportional basis. Proportional inclusion levels, coupled with the regression analysis of the data, allow some degree of assessment of associative effects and the point at which inclusion of the test food may alter voluntary food intake (VFI). Both methods are acceptable, but the degree of accuracy and flexibility of the proportional method make it the preferred choice when evaluating the digestibility of two foods fed simultaneously.

2.5.3. Indigestible markers as indicators of apparent digestibility

A less labour intensive alternative to the total collection digestibility trial is the use of markers to determine faecal output and AD. Markers can be external, that is a substance, which is added to the diet, or internal, a substance that occurs naturally in the diet. Commonly used external markers are chromic oxide (Cr_2O_3) (Chamberlain and Thomas, 1983; Haenlien *et al.,* 1966b; Householder *et al.,* 1976; Sauer *et al.,* 1979),

dysprosium (Dy) (Todd *et al.*, 1995b), ytterbium chloride ($YbCl_3$) (Pond *et al.*, 1989; Galyean, 1993; Musimba *et al.*, 1987) and chromium EDTA (Kotb and Lucky, 1972). Internal markers include lignin (Fahey, McLaren and Williams, 1979; Fahey and Jung, 1983), chromogen (Kotb and Lucky, 1972), and acid insoluble ash (Sutton *et al.*, 1977; Frape *et al.*, 1982; Cuddeford *et al.*, 1990). Whether internal or external, a marker should ideally fulfill most, if not all of the following criteria; They should:

1. be inert
2. be non toxic
3. have no physiological or psychological effects
4. not be absorbed
5. not be metabolised
6. be completely recoverable
7. have a low bulk
8. mix with the food
9. allow precise quantitative measurement

(Source: Kotb and Luckey, 1972)

Faichney (1975) narrowed this list down to numbers 3, 4 and 9, which are minimum requirements for all markers. The main advantages of using markers in place of total collection is the ability to correct for faecal loss, should coprophagy occur, and to reduce the labour required for total faecal collection. When using markers, spot grab samples can be taken and used to calculate total faecal output from the following formula.

$$\text{Faecal DM output (g/day)} = \frac{\text{Marker dose (g/day)}}{\text{Concentration of marker in faeces (g/g DM)}}$$

(source: Cochrane and Galyean,1994)

This method is most suitable when food intake is known and when using an internal marker, or a continuously fed external marker. The accuracy of the data obtained in all marker studies depends upon the recovery rate and the accuracy of detection of marker in the food and faeces. Yb and AIA seem to be relatively successful external internal markers respectively, when used to determine faecal output in horses. Cochrane *et al.* (1987) found faecal output values to be similar when comparing values determined from total collection and from Yb analysis of spot grab samples. This finding is supported by Krysl, McCollum and Galyean (1985), Ellis, Matis and Lascano (1979) and Mader Teeter and Horn (1984) who note Yb to be a more accurate marker for this process than Cr. All the previously mentioned markers have been widely tested by numerous researchers across a variety of animal species, and although some markers are better than others in certain conditions, no single internal or external marker is ideal (Kotb and Luckey, 1972; Owens and Hanson, 1992).

2.5.3.1. External markers

External markers can be difficult to administer, costly to detect and recovery rates variable. Numerous reviews have been published on the suitability of different external markers for use as AD indicators in ruminants (Ellis *et al.*, 1982; Galyean *et al.,* 1987; Pond *et al.,* 1988; Owens and Hanson 1992), but to-date only limited work has been published on the accuracy of different external markers for predicting AD in horses (Haenlein, Smith and Yoon, 1966; Knapka *et al.*, 1967; Veiga *et al.*, 1976).

Chromium (Cr) was first used in 1918 by Edin and although problems with complete recovery are still encountered with humans, recovery rates in ruminants are good (Kane, Jacobson and Moore 1950b). However, when compared with total collection data, Cr tends to underestimate AD in horses (Cuddeford and Hughes, 1990; Parkins *et al.,* 1982). Collection times and diurnal variation in faecal excretion patterns are blamed for the lower AD obtained, so researchers recommend a 24-hour faecal collection to combat this noted variation. A 24-hour faecal collection rather defeats the purpose of using

markers (as a labour saving device) to determine AD. The data presented by Uden, Colucci and Van Soest (1980) also indicate a lower AD when using Cr compared with total faecal collection, and additionally demonstrates a further problem of decreased digestibility of the Cr mordanted food samples. Therefore, when deciding on faecal collection times and comparing experimental results, diet and dosing method should be taken into account, although taken overall, Cr would appear to be of limited use as a digestibility marker in horses unless complete faecal samples are taken.

Rare earth elements such as cerium (Ce), dysprosium (Dy) and ytterbium (Yb) have been used extensively as particulate markers for determining digestibility because they bind well with foodstuffs (Ellis and Huston, 1968; Miller and Bryne, 1970; Miller *et al.,* 1971 and Young *et al.,* 1975). Yb in particular has a great binding affinity for EDTA, gluconic acid, glycolic acid, glyoxalic acid, hydroxyisobutyric acid, lactic acid, malic acid and acetylacetonate (Sinha, 1966), thus reducing the extent of migration during transit through the gut. Yb is also relatively cheap and has the advantage of being reasonably simple to measure by atomic absorption spectrometry or atomic emission spectroscopy (ICPMS) (Teeter *et al.,* 1979).

Prigge *et al.,* (1981), compared total faecal collections with single doses of Cr and Yb and reported a more constant output of Yb over a 24 hour period. Yb also gave faecal output levels very similar to the total collection levels whereas Cr underestimated faecal output, thus Yb proved to be the more accurate marker for determining faecal output. When marker dosing was increased to twice daily dosing, the diurnal variation seen to occur with a single dose was reduced, although some variation between Yb recovery and total collection was still noted at certain times. This study shows the importance of establishing diurnal faecal outputs so that marker collection is representative of faecal excretion patterns through the day in order that digestibility can be calculated accurately.

2.5.3.2. Internal markers

Internal markers are easier to administer than external markers as they are an integral part of the food itself, and therefore they closely mimic food behaviour within the gut. However, the recovery rates of internal markers across a wide variety of diets is variable (Kotb and Lucky 1972.; Cochran *el al,* 1986). Penning and Johnson (1983a) compared indigestible cellulose, 4N-HCI acid insoluble ash (AIA) and DMD values obtained from *in vitro* Tilley and Terry (1963) incubation, with total collection data from ruminants fed pelleted rye grass and Lucerne hay diets. Both markers produced good agreement with the total collection OMD values for rye grass, but AIA showed poor agreement when the Lucerne diet was fed. This was attributed to the slower rate of passage of Lucerne and with only a 5-day adaptation period, an insufficient time was allowed for the marker to reach equilibrium. On the strength of the Lucerne data, the authors recommended the preferential use of indigestible cellulose as an internal marker for ruminants. However, AIA has been used successfully as an internal marker in pigs (McCarthy *et al.,* 1974). These workers reported that AIA produced comparable figures to total collection data when cereal based starter diets were fed to 14 pigs in 2 separate experiments. Although the AIA content of this diet was low at only 0.194%, the AIA still gave a more accurate value for faecal output than Cr_2O_3, indicating that AIA was a superior marker to Cr_2O_3, when used to calculate AD in pigs. Sutton *et al.* (1977), completed three experiments with 4 geldings fed a grain : hay diet containing an AIA content of 2% and an alfalfa : oat diet with an AIA content of 1.2% and reported no significant differences between AIA and total collection for determining AD of the two diets. The AIA tended to produce a non-statistically significant higher value for faecal output than total faecal collection, indicating that AIA can replace total collection when determining AD in horses fed either an all forage diet (Cuddeford and Hughes, 1990), or a cube and hay diet, (Frape *et al.,* 1982) with no statistical loss of accuracy.

2.5.4. The in situ technique for determining apparent digestibility.

The *in situ* technique, first used in sheep by Quin, Van der Wath and Myburgin in 1938 as a rapid alternative to total collection digestibility trials for determining the apparent digestibility of foods *in vivo,* and has the advantage of yielding results more rapidly than total faecal collection methods. The technique involves suspending porous bags containing ground food in the rumen or caecum of the animal for a pre-determined period and measuring the nutrient disappearance by chemical analysis from the food residues (Stern, Bach and Calsamiglia, 1997). The results obtained yield information on the speed and extent of degradation, thus offering additional data to the end-point values obtained from conventional total collection digestibility trials. The *in situ* technique has limitations as it necessitates the use of surgically modified animals and only produces AD values from one chamber of the gastrointestinal tract, the caecum or rumen, thereby ignoring the physical and chemical digestive processes of the small and large intestines. Furthermore, values obtained from this technique merely reflect the disappearance of food particles from a bag and do not necessarily represent degradation to a simple chemical compound (Ørskov, Hovell and Mould, 1980). However, despite these limitations the *in situ* technique is a useful tool for grading the digestibility of foods and although a high proportion of the published *in situ* studies have been concerned with protein degradation in ruminants (Weakley *et al.,* 1983; Ørskov *et al.,* 1980; Nocek, 1985; Kandylis and Nikokyris, 1991; De Boer, Murphy and Kennelly 1987; Freer and Dove 1984) the technique is transferable, so that different foods can be tested for a wide range of parameters in both ruminants and hindgut fermenters. The degradability of foods is generally described using a modified version of the Ørskov and McDonald (1979) regression model, where degradation losses are plotted against time (McDonald, 1981; Dhanoa, 1988; Stefánsdóttir, 1996). The model of Dhanoa (1988) incorporates a lag phase, which can improve the accuracy of the fitted line to the observed data as it takes into account the time taken for physical and chemical alterations in the food before degradation begins.

Several comprehensive reviews on the *in situ* Technique (Huntington and Givens, 1995; Lindberg, 1985; Michalet-Doreau and Ould Bah, 1992) emphasise the importance of standardising several factors within the procedure to avoid unnecessary variations between studies for similar foods. Many of the modifications have been applied to the construction, and types of bags used and are applicable to both *in situ* and mobile bag studies and are detailed below.

2.5.5. The mobile bag technique

The mobile bag technique (MBT), as the name implies, is an *in sacco* technique which involves placing the test food into small porous bags, introducing these bags into the animal and measuring the disappearance of foodstuffs after the bag has travelled through all or part of the gastrointestinal tract. The Technique was first performed in 1756 by de Reaumur (Sauer *et al.*, 1983), who administered small-perforated metal tubes, filled with grass, to sheep. Spallanzani (1782) modified the technique for use in humans. He replaced the metal tubes with linen bags and filled them with bread and meat; the empty bags appeared in the faeces in less than 24 hours after swallowing. Despite this success Spallanzani was unable to repeat de Reaumur's experiment with animals, encountering difficulties with the passage of tubes through the pyloric sphincter. This appeared to discourage others from using the technique, as it was not resurrected until Petry and Handlos (1978) used small nylon bags (pore size 5 μm) to study digestion in pigs. Although their results were extremely variable and did not compare well with total tract trials, the potential of the technique was clearly recognised, as in the 1980's numerous mobile bag studies were performed in pigs and ruminants (Sauer *et al.*, 1983; Graham *et al.*, 1985; Cherian, Sauer and Thacker 1989).

One of the main advantages of the mobile bag technique over the *in situ* procedure is that intact animals can be used, as the bags can be administered naso-gastrically and collected in the faeces. Moreover, as the bags travel through all segments of the tract the AD values obtained reflect disappearances resulting from both enzymatic and microbial

digestion, whereas *in situ* bags give disappearances from one chamber only. In comparison with the total collection method, the mobile bag technique allows many more foods to be tested *in vivo* within a given time period (Cherian *et al.,* 1989), and the results obtained yield information on degradation dynamics as well as an end-point disappearance value. Additionally, the digestion of many foods such as cereals and protein supplements, which cannot be fed as sole diets, can be measured using mobile bags without the possible confounding associative effects which could result from a 50:50 basal food : test food diet in a total collection experiment (Metz and Vander Meer 1985). Overall, the mobile bag technique can save time and labour by reducing the time required to measure the AD of each food, and as a consequence experimental costs are reduced.

However, several workers have commented on the variable nature of experimental results obtained from mobile bag studies in pigs, which are generally due to different pre-digestion times and lack of consistency in bag size, bag pore size (Lindberg, 1985; Nocek, 1988) and sample weight to surface area ratio (Vanhatalo, 1995). Similar variations have been noted in ruminants (Kirkpatrick and Kennelly, 1984; Kandylis and Nikokyris, 1991). Therefore, if the full potential of this technique is to be realised, a degree of uniformity in experimental protocol must be embraced.

2.5.5.1. Factors affecting mobile bag digestibility values.

The pore size of the material used to construct mobile bags can have a significant effect on DM disappearance during *in sacco* studies, as pore size regulates the flow of microbes and solid particles into and out of the bag. The ideal pore size will depend upon the type of food under study but from the studies detailed below, pore size should allow as many as possible of the following criteria to be met:

1. Allow microbial colonisation of the sample and free exchange of fluid between the bag and the rumen / caecal contents.
2. Allow passage of fermentation end products from the bag.
3. Retain small un-degraded particles.
4. Prevent entry of particles from the basal diet into the bag.

Graham *et al.* (1985), found that 40 X 25mm bags with pore sizes of 10, 20 or 36 μm did not affect DMD in pigs from bags containing 250 or 1000mg of barley or whole crop peas. Conversely, Cherian *et al.* (1989) despite using a similar 70-hour incubation time, found that protein digestion of Soya bean meal, meat and bone meal and cottonseed meal in pigs increased as bag pore size increased from 10 to 70 μm. Moreover, the disappearance from bags with 10μm pores was lower than the values obtained from conventional collection methods whereas corresponding values from the 70μm pore bags was higher. These differences were attributed in the first instance to compromised enzyme penetration and in the second, to loss of undigested particles from the bag. These findings are in close agreement with the results reported from *in situ* studies where bags with a pore size of less than 10μm tended to restrict entry of protozoa and the contents had a lower pH and ATP level than bags with pores of >10μm (Nocek, 1988; AFRC, 1992; Michalet-Doreau and Ould Bah, 1992: Lindberg, Kaspersson and Ciszuk, 1984). Uden and Van Soest (1984), Weakley *et al.* (1983) and Varvikko and Lindberg (1985) all agree with these findings, adding that a very small pore size restricts both bacterial ingress and the outward transport of VFA and bicarbonate. Although all of the above studies recognise that no one pore size exists, which is ideal for all foods and species, the best diameter for both *in situ* and mobile bag studies in pigs and ruminants is generally accepted to be between 40 and 60μm (Cockburn *et al.,* 1993) and thus should be the size employed for studies in horses.

Bag pore size and sample particle size must be complimentary in order to achieve precise results with *in sacco* studies. However, sample particle size alone can influence the results of conventional digestibility studies. Milling samples for ruminants is deemed necessary as it ensures a greater uniformity between bag contents, compared with un-milled samples. This attempts to present the fermentation chamber with food material in a similar state to that of masticated food (Ørskov *et al.,* 1980) and additionally, increases the surface area available for microbial fermentation (Huntington and Givens, 1995). Milling may be less relevant for studies with pigs and horses as the large particles voided in the faeces clearly indicate that the extent of particle-size reduction is considerably less than in ruminants. The type of study-undertaken will to some extent, dictate the degree of milling. If for example the degradation of two supplements is to be compared, then the samples should be presented in the form in which they would be offered to the animal. On the other hand, if the objective is to determine protein degradation in both supplements, then milling will help reduce any differences that may occur due to particle size (Ørskov *et al.*, 1980). When Cherian *et al.* (1989) investigated the effect of sample particle size on the disappearance of protein from food contained in mobile bags, a decrease in protein digestibility was observed as mesh pore size (and therefore particle size) increased from 0.5mm to 2.0mm. The effect of grinding on the size of particles placed in the bag depends on the nature of the foodstuff under investigation. Different plant parts shatter at different rates, eg. leaf material shatters into smaller particles more readily than stem; thus a sample with a normal distribution skewed to the left, due to screening through a mesh, will contain a higher proportion of leaf and will consequently be more degradable than a sample with a higher mean particle size, which will contain a higher proportion of stem (Huntington and Givens, 1995). The milling and screening of foods could therefore produce a sample, which is not truly representative of the food offered in conventional *in vivo* trials.

It would thus seem extremely important to consider particle size when examining experimental results, derived from *in sacco* procedure for comparing the degradation

kinetics of animal foods. The recommended procedure for pigs is to grind Soya bean meal through a 0.5mm screen and the lower fibre meat and bone meal, through a 1mm screen Cherian *et al.* (1989). These recommendations gave similar digestibility results to conventional ileal-digesta collection methods.

Akin to the *in situ* studies in ruminants (Lindberg, 1981c; Mehrez and Ørskov, 1977; Nocek, 1985), increasing the sample size in mobile bags given to pigs had the effect of reducing protein digestion of Soya bean meal. A high sample weight to surface area ratio tends to obstruct enzyme penetration and interferes with the removal of digested particles from the bag. In order to increase the accuracy of mobile bag studies Cherian *et al.* (1989) proposed the following recommendations:

1) Samples should be ground through a 0.5mm mesh
2) Bag pore size should be between 48 to 63 μm
3) Between 0.5 and 1g of sample should be placed in each bag
4) Bag dimensions should be 2.5 to 4cm.

The diversity and composition of both rumen and caecal micro-flora are known to be strongly influenced by the diet fed to the host animal (McDonald *et al.,* 1981). This being so, the basal diet should be complementary to the samples *in sacco* to achieve accurate degradation values. Additionally, a complementary basal diet should supply adequate carbohydrate and nitrogen to support an active microbial population. Weakley *et al.* (1983) also stressed the importance of the abrasive effects of the basal diet and the gut wall, both of which help to prevent blockage of the bag pores by bacterial slime. Associative effects between the basal diet and the samples *in sacco* can occur in both *in situ* and mobile bag studies, and although these are greater in foregut fermenters than hindgut fermenters, Koller *et al.* (1978) recorded an increase in degradation when samples of Timothy hay were incubated in the caecum of ponies fed a basal diet of hay, compared with those fed a hay plus grain basal diet. Kern *et al.* (1973) also noted that a basal diet, which included grain increased the overall bacterial numbers and altered VFA

proportions in caecal and bovine fluid, but did not alter the population of cellulolytic bacteria, although cellulolytic activity was reduced. De Lange *et al.* (1991) investigated the effect of basal diet on porcine digestion of protein from mobile bags and in contrast to the findings of Sauer *et al.* (1989), found no detrimental effects on digestion when the protein content of the basal diet ranged from 111 to 169 g/kg CP. Low levels of dietary protein can reduce the secretion of pancreatic proteolytic enzymes (Corring and Saucier, 1972), however, clearly the 111 g/kg of CP was not low enough to induce any such effect in this experiment. High levels of dietary fibre can hinder the access of proteolytic enzymes in to the bags (Saunders *et al.,* 1969), increase endogenous protein loss (De Lange *et al.,* 1989), bind amino acids and increase digesta rate of passage (Den Hartog *et al.,* 1985), all of which have the effect of reducing protein digestion and absorption. Although none of these effects were noted in the experiment conducted by De Lange *et al.* (1991), clearly basal diet can have significant effects on bacterial species and numbers and should be carefully considered when comparing data from different experiments.

2.5.5.2. Expression of results obtained from *in sacco* studies.

Results from *in sacco* experiments can be used to determine the rate and extent of food disappearance and thus give an additional aspect to food degradation measurements which cannot be obtained from total collection trials. The results are normally expressed in degradation curves, where the % or g/kg loss of different components are plotted as the dependent variable and time (hours) as the independent variable (see Figure 2.5.1.). The line is described by the Ørskov and McDonald (1979) equation, of:

$$p = a + b (1 - e^{-ct}) \qquad \text{(equation 2.5.1)}$$

p = potential degradability of each component
t = incubation time (hours)

a = intercept of degradation curve at time zero and represent the soluble component of the food, which is rapidly washed / degraded.

b = insoluble but potentially degradable food component which will in time be degraded by the micro-organisms according to first-order kinetics (exponential).

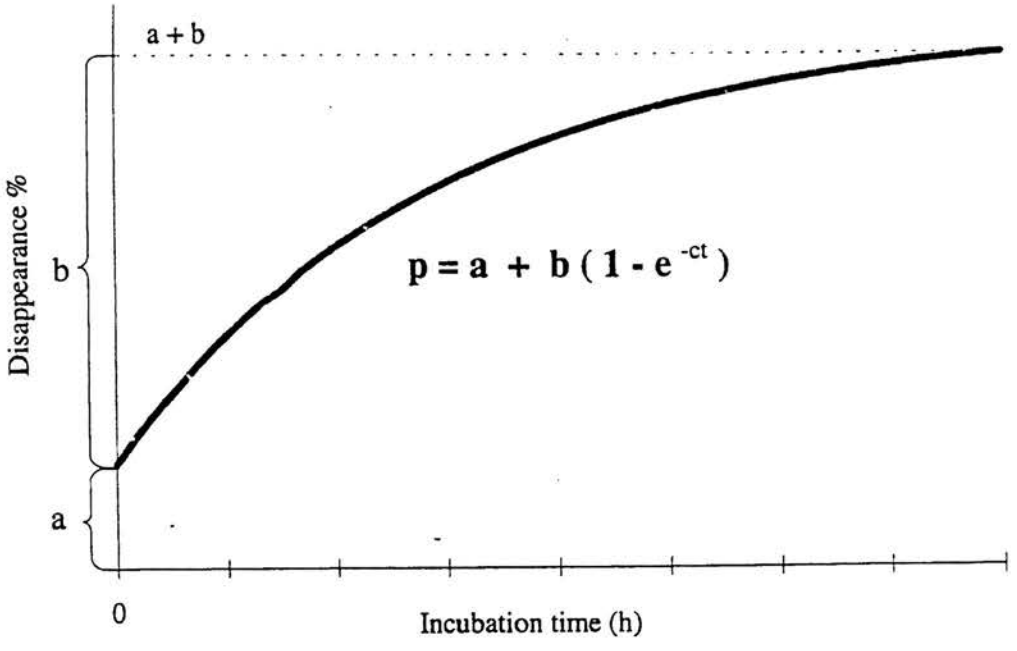
c = rate constant for degradation of b

a + b = the total degradability of the sample ie. the asympote

e = exponential.

1 – (a + b) = the undegradable fraction of the sample.

Figure 2.5.1. A typical degradation profile of the fitted curve derived from the model of Ørskov and McDonald (1979).



The constants a, b, and c are calculated by an iterative least squares procedure but may be obtained by fitting a curve by eye and calculating constants by simple algebra (Øsrkov *et al.,* 1980). If a is positive then the food has a rapidly degradable component,

if negative then a lag phase is present which is the period of time taken before degradation starts. The amount of rapidly degraded constituent could be measured by putting some bags into a washing machine to measure soluble losses. This however would only account for soluble components and would not measure components such as starch, which although insoluble, are rapidly degraded in the caecum. Thus, washing bags can help to differentiate between a soluble fraction and a rapidly degraded fraction (Ørskov *et al.,* 1980).

Equation 2.5.1. does not however, account for the fractional rate of digesta passage through the gut. This is an important consideration, as passage rate through the digestive tract is known to have a major affect on food degradability. In order to account for this passage rate factor, Ørskov and McDonald (1979) devised a model (equation 2.5.2.), which used the same parameters as equation 2.5.1, with an additional estimate for ruminal outflow rate (k), calculated using marked food particles. This they termed effective degradability.

Effective degradability (ED) is the amount of substance that will actually be degraded and is defined by the time the substance is present in the fermentation chamber. ED is calculated from the following equation:

$$ED = a + \frac{bc}{c + k} \qquad \text{(equation 2.5.2.)}$$

ED is therefore variable and depends on rumen / ceacal outflow rate, thus the longer digesta remains within the fermentation chamber, the greater the ED.

Although these equations are based on rumen degradation kinetics, the principles are the same for equids. A greater degree of accuracy can be obtained by substituting the rumen out flow rate (k) for a specific equine digesta flow rate, which can be pre-determined with the use of indigestible food makers in the gastrointestinal tract of the horse. If

actual rates have not been determined, then a standard rate of digesta passage of 5, 10 and 20 % per hour are deemed appropriate (Hyslop, pers. Comm).

2.5.5.3. Use of the mobile bag technique in pigs and ruminants

The MBT has been widely used to determine protein digestion in pigs (Sauer *et al.*, 1983). It has been used in preference to sampling digesta straight from the small intestine through a cannula, because it saves time and individual housing of animals is unnecessary. For protein analysis, bags are generally administered through a duodenal cannula and retrieved from an ileal or caecal cannula, enabling digestion of foodstuffs in the small intestine to be measured. A pre-digestion step is necessary and can be undertaken either *in vivo* or *in vitro,* but to achieve normal N digestion, samples must be incubated in pepsin HCl, for approximately 6 hours (Wilson and Leibholz, 1981). A potential draw-back to the Technique, which was experienced by Spallanzani in 1782, is the difficulty in getting bags to pass through the pyloric sphincter (Sauer *et al.*, 1983). However, if bags are inserted through a duodenal cannula and retrieved through an ileal cannula 120 to 170 minutes later, the values obtained are similar to those recorded from digesta collection trials. Studies by Leibholz (1991), found good agreement in digestibility for low fibre protein sources between direct ileal sampling and nylon bag methods. However, when using high fibre foods, the food residues in the bag contained more N than the ileal digesta. This difference was attributed to the shorter retention time of the bags in the small intestine and to the high sample weight to surface area ratio, both of which could compromise enzyme action.

Success with the MBT in pigs encouraged researchers to use the Technique for determining food digestion in ruminants. One of the earliest studies was performed by Kirkpatrick and Kennelly (1984), who used mobile bags to determine protein and DM digestion of a number of foodstuffs in growing heifers. They reported CPD that were comparable with results from conventional collection Techniques, although DMD was notably lower. This pioneering study in ruminants indicated that the MBT was a

promising method for measuring protein digestion in fore gut fermenters. However, DMD values required further modification, such as reduction of sample weight to surface area ratio and correction equations, similar to the regression analysis proposed by de Lange *et al.* (1991) for pigs, which would correct for the noted under estimation of DMD from mobile bags in ruminants.

2.5.5.4 Use of the mobile bag technique in equids.

Determining AD in equids, using the mobile bag technique is a relatively new phenomenon. The first published account is by Macheboeuf *et al.* (1995) who used 6 X 1 cm nylon bags to study pre-caecal and total tract digestion of nitrogen from hay. Results of DMD and NDFD obtained from bags collected with the faeces showed good agreement with *in vivo* total collection data, although nitrogen disappearances were notably higher *in sacco*. Nitrogen digestibility values determined from bags that were collected from the caecum, used to represent foregut disappearances, were high and varied little among hays. The high disappearances could be attributed to the manner by which bags were collected at the caecum. Two to six hours after naso-gastric administration, bags were recovered from the caecum by opening the cannula and inserting a hand into the caecum. However, this method left no way of telling how long bags had been immersed in caecal chyme. Thus, the data presented as foregut disappearances may in fact be confounded by some degree of caecal fermentation. Moreover, continually opening the cannula is undesirable, as it makes normal anaerobic conditions in the caecum difficult to maintain, which could compromise caecal fermentation. A continuation of this work was presented at the 47th Annual Meeting of the European Association for animal Production in Lillehammer, Norway (Macheboeuf *et al.,* 1996), where a list of recommendations for using the mobile bag technique in horses was presented. The recommendations closely resemble those made for pigs and ruminants and include guidelines on particle size (which should be 1.5mm), sample weight to surface area (10mg / cm^2 for fibre foods and 20mg /cm^2 for concentrate foods), pore size (48 μm) and a post-digestion washing machine treatment, to ensure

removal of endogenous enzymes and mucous from the bags. In addition to these recommendations, Macheboeuf *et al.* (1996) also recommended that physiological transit time is taken into account when evaluating nitrogen disappearances from bags collected at the caecum as disappearances from a variety of fibre and concentrate foods were significantly affected by pre-caecal transit time.

Although many of the mobile bag studies in pigs, ruminants and horses have been carried-out using fistulated animals, the technique can be used as an alternative to total faecal collection to determine total tract digestibility. Total-tract mobile bag studies are cheaper to do than total collection trials, because they require less food and reduce the time factor by dispensing with long dietary adaptation phases. Additionally, as in the study of Tomlinson (1997) total tract mobile bag studies can be used to calculate degradation kinetics using the standard *in situ* mathematical model of Ørskov and McDonald (1979). Transit time of bags through the gastrointestinal tract can be manipulated by altering bag size, which allows effective degradability (ED) (degradability weighted according to digesta mean retention time) to be calculated. Tomlinson (1997) found a positive correlation between bag size and DM and NDF disappearances from fibre foods contained in 6x1cm and 4x1cm polyester mesh bags. Hyslop and Cuddeford (1996) agree, with NDF disappearances (NDFD) from food in bags incubated for 32 hours loosing 236 g/kg, whereas those incubated for 117 hours lost 542 g/kg. Tomlinson (1997) also found the mobile bag technique to be sufficiently sensitive to differentiate between the degradation of dehydrated grass, dehydrated alfalfa, rye grass hay and grass chaff, although the DMD and NDFD obtained from the *in sacco* studies were notably lower than the results obtained from the total collection method. De Lange *et al.* (1991) also found lower disappearances from cereal grains *in sacco* compared with total collection in pigs and attributed these findings to the ingress of material into the bags from the basal diet. This may have occurred in the study by Tomlinson (1997) where particles from the basal diet of poor quality grass chaff could have entered the bags.

The above studies indicate that the mobile bag technique can be successfully used in equids to measure both foregut and total tract degradation of fibre foods. Manipulation of bag mean retention time (MRT) by altering the size of bag incubated, is a particularly useful skill when examining fibre degradation in equids as digesta MRT can vary considerably between individual animals (Tomlinson, 1997). However, before this technique can be routinely used to determine the AD of equine foods, the relationship between total collection AD and *in sacco* AD should be determined by regression analysis, so that food degradation can be obtained using the mobile bag technique with some degree of accuracy.

2.6. Measurement of digesta passage rate through the gastrointestinal tract

2.6.1. Introduction

The period of time that a food remains in contact with the enzyme secretions in the SI and the microbial population in the fermentation chamber, will have an influence on both the rate and extent of it's digestion. To fully appreciate the dynamic interactions between the diet, the enzymes and the microbial population, it is desirable to be able to measure the rate of passage of digesta through the different segments of the gastrointestinal tract. To measure digesta flow directly *via* re-entrant cannulae (Faichney, 1980) is laborious and requires surgically modified animals, thus the alternative method of using inert markers and appropriate mathematical models fitted to faecal excretion data from intact Animals, is now the method of choice (Grovum and Williams, 1973; Ellis *et al.*, 1984).

2.6.2. Use of indigestible markers as indicators of digesta passage rate in animals.

Markers have been used in rate of passage studies in both man and animals for many

years, one of the earliest being performed in 1904 by Elliott and Barclay-Smith using glass beads in rabbits. They recognised that in order to estimate digesta passage rate by marker collection, the marker concerned must be indigestible as it is only the undigested entity that is voided in the faeces (Ellis *et al.,* 1994). However, glass beads and other markers such as, seeds, charcoal and thread knots were found to move independently of the digesta, thus today non absorbable markers which associate with the digesta such as metal oxides, rare earths and radioactive substances are preferred, as they mimic the behaviour of the food passing through the gastrointestinal tract (Kotb and Luckey, 1972). By tracking marker progress using faecal collection or radioactive scanning, rate of digesta passage, mean retention time (MRT) and digestive capacity can be calculated. Since the mid seventies, the accuracy with which markers reflect the passage of digesta through the ruminant gut has received much attention. Although imperfections exist, it is now generally accepted that variations due to marker behaviour are small when compared with other variables such as environment, food intake and differences in gut physiology. Therefore, markers, although not necessarily precisely reflecting digesta flow, provide data on the variation of digesta kinetics induced by different treatments and are thus extremely useful tools in studies of digestive physiology (Owens and Hanson, 1992). A plethora of scientific literature reporting the measurement of digesta flow through the ruminant gut are available for reference, but the terms used for describing digesta kinetics are not always consistent so in the interests of clarity, the following definitions will be used in this review.

Transit time (TT) = the time taken for the digesta to pass through the gastrointestinal tract, or part of the tract expressed as time post dosing, until first appearance of marker.

Flow rate = the volume of digesta to pass through the tract per unit time expressed in litres per hour.

Rate of passage = the proportion of digesta that passes along the tract per unit time, expressed as % per hour.

Mean retention time (MRT) = The average time digesta is retained in the gut expressed in hours. [MRT is calculated from the reciprocal of the fractional flow rate (Warner, 1981)]

2.6.2.1. Desirable characteristics of markers

The properties required of a marker for use in rate of passage measurements are more critical than those demanded for a digestibility study. When determining digesta rate of passage, it is essential to choose a marker that has a high binding affinity for the food to be monitored, so migration of marker to other food particles is minimal. Additionally, the marker must be non-toxic, easily detectable (Uden, Colucci and Van Soest, 1980) and should closely mimic the passage of the digesta through the tract (Teeter, Owens and Mader, 1984). Inert metals such as chromium (Cr) have been successfully used as particulate markers for many years (Ellis and Huston, 1968; Huston and Ellis, 1968; Miller and Bryne, 1970). More recently, rare earths have been chosen, as they are indigestible and have an affinity for plant cell walls, which is particularly useful when measuring rate of passage of fibrous foods (Hart and Polan, 1984).

Many different markers have been used in rate of passage studies in ruminants, but the Cr mordanting Technique of Uden *et al.* (1980) has proved particularly popular. Cr mordanted onto food produces little migration and allows the flow of particulate matter to be measured with some degree of accuracy (Mader, Teeter and Horn, 1984).

The mordanting technique involves impregnating the Cr into the fibre portion of the cell wall. Recovery of the marker is generally good with 66 to 103% recovered by Martz *et al.* (1974) from *in vitro* and neutral detergent analysis, while Uden *et al.* (1980) recorded *in vivo* recoveries of 99.5% after a 10-day collection period. Food mordanted with Cr

therefore fulfils the two most important requirements of a good particulate marker, in that it is fully recoverable and migration from labelled particles is low (Lindberg, 1985). However, mordanting affects digestibility and can render the marked food particles totally indigestible (Elimam and Ørskov, 1984). Mader, Teeter and Horn (1984) found that the *in vitro* dry matter digestibility coefficient of sorghum-Sudan hay to be 0.55 for unlabelled food, but this decreased to 0.35 and 0.09 when labelled with Yb and Cr respectively. Uden *et al.* (1980) reported a very low digestibility (0.04) when Cr mordanted food contained a marker concentration of 8%. Additionally they found an increase in the particle density and reduction in particle size, which in turn could alter the turnover rate, bringing into question the accuracy with which the marked particles reflected the passage rate of the food. Pond *et al.* (1989) and Moore *et al.* (1992) compared food labelled with Cr, ytterbium (Yb) and terbium (Tb) for determining rate of passage in cattle and found no significant difference between the markers, although MRT with Cr tended to be longer. However, their study did not document the effect of each marker on digestibility of the food samples. As one of the essential criteria for flow markers is that the marked particles are digested to a similar extent and thus flow at a similar rate to the unmarked particles, it is essential to measure both characteristics in order to have confidence in the results obtained using this method.

Due to their high absorptive properties, the rare earths Ytterbium (Yb), cerium (Ce) and dysprosium (Dy) have been used extensively as particulate phase markers in rate of passage studies (Ellis and Huston, 1968; Miller and Byrne, 1970; Younge *et al.*, 1975). Yb is usually preferred, as it is relatively cheap and easy to use and detection at low concentrations can be performed by both atomic absorption and atomic emission spectrometry (Teeter *et al.*, 1979). Information for the preparation and analysis of samples are detailed in papers by Ellis *et al.*, (1982), Hart and Polan (1984), and Teeter *et al.* (1984).

2.6.2.2. Methods for marking foods

The method of marking the food is critical to the performance of the marker within the gastrointestinal tract. Mader *et al.* (1984) examined the relative success of the spray versus the immersion techniques for labelling wheat forage. The persistent washing during the immersion process, which removed the un-bound and loosely bound Yb from the samples, produced a concentration of 15.6mg Yb/g DM, whereas the corresponding sprayed samples contained 48.6mgYb/g DM. The food marked using the immersion process had a lower marker migration during passage through the GIT, than the food marked by spraying. However, the repeated washing (immersion technique) resulted in a reduction in *in vitro* digestibility of the marked food. This was possibly due to the loss of soluble cell contents during the rinsing process. However, taken overall Mader *et al.* (1984) concluded, that to avoid unwanted marker migration, the immersion Technique was preferable to spraying as it allowed a greater exposure of binding sites and therefore a better distribution of Yb on the marked food. Both Yb acetate (Siddons *et al.*, 1985) and $YbCl_3$ (Coleman, Evans and Horn, 1984) have been used to measure digesta turnover and flow-rate in ruminants. $YbCl_3$ is more stable than Yb acetate at pH 4 and above, and has no deleterious effects on *in vitro* DM disappearance at marker concentrations of 40mg /g; recovery of $YbCl_3$ is high at 90-99% making it a more reliable marker than Yb acetate for use in rate of passage studies.

2.6.2.3. Administration of marked food and faecal sampling procedures.

Two methods of administering markers are commonly used, continuous infusion and pulse dosing. Both systems can be used to measure digesta passage rate through the total tract, or a segment of the tract. For total tract studies the marker is generally given in the food, whereas in segmental studies, the marker is infused into a particular segment of the gut, and samples of the faeces and digesta are made accordingly.

Continuous infusion of the marker coupled with time sequence sampling, allows

measurement of digesta passage rate. On cessation of marker infusion, sampling can measure MRT and compartmental mass of digesta in the subsequent segments of the GIT. Segmental studies do, however, necessitate the use of fistulated animals. When equilibrium is reached ie. when a constant concentration of marker is recorded, flow rate = infusion rate / marker concentration (Faichney, 1975). The difficulties encountered with sampling of digesta can be overcome if both a liquid phase marker and a particulate phase marker are used together. The digesta can then be re-constituted either physically or mathematically to measure the total rate of passage of digesta.

A single pulse dose of a marker (generally administered orally) is the most common method used to determine compartmental digesta mass and MRT in intact animals. To achieve the maximum benefit from this procedure, frequent sampling is required, particularly in the early stages of the experiment, to facilitate the mathematical modelling of digesta passage rate (Faichney1975). Pond *et al.* (1989) found that the timing of administration of the pulse dose to ruminants in relation to meal time had a significant impact on passage rate estimates. Particles administrated at the beginning of the meal had a passage rate 42% higher than for post-prandial administration. These passage rate characteristics were the same whether measured using Cr mordanted particles, or rare earths. Pulse dosing is also frequently used to estimate faecal output, which in turn can be used to predict food intake in grazing animals (Galyean, 1993).

2.6.2.4. Expression of results

Partitioning and describing the digestive processes with equations and mathematical models, clarifies our understanding of digestion kinetics. This can be done algebraically, with equations that yield MRT and digesta volume figures, or by using models that compartmentalise the digestive tract into two or more sections and thus give 'rate' parameters as well as MRT values.

2.6.2.4.1. Algebraic models

Balch (1950) used a stained particle technique in cows, and from the data collected produced a cumulative excretion curve by totaling the concentration of marker excreted in the faeces and plotting the concentration of each sample as a percentage of the total against time post-dosing, taken as the mid-point between dosing time and the ith collection. He calculated the MRT in the reticulo-rumen as the interval time between excretion of 5 and 80% of the marked particles. Cumulative excretion curves are commonly used today, and as well as allowing MRT to be calculated, they allow marker recovery rates to be determined, enabling the reliability of the data to be checked. Castle (1956a) suggested that the MRT could be calculated from the cumulative excretion curve by calculating a value R which is directly proportional to the area left of the curve and is calculated by adding the time of excretion from 5 to 95% at intervals of 10% and dividing by 10. When determining the MRT in monogastrics Kotb and Luckey (1972), in their extensive review of the use of markers for digesta passage rate studies, favoured the method of Brandt and Thacker (1958) who used marker half life, which is the time taken for 50% of the marker to be excreted.

Although a number of equations can be used to calculate MRT, the equation produced by Faichney (1975) (equation 2.6.1) has become the standard by which all other formulae and models are compared when using pulse-dosing administration of markers.

$$MRT = \sum ti\ Mi \qquad \text{(equation 2.6.1.)}$$

ti = time (hrs) post-dose to the mid-point of the ith defecation.
Mi = Marker excreted in the ith defecation as a fraction of the total amount of marker excreted.

This equation is preferred to the methods of Castle (1956a) and Patton and Krause (1972), as it uses the observed data (ie. the amount of marker recovered in the faeces for

each post-dosing time point) rather than a smooth curve obtained by calculation, which implies continuous faecal output.

Thielmans *et al.* (1978) produced equation 2.6.2, where the observed data is also used to obtain total tract MRT (MRT_T). This equation uses faecal marker concentration rather than the amount of marker per sample and so can be used in conjunction with spot-faecal sampling.

$$(MRT_T) = \Sigma\, t_1\, C_1\, \Delta\, ti \,/\, \Sigma\, C_1\, \Delta t_1 \qquad \text{(equation 2.6.2.)}$$

where:

t_1 = time (hrs) elapsed since dosing to the mid-point of the ith collection

C_1 = concentration of marker in the ith sample (as a proportion of total marker excreted)

Δt_1 = faecal collection interval (hrs)

n = number of faecal samples

Thielemans *et al.* (1978) compared the above formulae with that of Blaxter, Graham and Wainman (1956) (equation 2.6.3.), which has a similar mathematical form to that of Faichney (1975), and found similar MRT were obtained irrespective of the calculation method used.

$$MRT_B = \Sigma mi\ ti \,/\, \Sigma\, mi \qquad \text{(equation 2.6.3.)}$$

where:

mi = amount of marker excreted in the ith defaecation

ti = time (hrs) elapsed since dosage of marker

All three equations use the actual data collected and are thus equally accurate methods by which to calculate MRT. However, if total faecal collection is impossible, the method of Thielmans *et al.* (1978) allows MRT to be obtained from spot-faecal

sampling, thus rate of passage data can still be calculated when labour is limited or the number of animals in the experiment makes total faecal collection untenable.

2.6.2.4.2. Compartmental mathematical models

Blaxter, *et al.* (1956) used a cumulative excretion curve to obtain two rate constants from faecal marker data collected after a pulse dose was administered into the reticulo-rumen of sheep. Although such rate constants were potentially useful it was unclear as to which compartment of the GIT each constant applied. Subsequent work has concentrated upon describing digesta passage and the factors affecting the flow of digesta through the individual compartments of the ruminant GIT, and these models can be broadly categorised into time-independent and time-dependent models.

2.6.2.4.2.1. Time-independent models.

The early models of Blaxter *et al.* (1956), Brandt and Thacker (1958), Hungate, (1966) and Grovum and Williams (1973), are time-independent models and make the following three assumptions:

1. The mixing of in-flowing fragments occurs instantaneously.
2. Equal opportunity exists for escape for all particles, regardless of their residence time within that compartment.
3. There is a constant flow and compartmental mass therefore steady state conditions exist.

These three assumptions lead to an excretion curve that is described by an exponential distribution of residence times (ie. time-independent) (Ellis *et al.*, 1994). Data treated in this manner leads to a curvilinear plot on an arithmetic scale, or a linear plot on a log scale. Such models are referred to as deterministic, which means they model digesta flowing irreversibly through a fixed number of sequential compartments, according to

first order kinetics (Lalles *et al.*, 1991). The models of Blaxter (1956), Grovum and Williams (1973), Dhanoa (1985) and France *et al.* (1985) all fit into this category. Blaxter *et al.* (1956) who was one of the first workers to use models considered the passage of digesta in sheep to be a kinetic process and described it thus:

A (rumen)----^{k1}--------B (abomasum)-------^{k2}--------C (duodenum)---- τ------R(faeces)

k1 and k2 = rate constants

τ = time delay, which is the time post-dosing until first appearance of marker.

Although no direct *in vivo* experiment was carried out by Blaxter *et al.* (1956) to validate all the specific compartments, validation was performed by Brandt and Thacker (1958) using rabbits and cattle. The Blaxter *et al.* (1956) model thus formed the basis of the most widely used deterministic model in ruminant nutrition, that of Grovum and Williams (1973) (equation 2.6.4)

$$Y = A\, e^{-k1(t-TT)} - A\, e^{-k2(t-TT)} \qquad \text{(equation 2.6.4)}$$

where:

Y= adjusted marker concentration in faecal DM

A = adjusted marker concentration in faecal DM and is the anti natural log calculated from the mean of (TT x k1) + C and (TT x k2) + C1.

k1 and k2 are rate constants.

t = sampling time in hours (taken as the mid point between successive samples)

TT = transit time in non mixing compartment [time taken until first appearance of marker in the faeces (referred to in other models as time-delay or tau)]

Grovum and Williams (1973) reversed Blaxter's (1956) order of k1 and k2, thus in equation 2.6.4, k1 denotes the slow compartment (rumen) and k2 the fast compartment (abomasum, SI and LI); TT is the transit time of the marker through the omasum, SI and LI. The TT through the reticulo-rumen should be close to zero as mixing is fast in this

organ, causing immediate departure of some of the marker. These assumptions probably do not apply in the hindgut fermenter as mixing is likely to be incomplete in the tubular small intestine and colon.

The models mentioned above all contain two sequential time-independent mixing compartments with a discrete time delay (TD) otherwise known as tau (τ). Other investigators have proposed three or more exponentially distributed mixing compartments, claiming such a model gives an improved statistical fit. An example of one such model is that of Dhanoa *et al.* (1985), who derived the following equation:

$$y = A\, e^{-c1\, t} \exp\left[-Be^{-c2\, t}\right] \qquad \text{(equation 2.6.5)}$$

y = adjusted marker concentration in faecal DM.
A = anti-natural log of faecal marker concentration.
c1 = rate parameter equivalent to k1.
B = number of compartments.
c2 = rate parameter (k2 - k1).
t = time post dose, taken as the mid-point between successive samples.

Improved fit is not always achieved and multi-compartmental models can result in near equal estimates for two of the time-independent rate parameters, suggesting that a) the data collected is inadequate, or b) that three distinguishable compartments do not exist or c) that digesta passage is in fact time dependent and so represents a single time-dependent compartment (Pond *et al.*, 1988; Mertens, 1989).

2.6.2.4.2.2. Time-dependent models

Alternative models to the two and multi-compartmental exponential models have been proposed by Matis (1972) and Matis *et al.* (1989). These are stochastic (ie. a process that involves a random variable) in nature and use gamma functions, which are

non - exponential residence time distributions, to describe time-dependent passage through different segments of the GIT. Time-dependent passage is sometimes incorporated into a model which uses both time-independent and time-dependent functions within the same equation, as used by Ellis *et al.* (1979); or used as the sole means of describing digesta passage through a non-mixing segment of the gut. When non-mixing and mixing compartments lie in sequence, the non-mixing compartment results in a time delay (τ) before first appearance of marker in the faeces. This is generally accepted to represent residence time, and accordingly, flow through the intestines. Some researchers, Matis, (1972), Uden *et al.* (1982), Matis, (1987) and Pond *et al.* (1988) feel that the particular restrictions that the ruminant gut architecture imposes on the passage of solids through the gut, results in a degree of uncertainty when describing the movement of digesta and therefore favour the use of stochastic models, which utilise the family of integer gamma functions to model this time-dependent process. Using such a model accommodates the imperfect mixing known to occur in the rumen and so improves precision in estimating passage rate parameters (Pond *et al.*, 1988). A gamma 1 (G1) function represents an exponential time-independent residence while G2 and above; all represent time-dependent distribution of residence times. Once the integer value of the gamma function increases beyond 2, the modelled effects of time slows the rate at which the time-dependent escape rate occurs, thus MRT is increased in the time-dependent compartment while the TD phase is reduced. Matis (1972) suggested that freshly ingested particles entering the rumen had extremely slow escape-rates, while those which resided in the rumen for longer periods and subjected to microbial fermentation, would be sufficiently reduced in size to have an increased chance of escape. The mean compartment residence time for the gamma distributions equals the numeric order of gamma function, divided by λ ; eg. for G2 model MRT = $2/\lambda$. ' The rate is a function of the past residence time t and the mean departure rate averaged over particles of all past residence times and is a constant function of λ (ie 0.59635 λ for G2).' (Pond *et al.*,1988).

[MRT = $2/\lambda$ thus $\lambda = 2/MRT$ and mean out flow rate = λ x 0.59635]

Matis (1972) combined the time-independent and time-dependent models, proposing a 2 compartmental model with sequential time-dependent and time-independent compartments. He designated the asymptotic λ to the time-dependent initial fast compartment, to distinguish it from the time-independent turnover rate k_2. λ_1 although an estimate of time-independent turnover after infinite time, is not comparable to k_1 and k_2, thus compartmental MRT must be computed differently than for time-independent compartments.

Time-dependent residence distributions have a number of advantages over the time-independent models (Pond *et al.*,1988; Matis, 1987). First, they are consistent with biological evidence on digesta flow, and secondly allow some lea-way from the rigorous assumption of instantaneous mixing, indicative of the time-independent models. In addition, Pond *et al.* (1988) felt that time-dependent models yield estimates of tau (τ) which are more consistent with the actual data collected from cows on straw diets and moreover, they 'resolve statistically valid estimates of turnover from compartments having different distributions of residence times which are not resolvable by the G1-G1-τ-0 model (rate λ_1 versus k_2 corresponding to Gn versus G1 respectively).' The Gn G1 (time-dependent / time-independent) models additionally allow flexibility for fitting data, which has a rapidly ascending early phase (G2 G1 model) while the higher G4 G1 fits data where initial passage rate ascends more gradually (Moore *et al.*, 1992).

The model proposed by Dhanoa *et al.* (1985) uses multiple sequential time-independent compartments to model an essentially time-dependent process (Ellis *et al.*, 1994). The output profiles and statistical fits for all these models are similar when fitted to good quality data, ie. high numbers of values collected in the early stages of excretion and at peak output. However, because each model operates on slightly different assumptions and consequently have different data requirements in terms of quality and quantity, estimation of individual parameters can be adversely affected and result in difficulties in fitting some models, if insufficient data points are collected at critical times (Dhanoa *et*

al., 1985; Mertens, 1989; Pond *et al.*,1988). The higher the order of gamma function, the more critical it becomes to ensure adequate data is collected during the early phase of marker excretion, so that clear differentiation can be made between τ and λ.

2.6.2.4.2.3. Comparison between models for predicting digesta passage rate

Cochrane *et al.* (1987) used Yb to determine the rate of passage in 12 grazing steers using three models: a) a one compartment time-dependent model; b) a two compartment time-dependent - time independent model; c) a two compartment time-independent model. Model b failed to fit some of the faecal profiles, while passage rate for the c model was greater than from the b model. Faecal output values estimated from model a, resembled those obtained from total collection. Mader *et al.* (1984) however, reported considerable variation in faecal output prediction when using one and two time-dependent compartment models. Lalles, Delval and Poncet (1991), broadened the comparison to include the two algebraic methods of Faichney (1975) and Thielemans (1978), as well as looking at the models of Grovum and Williams (1973), Ellis *et al.* (1994) and Dhanoa *et al.* (1985) for estimating total tract MRT. Comparisons between these three methods were made using residual standard errors. Best fits were obtained from the Dhanoa *et al.* (1985) model, followed by Ellis *et al.* (1979) and lastly that of Grovum and Williams (1973). Generally any difficulty encountered with fitting a model was experienced across all models. Both algebraic methods provided similar estimates for MRT within each diet, although the Thielemans (1978) method significantly underestimated hay and concentrate MRT when MRT was long. All MRT from the three models produced similar values. Estimates for MRT (compartment 1) were similar when applying Grovum and Williams (1973) and Ellis *et al.* (1979), whereas the Dhanoa *et al.* (1985) model seemed to underestimate this value. The main difference between the models arose from residual MRT (MRT - MRT1) and between MRT2 and TT. Again Grovum and Williams (1973) and Ellis *et al.* (1979) were close for MRT2; but on the hay diet the Dhanoa *et al.* (1985) model generated 50% lower value than the other

two models. This in turn, as expected, gave a higher TT value for the Dhanoa *et al.* (1985) model than the values recorded from the other two models.

The results from these three research groups support the comments of Dhanoa *et al.* (1985), Mertens (1989) and Pond *et al.* (1988) who indicated that the type of data collected should be synchronised with the type of model used before collection commences, so that the chosen model predicts the parameters to be measured with a high degree of accuracy. However, there are still numerous physiological processes occurring within the GIT, which are poorly understood and undoubtedly contribute to some of the variation between models. No one model appears to be superior and generally if the data collected is plentiful then all the models will fit the data well. Total tract MRT can be reliably obtained from both deterministic and stochastic models with a high degree of correlation, although generally stochastic models are more flexible when modelling rate of passage in ruminants (Lalles *et al.*, 1991).

2.6.3 The use of markers for passage rate studies in Horses

Berton *et al.* (1989) used Yb marked food to describe digestion kinetics in the horse. They applied first order kinetics to data collected from normal and colon-resected horses and identified two compartments. The passage rate through the first compartment (k1) was not altered by colon resection. Additionally this compartment had a pool size of 17 litres, which is similar to published values (DeBoom, 1975) for the caecum, thus k1 was identified as the caecum. The k2 compartment produced a significantly faster passage rate in resected horses in comparison with non-resected Animals, so k2 was identified as the colon. Six months after the resection operation, passage rate through the colon was noted to be similar to the values obtained before the operation. The capacity of the k1 compartment also increased during this period, indicating a degree of adaptation had occurred in those animals with a resected tract. In this study, the objective was to measure the overall passage rate and $YbCl_3$ proved an effective marker for this purpose. However, it is recognised that $YbCl_3$ can dissociate and migrate in the low pH

conditions of the stomach and consequently may be of limited use when trying to determine MRT of a particular dietary ingredient. Additionally in hot water $YbCl_3$ can be hydrolysed to the insoluble un-binding basic salt $Yb0HCl_3$ so water temperatures during marking should be kept to 80°C to minimise this reaction.

Cr_2O_3 has been used as a flow marker and will yield information on the relative passage of digesta, however, it use is limited since it does not associate with either the particulate or liquid phase of the digesta. This can result in sedimentation and sporadic transfer of marker, thus Cr_2O_3 lacks the necessary accuracy for modelling digesta flow in the horse (Bertone, *et al.*, 1989).

2.6.3.1 Mathematical modelling of digesta passage rate data in horses

There are very few published reports on the modelling of digesta passage rate in horses and those that do exist, tend to concentrate on establishing digesta MRT for different diets. Nyberg *et al.*(1993) used a variation of the Faichney (1975) equation (equation 2.6.5) to estimate MRT in the small intestine of ponies:

$$MRT = \Sigma t1\ C1 / \Sigma\ C1 \qquad \text{(equation 2.6.5)}$$

where:

t1 = time each sample was taken

C1 = concentration of marker in faeces at that time.

Orton Hume and Leng (1985b) used the Blaxter *et al.* (1956) equation (equation 2.6.6.) of:

$$MRT = \Sigma\ mi\ ti / \Sigma\ mi \qquad \text{(equation 2.6.6)}$$

where:

mi = amount of marker excreted at the ith defaecation voided at time ti post dose.

Pearson and Merritt (1991) simplified this equation by making mi denote the amount of marker excreted at time ti, although how the amount was calculated is unclear. This equation was also used by Cummings *et al.* (1976) and Mathers and Blake (1983). All three equations although having a slightly different form are based on the Faichney (1975) equation and produce similar values for MRT.

Two and multi-compartment time-independent and time-dependent models have been widely tested on ruminant faecal excretion data, but no information is published on the application of such models to horse data. The advantage of the compartmental models over the algebraic models is their ability to determine digesta passage rate through the different regions of the gut, and thus enable predictions to be made on the extent of digestion in different regions of the tract (Mertens, 1993). Such information would be very useful when compiling diets for horses, particularly when feeding cereal-based diets, whereby the application of a certain feeding regime or dietary combination could reduce passage rate and thus minimise the acidosis caused by the 'dumping' of undigested starch into the caecum. When modelling faecal excretion data Moore *et al.* (1992) suggest that a variety of time-independent and time-dependent models should be tested on the data in order to find which model fits most accurately. Thus, at present describing faecal excretion data using different mathematical models is relatively easy, and provided adequate data is collected, a variety of time-independent and time-dependent models fit the data equally well (Lalles *et al.,* 1991). However, despite the fact that the compartmental models detailed above have been specifically developed for measuring digesta rate of passage in ruminants, the biological interpretation of these models when applied to ruminant faecal excretion data has been notoriously difficult (Mertens, 1993; Dhanoa *et al.,* 1985). Despite these difficulties of interpretation, mathematical modelling is a procedure worth developing for horses as being able to predict digesta passage rate through the different regions of the gut could greatly improve ration formulation for all categories of equids.

2.7. *In vitro* Techniques for measuring the apparent digestibility and nutritive value of horse foods.

2.7.1. Introduction

In vitro techniques are becoming increasingly popular research tools, for assessing the nutritive value of foods, as they are cheap, rapid and easily repeatable alternatives to *in vivo* digestibility trials. However, for such techniques to be accurate substitutes for *in vivo* experiments they must produce consistent and accurate predictions of *in vivo* values (Adesogan, *et al.*, 1997). Thus, to be successful, *in vitro* techniques must simulate the 'real' caecal/ rumen environment by operating at the appropriate pH and temperature, by maintaining normal microbial populations, attaining normal degradation rates and additionally, have the ability to predict *in vivo* results (Nocek, 1988; Warner, 1956). Moreover, when using *in vitro* results for predicting *in vivo* digestibility, an *in vivo* calibration data set is required. The *in vitro* results can then be regressed against this data set, to derive prediction equations, from which *in vivo* digestibility can be determined (Goldman *et al.*, 1987). This exercise has been done many times for ruminants (Tilley and Terry, 1963; Omed *et al.,* 1989; Johnson, 1966; Huntington and Givens, 1995), and provided separate equations are used for different forage types and each laboratory derives it's own calibration data set, the equations are considered an accurate prediction of *in vivo* values (Weiss, 1994).

The most widely used *in vitro* techniques are the Tilley and Terry (1963) and Gas production (Theodorou *et al.,* 1994) systems, which involve the use of microbial inoculum prepared from rumen / caecal fluid or faeces. The latter form of inoculum is becoming increasingly popular (Harris, 1995), as it dispenses with the need for fistulated animals, which is both expensive and unpopular in today's research environment (Mauricio *et al.,* 1997).

2.7.2. The Tilley and Terry technique.

This technique is a two-stage process. The first involves incubating (anaerobically) *ca.* 0.5g of ground food with 10 ml of rumen fluid, and 40 ml of bicarbonate buffered salt solution for 48 hours. The second step simulates enzymatic digestion, by addition of HCl until pH 2 is attained and incubating with pepsin for a further 48 hours. This technique yields an end-point value for DM disappearance, but does not indicate the rate at which degradation has occurred.

As enzymatic digestion precedes microbial degradation in the equid, this technique would not accurately simulate the digestive processes of the horse. Additionally, horses have a much faster digesta passage rate than ruminants (Uden *et al.,* 1982), so information on the speed of degradation is particularly useful, to equine nutritionists. The Tilley and Terry (1963) technique would therefore be of limited use when studying the suitability and characteristics of different foods for horses.

2.7.3. The Gas Production technique.

The manual gas production technique, developed by Theodorou *et al.* (1994) is a relatively cheap method for measuring the rate and extent of fermentation of food and thus provides detailed information on degradation kinetics. The technique is based upon the measurement of gas (mainly CH_4 and CO_2) produced by the fermentation of food in a bicarbonate buffered medium, inoculated with rumen / caecal fluid or faeces. As the fermentation proceeds, gas collects in the headspace of the sealed culture bottles. The pressure and volume of this gas is periodically measured using a syringe and pressure transducer over an incubation period of *ca.* 140 hours. The speed and the amount of gas produced are determined by the characteristics of the food, thus the pattern of gas production is closely correlated with the pattern of food degradation (Jessop and Herrero, 1997). The process by which gas is produced within the culture bottles is not fully understood. In addition to the direct production of CH_4 and CO_2, indirect gases are

emitted which are believed to be produced by the fermentation acids reacting with the carbonates in the buffer and producing CO_2 (Rymer *et al.,* 1997). This can cause confusion when relating *in vitro* to *in vivo* information (Theodorou *et al.* 1997), although it is less problematic when using the technique to compare the *in vitro* degradation of different animal foods, as all the foods will be subject to the same conditions (Longland, pers. Comm.). At the end of the incubation, the fluid medium can be sampled for VFA analysis and the remaining substrate filtered and weighed to determine end-point DM loss.

This technique thus measures DM degradation indirectly (by the measurement of gas production) and directly by measuring substrate disappearance. The early measurements of gas production are particularly useful when studying horse-foods, as the speed of degradation is important to an animal that has a high rate of passage (Warner, 1981). However, several factors (ie. temperature, ambient pressure and inoculum source) can influence the early phase of gas production, and these must be taken into account when performing gas production experiments.

The temperature of the rumen is usually maintained between 39 and 40.5°C, which is optimal for microbial growth and activity (Johnson, 1966; Lowe *et al.,* 1987). Lowman (1998) found lower rates of gas production when bottles containing *Lolium perenne* grass were incubated at 25, 30, and 45°C. The lower temperatures affected microbial activity and may also have influenced the attachment of micro-organisms to food particles, while the high temperatures undoubtedly affected microbial growth and possibly denatured or degraded mediating enzymes (Johnson, 1966). Therefore the optimum temperature for *in vitro* experiments is 39°C, which maintains a critical balance between increased reaction rate and thermal inactivation of mediating enzymes (Mandelstam and McQuillen, 1973). Ambient pressure will affect the volume of gas produced, which must be considered when comparing experimental results from different regions across the country. However, the most important factor to influence the early rates of gas production is inoculum source.

2.7.3.1. Inoculum source.

The gas production technique was originally developed using rumen liquor as the microbial inoculum. However, Mauricio *et al.,* (1997) recently reported similar cumulative gas profiles when using both rumen fluid and ruminant faeces as inoculum. The lag time was greater with the faecal inoculum but all other parameters were similar indicating the suitability of faeces as a source of microbes for this technique.

Although the gas production system has not been widely used for measuring the *in vitro* degradation of horse foods, the suitability of this technique for measuring horse-food degradation should not be over-looked. The horse's gastrointestinal tract, like that of the ruminant, contains anaerobic bacteria (Kern *et al.,* 1973, 1974; Mackie and Wilkins, 1988), whose objective is to sustain their own population by utilising structural carbohydrates and producing volatile fatty acids (VFA), as waste products, which in turn are utilised by the animal (Janis, 1976). Therefore, an *in vitro* system, based upon fermentation, is eminently suited for studying the degradation of horse foods. Additionally, because the horse is a hindgut fermenter, their faeces contain high numbers of viable microbes (Uden *et al.,* 1982), which act as an ideal inoculum for such a process. Moreover, horse faeces are produced in large faecal balls, which generally remain anaerobic for some time after voiding, thus the microbes adhering to the voided food particles (Theodorou *et al.,* 1993; Van Soest, 1982) remain viable for several hours after excretion (Holter, 1991). Macheboeuf and Jestin (1997) used equine faecal inoculum for predicting OMD of forages in horses and found the results agreed well with the data obtained from caecal-inoculated forages. Lowman (1998) also used equine faeces to measure *in vitro* degradation of naked oats, Soya hulls and sugar beet pulp, and found that although significantly less gas was produced from faecal inoculated naked oats and Soya hulls, compared with caecal inoculated samples, the gas produced from the sugar beet did not differ between the two inocula. Lowman (1998) thus concluded that equine faeces is a suitable alternative to caecal fluid as an inoculum for gas production studies, although separate predictive equations must be derived for each type

of inoculum if the system is to be successfully used to predict the nutritive value of horse foods.

The main advantage of faecal inoculum as opposed to caecal inoculum is that intact animals can be used as donors. This reduces experimental costs and affords a degree of flexibility as different donor animals can be used. Moreover, the low cost and portability of the manual pressure transducer technique allows the experiment to be carried out in all types of laboratories, thus the hindgut microbial activity of an animal with a compromised gut could be assessed, or a specific feeding regime could be tested using faecal inoculum from the horses concerned.

2.7.3.2. Donor animal

The microbial inoculum, whether of caecal or faecal origin, provides the active ingredient in an *in vitro* system. Since diet affects the profile and numbers of gastrointestinal microbes, it follows that the diet of the donor animal will influence the activity of the inoculum (Weiss, 1994). Therefore, if the objective of the experiment is to predict *in vivo* digestion from the *in vitro* data, the diet fed to the donor animal should be similar to the substrate in the bottles. When determining the DMD of forages, the type of forage fed to the donor animal does not have a significant effect on gas production (Weiss, 1994). The only exception to this is when low protein forages are fed (particularly those < 10%CP), which can compromise microbial activity (Demment and Van Soest, 1983). Dhanoa and Deciaz (1984) reported improved precision when *ca.* 10mg of N was added to the incubation mixture. Thus careful consideration of the objectives of the experiment is required, before the donor animal is chosen.

2.7.3.3. The use of mathematical models to describe gas production data.

Commonly, gas production data is presented as cumulative gas profiles, which show the amount of gas produced from the fermentation of *ca.* 1 g of foodstuff over an incubation period of *ca.* 140 hours. However, comparison of gas production profiles is only possible if foods are fermented under similar conditions within the same experiment. Mathematical modelling of these profiles produces rate parameters, which allows comparisons to be made between foods across different experiments. All gas models work on the principle that a substrate S is fermented to yield a volume of gas Y at a rate μ (μ = fractional rate of degradation per hour at time t), and are based on 4 types of expression;

1. Mitscherlich
2. Michaelis-Menten
3. Logistic
4. Gompertz

(France and Thornley, 1984).

Schofield, Pitt and Pell (1994) developed a dual-pool logistic equation with a single lag value to be used in conjunction with the automated system of Pell and Schofield (1993), whereas the multi-phasic model produced by Groot *et al.* (1996) was developed for use with the automated gas production system of Cone *et al.* (1994).

The model of France *et al.* (1993), (equation 2.7.1) was developed specifically for the pressure transducer Technique of Theodorou *et al.* (1994) and models the sigmoidal type of response frequently encountered when fermenting foods *in vitro.*

$$Y = A \{1 - \exp[-b (t - L) - c(\sqrt{t} - \sqrt{L})]\} \qquad \text{(equation 2.7.1.)}$$

Y = gas produced (ml) at time t.

A = asymptotic value for gas pool size.

b = rate constant (h^{-1}) and is independent of time

c = rate constant ($h^{-0.5}$) and whose influence decreases with time

t = time

L = lag time (hours)

Equation 2.7.1. can also be written as: $Y = A - B\,Q^{t}\,Z^{\sqrt{t}}$

Where:

$B = A\,e^{bL + c\sqrt{L}}$

$Q = e^{-b}$

$Z = e^{-c}$

The fractional rate of gas production, (FRGP or μ) ie. the amount of gas produced at t_{50} can be calculated from

$$FRGP = b + (c / 2\sqrt{t_{50}}) \qquad \text{(Equation 2.7.2.)}$$

The rate constants b and c both influence the FRGP. As the c rate changes with time it influences the shape of the curve. When c is a negative figure, the resulting curve is sigmoidal in shape and thus the quadratic equation 2.7.2 is used to derive the fitted data. However, when c = 0 an exponential rate of gas production has occurred and the simpler exponential equation 2.7.3 is used to obtain the relevant parameters.

$$Y = A - B\,Q^{t} \qquad \text{(equation 2.7.3.)}$$

The extent of degradation can be calculated from equation 2.7.4

$$Ext.D = So\,e^{-kL}\,(1 - kI) / (So + Uo) \qquad \text{(equation 2.7.4)}$$

Where:

S = quantity of degradable food at t = 0

U = quantity of undegradable food at t = 0

k = passage rate constant (h^{-1}) usually estimated at 2-3%.

I = integral ($I = \int^{\infty}_{L} \exp \{- [(b + k) (t - L) + c (\sqrt{t} - \sqrt{L})]\} dt$

L = lag time (hours)

The advantage of the France *et al.* (1993) model over those of Schofield *et al.* (1994) and Groot *et al.* (1996) is that the extent of degradation can be calculated, thus taking into account the important factor of digesta flow rate (k). The France *et al.* (1993) model has been used in this thesis, because it not only enables the calculation of the extent of substrate degradation, but it also works using two rate constants, from which the time taken to produce 50% of the total gas (t_{50}) can be calculated. This is a particularly useful estimate when measuring the degradability of foods for Animals like the horse because the proportion of food to be degraded in the early stages of the incubation is more relevant to an animal with a low digesta MRT of *ca.* 25 hours (Pearson and Merritt, 1991; Morrow, 1998) than to a ruminant which has an MRT of *ca.* 57 hours Uden *et al.* (1982b).

2.7.4. Near Infrared Reflectance Spectroscopy for estimating digestibility.

Infrared reflectance spectroscopy (NIRS) can be used to predict the digestibility of animal foods *in vitro,* by measuring the chemical content of the food and applying the data to multivariate mathematical models derived from data sets calibrated by 'wet chemistry' methods (Baker *et al.,* 1994). However, as with estimating the chemical composition of food (section 2.2.4) lack of precision or inaccuracies in the calibration data can result in highly variable results. The inaccuracies associated with the derivation of an equation to predict digestibility from chemical content, is added to that associated with the production of a robust validation data set for determining chemical composition, thus the accuracy of the prediction process can be severely compromised. In order to improve the ability of NIRS to predict OMD of silage, Barber *et al.* (1990) used data from 122 *in vivo* silage samples to develop prediction equations using multi-

variate linear regression analysis of the *in vivo* and NIRS data. When tested on an additional 48 data sets, the equation produced good agreement with the *in vivo* data, thus other workers Park *et al.* (1997) and Gordon *et al.* (1998) have adopted this approach when using NIRS to predict OMD of silages for ruminants.

Although NIRS is used on the production line in horse-food companies to measure chemical composition of the food (Orme, pers comm.), no data exist on the prediction of digestibility for horses using NIRS. A possible reason for this is the lack of *in vivo* data on horse foods, which would make accurate validation impossible. While the initial costs of installing NIRS are high, there is little doubt that this procedure, which is fast, accurate, reproducible and non-destructive, has great potential as a tool for predicting the nutritive value of horse foods.

2.8. The scope of this thesis

The aim of this thesis was to investigate the *in* vivo apparent digestibility and nutritive value of botanically diverse fibrous foods in ponies. Firstly, the intake and apparent digestibility of several types of conserved forage and dried, processed high-fibre food by-products, were evaluated, together with their effect on intra-caecal fermentation parameters in order to establish if these were suitable foods for inclusion in equine diets. Secondly, the suitability of pig and ruminant *in sacco* and indigestible marker Techniques, were evaluated as methods for determining small intestine and total tract apparent digestibility and digesta rate of passage in equids. Finally, the potential of the *in vitro* gas production Technique of Theodorou *et al.* (1994) was evaluated as a tool for the routine prediction of the nutritive value of fibre foods for horses.

Chapter 3. Experimental Work

3.1. Intake and apparent digestibility of four types of conserved forage by ponies.

3.1.1. Introduction

Grass hay is the traditional conserved fodder fed to horses in the UK. However, good quality hay is often scarce and even the best samples contain high levels of dust (Woods *et al.*, 1993). This dust is largely composed of fungal spores, plant fragments, mites and bacteria, all of which are 'respirable' and thus potentially allergenic to horses (Clarke, 1992). Exposure to this dust, even in well-ventilated stables with up to five changes of air per hour, can initiate the debilitating disorder chronic obstructive pulmonary disease (COPD) (Cook, 1976; McPherson and Thomas, 1983; McGorum *et al.*, 1993; Webester *et al.*, 1987). Horses that have COPD remain sensitised to stable dust for the rest of their lives and cannot be fed hay without developing the disease (Lawson *et al.*, 1979). In order to maintain these animals in an a-symptomatic condition, owners are forced to seek alternative low-dust forages and many choose commercially produced haylage, which although expensive, is the most widely used replacement for hay (Todd and Newsum, 1991).

Other conserved forages commonly fed to farm animals, such as clamp and big bale silages, are seldom fed to horses. Clamp silage is considered too wet, too acidic and therefore unpalatable (Frape, 1986), whilst big bale silage, although conserved in a similar manner to haylage, is considered a high-risk food by horse owners, because it can contain *Clostridium botulinum and Listeria* spp (Ricketts *et al*, 1984; Gudmundsson, 1997) which can cause the fatal diseases Botulism and Listeria in all Animals. Both clamp and big bale silages are conserved at an earlier stage of growth than hay, and as a consequence, the nutritive value of these foods is generally higher than hay, with typical ME values determined for ruminants ranging from 12-14 MJ/kg DM, compared with an

average 8 MJ/kg DM for hay (MAFF, 1992). This additional 4-6 MJ/kg DM for silage, allows the productivity of farm Animals to be maintained with less reliance on supplementary concentrates than would be the case if the basal diet was hay. As hindgut fermenters, horses should theoretically gain similar benefits from these high quality fibre foods as ruminants. In order to determine whether silages are suitable forages for horses, detailed measurements of their nutritive value and apparent digestibility should be conducted.

The fibre content of forages is commonly determined gravimetrically using ADF and NDF analysis. However, these techniques measure the residues remaining after sequential treatment in various detergent solutions, which yield a figure for total fibre content rather than a quantitative measurement of individual fibre constituents. In addition, the gravimetric techniques can result in variable losses of polysaccharide fractions, such as pectins, arabinans and galactans and can contain non-fibrous components such as Maillard products (Van Soest and Mason, 1991), thereby compromising the accuracy of the value obtained. Non-starch polysaccharide (NSP) analysis is an alternative, more accurate method for determining fibre content in animal foods. NSP analysis involves breaking the potentially degradable fibre into its constituent monomeric units, yielding precise information on fibre composition as well as a value for the total carbohydrate content of the fibre (Englyst and Cummings, 1988). NSP analysis would therefore be a more accurate method for determining the potentially degradable fibre content of silages and could be used with benefit when seeking to determine the nutritive value of these forages for horses.

The aim of this experiment was to: a) to determine the intake and apparent digestibility of four types of conserved forage; grass hay (H), haylage (HY) big bale silage (BB), and clamp silage (CS) by ponies and b) to determine if silage is a suitable replacement for hay as the basal forage in equine rations.

3.1.2. Materials and Methods

Four mature Welsh cross pony geldings (LW *ca* 320-370 kg) were used in a 4x4 Latin square changeover design experiment, consisting of four 28-day periods. The ponies were individually housed in10x12 ft boxes, with Equimat rubber matting (Davies & Co. Kettering, UK) covering the floors and where water was available *ad libitum*. One of the four forages hay (H), haylage (HY), big bale silage (BB) or clamp silage (CS) were offered at *ca.*1.65kg DM / 100kg LW per day. The diets were fed in two equal meals per day, at 08:00 and 20:00 hours. Each 28-day period consisted of a 23-day adaptation phase, and a five-day collection phase. Pony LW was recorded weekly at 11:00 hours throughout the experimental period using a weigh-bridge (Basic Weigher, Tyler Farm Equipment, Grantham, Lincolnshirs UK). During adaptation weeks, ponies were turned loose in a 60 X 30 m out-door sand arena for *ca.* 1 hour of exercise, while during collection weeks they were hand-walked for 0.5 hours twice a day.

3.1.2.1. In vivo apparent digestibility and nutritive value measurements.

During the five day collection phase *in vivo* apparent digestibility (AD) of dry matter (DMD), organic matter (OMD), crude protein (CPD), acid detergent fibre (ADFD), neutral detergent fibre (NDFD), gross energy (GED) along with total and individual non-starch polysaccharide (NSP) constituents were determined by total faecal collection. Boxes were checked every 2 hours for faeces, which when excreted were collected using a dust-pan and brush, transferred into a pre-weighed plastic bucket and weighed before being placed into a large plastic bin. At 4 pm each day the daily faecal samples were taken to a cold-room and stored in covered plastic bins at 2°C until the end of the collection period. The total 5-day faecal outputs of each pony were then tipped onto a flat, scrubbed, concrete pad and three people thoroughly mixed the faeces using clean shovels and forks. A sub- sample of 600g was put into a pre-weighed foil tray and stored

at -20°C until freeze-dried for laboratory analysis. Prior to the 08:00-hour food, any food refusals from the previous 24 hours were collected and weighed.

3.1.2.2. Chemical analysis

Freeze-dried samples of food and faeces were analyzed for DM, (using the difference in weight obtained between fresh weight and weight after freeze drying) OM (difference between dry weight and residue weight after burning at 500°C which = ash, 100 – ash = OM), according to the method of the Association of Official Analytical Chemists (1990). Acid detergent fibre (ADF), neutral detergent fibre (NDF), crude protein (CP), calcium (Ca), phosphorus (P), magnesium (Mg), starch and gross energy (GE) analytical methods are all detailed in appendix 1.

Total non-starch polysaccharide (TNSP) were determined as the sum of the individual monomeric constituents, the neutral sugars of which were quantified by gas chromatography of alditol acetate derivatives of acid hydrolysates of de-starched samples using the method of Englyst and Cummings (1984) detailed in appendix 1.

3.1.2.3. Calculation of theoretical energy and crude protein intakes.

The intakes of digestible energy (DE) in MJ/kg DM and digestible crude protein (DCP) g/kg DM of the foodstuffs offered were calculated using the following formulae (Cochrane and Galyean, 1994):

DE (MJ/kg DM) = mean GED X GE content of the food.

DCP (g/kg DM) = CPD coefficient X CP content of food g/kg DM

DE requirements (DE req) MJ/d, and DCP req (g/day) were calculated from NRC (1989) recommendations, using the following formulae:

$$\text{DE req (MJ/d)} = (0.021 \times \text{LW} + 0.975) \times 4.18$$

$$\text{DCP req (g/d)} = 0.6 \times \text{LW}$$

3.1.2.4. Data analysis

Pony LW and *in vivo* apparent digestibility and nutritive values were subjected to a two-way analysis of variance using Genstat 5 (Laws Agricultural Trust, 1993). Comparisons between foods were made using the least significant difference test (LSD = t value for the error degrees of freedom (df) x the standard error of difference (s.e.d) obtained from the analysis of variance).

3.1.3. Results

3.1.3.1. Food composition

The average chemical compositions of the four experimental forages are given in Table 3.1.1. The DM contents ranged from 337 g/kg for CS to 922 g/kg for H. The CP content of the hay at 44 g/kg was notably lower than the 70, 111, and 154 g/kg DM for HY, BB and CS respectively. ADF values for HY, CS and BB were similar at *ca.* 370 g/kg DM, whereas NDF values for CS were similar to H (557 and 529 g/kg respectively), but notably lower than for either HY or BB. Starch contents of the four forages were less than 100 g/kg DM, ranging from 33 g/kg in CS to 90 g/kg DM in H. Mineral levels were variable with levels of Ca, P and Mg in H being lower than in the other three forages.

All forages contained low levels of rhamnose and mannose. The pectic fraction which is composed largely of arabinose, galactose and uronic acids was 12 -16% of the TNSP content. HY had higher levels of glucose (720 g glucose/kg TNSP) than the remaining foods, which contained less than 600 g/kg TNSP as glucose. However, glucose and xylose together accounted for more than 850 g/kg of the TNSP present in all the foods. H and BB had the highest TNSP contents at 408 and 405 g/kg DM respectively, followed by CS with 353g/kg DM and HY with 100g less at 293g/kg DM.

Table 3.1.3.1. Average composition of the four experimental forages hay (H), haylage (HY), big-baled silage (BB) and clamp silage (CS) fed to the 4 ponies (g/kg DM).

	H	**HY**	**BB**	**CS***
DM (g/kg)	922	676	500	337
OM	946	934	937	916
CP	44	70	111	154
ADF	410	359	389	357
NDF	529	602	654	557
Starch	90	50	40	33
GE (MJ/kg)	17.4	17.5	17.8	18.6
Ca	3.1	5.8	4.7	5.5
P	1.3	2.0	3.7	3.3
Mg	1.4	1.8	2.4	2.6
Rhamnose	0.5	0.1	0	0.5
Arabinose	25	17	25	23
Xylose	93	49	99	69
Mannose	2	2	1	2
Galactose	9	6	8	9
Glucose	261	210	256	224
Uronic acids	20	11	17	25
Total NSP	408	293	405	353

*CS fermentation characteristics:- pH 4; NH_3 7% of total N; TVFA 86 g/kg DM; lactic acid 69 g/kg DM; acetic acid 17 g/kg DM; butyric acid <1g/kg DM.

3.1.3.2. Liveweight and food intake measurements

Table 3.1.3.2 details the average live weight (LW) and voluntary food intake (VFI) of the ponies when offered the four forages at *ca.* 1.65kg DM/100 kg LW per day. All ponies had food refusals on each diet, although the extent of refusals differed with each pony. Fresh weight intake (FWI) for H was significantly lower ($P<0.05$) at 5.37 kg/d than when ponies were consuming HY, BB and CS when intakes were 9.31, 11.78 and 8.85kg/d respectively. However, when comparing DMI (kg/d), both the H and CS diets were significantly lower ($P<0.05$) than that recorded for the HY and BB diets. The DM content of the HY was 12% higher than the 55% quoted in the trade literature (Marksway, HorseHage, Paignton, Devon) thus the ponies received *ca.*1.9 kg of HY DM /100 kg LW per day. Similarly, the DM content of the BB was also a little higher than the original analysis indicated therefore ponies received *ca.* 1.8 kg BB DM / 100 kg LW per day. When expressed per unit LW (g/kg LW) or on a metabolic live weight (g/kg $LW^{0.75}$) basis the DMI of CS continued to be significantly ($P<0.05$) lower than for the other three foods, whereas the DMI of the H was only significantly ($P<0.05$) lower than that of the HY when calculated per unit live weight. Average pony LW was significantly lower ($P<0.05$) at 317 kg when ponies were fed CS compared with when they were fed either the BB or HY diets, when LW increased to 340 kg and 341 kg respectively. No significant difference in LW was recorded when the ponies were fed H, HY, or BB.

Table 3.1.3.2 Mean live weight (LW) and voluntary food intake measurements of fresh weight intake (FWI), and dry matter intake (DMI), for 4 ponies consuming either hay (H), haylage (HY), big bale silage (BB) or clamp silage (CS) *ca.* 1.65 kg DM/ 100 kg LW.

	H	HY	BB	CS	s.e.d	Sig
LW (kg)	333^{ab}	340^{b}	341^{b}	317^{a}	8.99	*
Voluntary Food Intake						
FWI (kg/d)	5.37^{a}	9.31^{b}	11.78^{b}	8.85^{b}	1.226	*
DMI (kg/d)	4.95^{a}	6.30^{b}	5.96^{b}	2.95^{c}	0.444	*
DMI (g/kg LW)	14.7^{a}	18.4^{b}	17.3^{ab}	9.17^{c}	1.47	*
DMI (g/kg$W^{0.75}$)	62.9^{a}	79.2^{b}	74.6^{ab}	38.8^{c}	6.06	*

^{abc} Values in the same row not sharing common superscripts differ significantly.

3.1.3.3. In vivo apparent digestibility.

Table 3.1.3.3 details the *in vivo* apparent digestibility coefficients of DM, OM, CP, ADF, NDF, starch, GE, Ca, P and Mg, together with the DE and DCP contents of the four experimental forages. With the exception of starch, H was the least digestible of the four forages for all parameters measured, and these differences were significant ($P<0.05$). Although the digestibility coefficients of HY constituents were generally 0.14 to 0.59 greater than the corresponding values for hay, values for HY were still significantly lower ($P<0.05$) than those recorded for CS for CPD, ADFD, NDFD, and GED. Across all measurements, the digestibility of CS and BB were not significantly different, although the CS tended to be slightly more digestible than the BB diet. The digestibility of all of the organic parameters was in excess of 0.6 for CS. By contrast

mineral digestibilities of Ca and P were highest from HY, although the only significant difference ($P<0.05$) was between HY and CS for CaD.

The DE content (MJ/kg DM) of H (5.75 g/kg DM) was significantly ($P<0.05$) lower than from the other three forages. Furthermore, the DE of HY at 9.09 MJ/kg DM was significantly ($P<0.05$) lower than that of CS (11.98 MJ /kg DM).
DCP contents (g/kg DM) were significantly ($P<0.05$) different between all foods, with CS > BB > HY > H.

NSP digestibility coefficients are shown in Table 3.1.3.4 and indicate that the TNSPD, GD and XD of CS and BB were significantly higher ($P<0.05$) by 20 to 30% than for both the H and HY foods. Except for rhamnose, the digestibility of all monomers measured in BB and CS were greater than 0.64, which was more than 40% of the corresponding values for H. HY digestibility values were intermediate at 0.4-0.6, but these values were still significantly lower than the CS and BB in all but AD and GAD.

Table 3.1.3.3. *In vivo* apparent digestibility coefficients and calculated DE and DCP nutritive values of hay (H), haylage (HY), big bale silage (BB) and clamp silage (CS) when fed to the 4 ponies.

	H	HY	BB	CS	s.e.d	Sig
DMD	0.39^{a}	0.57^{b}	0.61^{b}	0.67^{b}	0.046	*
OMD	0.40^{a}	0.57^{b}	0.62^{b}	0.67^{b}	0.047	*
CPD	0.20^{a}	0.48^{b}	0.66^{c}	0.68^{c}	0.041	*
ADFD	0.31^{a}	0.45^{ab}	0.57^{bc}	0.67^{c}	0.062	*
NDFD	0.33^{a}	0.47^{ab}	0.58^{bc}	0.66^{c}	0.058	*
Starch D	0.67	0.70	0.63	0.62	0.064	NS
GED	0.33^{a}	0.52^{b}	0.55^{bc}	0.65^{c}	0.048	*
CaD	0.29^{a}	0.71^{b}	0.60^{b}	0.52^{c}	0.122	*
PD	-0.23^{a}	0.24^{b}	0.46^{b}	0.40^{b}	0.090	*
MgD	0.80^{a}	0.66^{b}	0.63^{b}	0.48^{b}	0.154	*
Nutritive Value						
DE (MJ/kgDM)	5.75^{a}	9.09^{b}	9.83^{bc}	11.98^{c}	0.927	*
DCP (g/kgDM)	9^{a}	34^{b}	74^{c}	104^{d}	5.02	*

[abc] Values in the same row not sharing common superscripts differ significantly ($P<0.05$).

Dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD), acid detergent fibre digestibility (ADFD), neutral detergent fibre digestibility (NDFD), starch digestibility (starch D), gross energy digestibility (GED), calcium digestibility (CaD), phosphorus digestibility (PD) magnesium digestibility (MgD), digestible energy (DE) and digestible crude protein (DCP).

Table 3.1.3.4. *In vivo* apparent digestibility coefficients of rhamnose, arabinose, xylose, mannose, galactose, glucose, uronic acids and total non-starch polysaccharides (TNSPD) by four ponies offered hay (H), haylage (HY), big-bale silage (BB), and clamp silage (CS).

	H	HY	BB	CS	s.e.d	Sig
Rhamnose	0.33	0.22	0.25	0.50	0.391	NS
Arabinose	0.53^{a}	0.59^{ab}	0.75^{bc}	0.81^{c}	0.066	*
Xylose	0.34^{a}	0.21^{a}	0.68^{b}	0.67^{b}	0.070	*
Mannose	0.76	0.93	0.96	0.73	0.086	NS
Galactose	0.58^{a}	0.64^{ab}	0.71^{b}	0.76^{b}	0.049	*
Glucose	0.38^{a}	0.47^{a}	0.64^{b}	0.76^{b}	0.066	*
Uronic acid	0.77^{b}	0.65^{a}	0.85^{bc}	0.92^{c}	0.038	*
TNSPD	0.41^{a}	0.45^{a}	0.67^{b}	0.76^{b}	0.065	*

^{abc} Values in the same row not sharing common superscripts differ significantly ($p<0.05$).

3.1.3.4. Energy and protein parameters

Average DE intakes in MJ/day (Table 3.1.3.5) of H and CS at 30.7 and 34.9 respectively were significantly lower ($P<0.05$) than those of both HY (56.6) and BB (58.7). This trend was similar for all calculations of daily energy intake, whether expressed on a pony live weight (LW) or metabolic live weight ($LW^{0.75}$) basis. As a result of the slightly lower LW recorded for ponies consuming the CS, the calculated DE requirement was similar to the H but slightly lower than that calculated for the BB and CS diets. The amount of DE consumed per day on the H diet was slightly below the calculated

theoretical requirements, whereas on all other forages the DE requirement was met or exceeded.

DCP intake (DCPI) for H was only 44 g/day and was significantly ($P<0.05$) lower than the intake recorded for the other three diets. The H diet provided only 20% of the theoretical daily DCP requirements; therefore the ponies were deficient in DCP when consuming this diet. BB provided the most DCP at 447 g/day, which amounted to twice the daily requirement. HY provided 3% over the requirement, whereas CS provided an excess of 1.5%.

Table 3.1.3.5. Energy and protein intakes and requirements of the 4 ponies when offered hay (H), haylage (HY), big bale silage (BB) and clamp silage (CS).

	H	HY	BB	CS	s.e.d	Sig
Energy Parameters						
DE intake (MJ/day)	30.7^{a}	56.6^{b}	58.7^{b}	34.9^{a}	5.59	*
DE intake (MJ/kg LW)	0.09^{a}	0.17^{b}	0.17^{b}	0.11^{a}	0.019	*
DE intake ($MJ/kgLW^{0.75}$)	0.39^{a}	0.72^{b}	0.73^{b}	0.46^{a}	0.079	*
DE req[1] (MJ/d)	33.4^{ab}	34.0^{b}	34.1^{b}	32.0^{a}	0.793	*
DEI / DE req	0.90^{a}	1.66^{b}	1.70^{b}	1.07^{a}	0.188	*
Protein parameters						
DCPI (g/day)	44^{a}	212^{b}	447^{c}	306^{b}	51.5	*
DCP req[1] (g/day)	200^{ab}	204^{b}	204^{b}	190^{a}	5.4	*
DCPI / DCP req	0.22^{a}	1.03^{b}	2.2^{c}	1.6^{bc}	0.231	*

[abc] Values in the same row not sharing common superscripts differ significantly.

[1] Calculated from NRC (1989) DE req (MJ/day) = (0.021 x LW + 0.975) x 4.18

DCP req (g/day) = 0.6 x LW

3.1.4. Discussion

3.1.4.1. Food composition and voluntary food intake measurements

The DM contents of the 4 forages fed in this experiment were notably higher than the 864, 368 and 242 g/kg recorded by MAFF (1992) for typical UK samples of H, BB and CS respectively. No comparative values could be found for HY, although the DM content recorded here was 12% higher than the 550 g/kg DM quoted in the trade literature for this forage (Marksway, HorseHage, Paignton, Devon). The CP content of the H at 44 g/kg DM was less than half the MAFF (1992) value of 107 g/kg DM quoted for average UK hay and is typical of the mature grass hay fed to horses (Frape, 1986). This low CP content resulted in a deficiency in DCP supply when ponies were consuming the H diet. Although the DCP contents of the other three forages were also lower than values presented for similar UK forages (MAFF, 1992), these three foodstuffs met or exceed the calculated dietary DCP requirements. ADF, GE, P and Mg contents of the four forages are similar to UK averages (MAFF, 1992) and reflect the physiological maturity of the grass at the time of conservation.

The NDF values of 529 g/kg DM detected in the hay in this experiment are slightly lower than the 546 to 695 g/kg DM quoted by Smoulders, Steg and Hindle (1990) and Dulphy *et al.* (1997a) for a range of grass hays fed to horses and sheep. The CS value of 557 g/kg DM, is similar to the UK average for this type of forage and is within the range of 403 to 569g/kg DM, cited by Smoulders *et al.* (1990). Ca levels at 3.1, 4.7, and 5.5g/kg DM for H, BB and CS respectively, were notably lower than UK average values of 5, 6.3, and 6.4g/kg DM (MAFF, 1992) and reflect the low Ca content of the soil in West Wales where these forages were grown.

Rhamnose and mannose levels are known to be low in the *gramineae* and levels in all four forages were indeed low (<0.5 g/kg) and at the limits of detection by NSP analysis. The variability in AD of this sugar was therefore probably due to the low detectability of

rhamnose by the NSP analysis rather than any animal factor *per se*. In all four forages the xylose and glucose together comprised > 830 g/kg DM, with arabinose and uronic acids making up the remaining 150 g/kg DM. HY contained notably lower amounts of xylose and glucose than the other three forages, and this resulted in a 60 to 115 g/kg less total NSP than H, BB and CS. The NSP profile of the HY suggests that this forage contained less cell wall material than the other foods, suggesting that it was harvested at an early stage of growth. This undoubtedly was the main factor influencing the DMD of this forage, as it is well known that the growth-stage of a crop at harvest is the main factor influencing the subsequent digestibility of that forage post-conservation (McDonald *et al.,* 1991).

The amount of food offered was designed to meet the maintenance requirements of the ponies, having been pre-determined in a preliminary experiment in which LW and food refusals were recorded. However, over the 12-week experimental period, when each diet was offered to only one pony in each period, food intakes varied between ponies and between diets, with some ponies showing marked preferences for certain food. Significant food refusals were collected in 13 out of the 16 measurements taken, thus the ponies exercised a degree of choice. Ponies 1 and 2 had food refusals on all four diets, with particularly high refusals recorded for pony 1 on CS (52%) and pony 2 on H (49%). These values had a large effect on average DMI values, skewing the data in favour of lower intakes. Ponies 3 and 4 ate virtually all of the H, HY and BB on offer but refused 13 and 23% respectively of the CS. The preference for the BB and HY is reflected in the LW figures, which show a significant increase when ponies were consuming these two diets in comparison with the LW recorded when CS was fed. Fresh weight intakes (FWI) were similar for the HY, BB and CS diets, although the ponies did consume 2.93 kg/d more BB than CS, indicating that gut capacity was not a limiting factor in controlling the intake of CS.

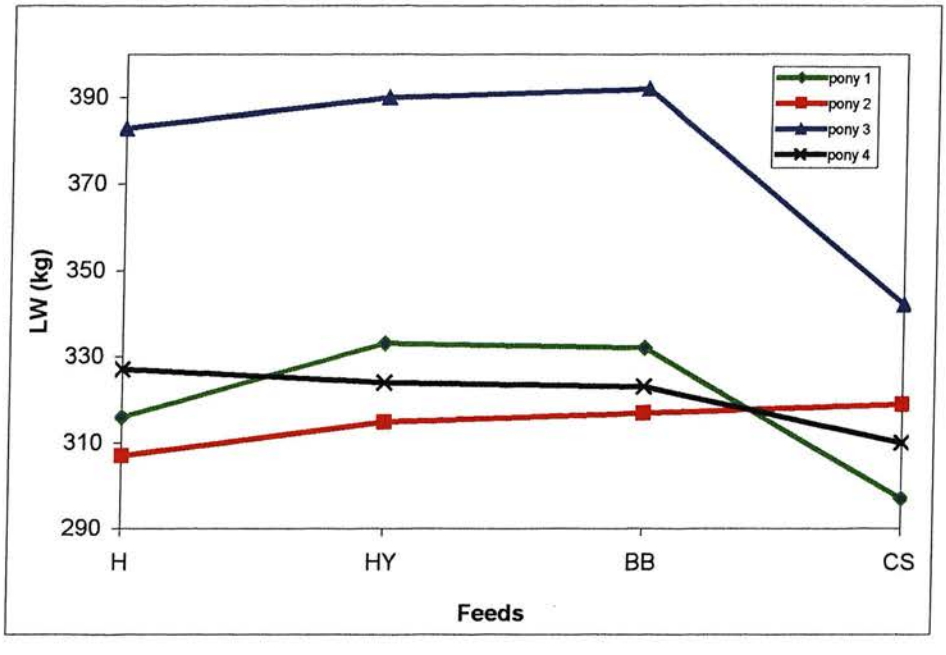
When considering the DMI of CS, the 2.95 kg DM /d consumed was significantly ($P<0.05$) lower than values recorded for the other three forages. Hay DMI at 4.95 kg/d

(which was strongly influenced by the 49% refusal of one pony) was also significantly ($P<0.05$) lower than the average DMI for both the HY and BB (6.3 and 5.96 kg/d respectively) and would suggest that the DM content of the foods did not play a major role in determining forage intake in this experiment. The DMI of BB and H noted here are higher than the 4.46 and 3.88 kg DM/d noted for big bale silage and hay by Morrow (1998) when feeding two different chop lengths of silage and hay to ponies. Morrow (1998) also recorded significantly lower DMI for hay than for both chop lengths of big bale silage. Calculation of DMI on a metabolic live weight basis (DMI g/kg $W^{0.75}$) allows comparisons to be made between DMI of animals of different live-weights. The DMI of 62g/kg $W^{0.75}$ for H obtained in this experiment is at the lower end of the 63 to 99g/kg $W^{0.75}$ range quoted in the literature for a variety of grass hays (Cymbaluk, 1990; Martin-Rosset and Dulphy, 1987; Pearson and Merritt, 1991). The DMI of BB and HY were similar at 74.6 and 79.2 (g/kg $LW^{0.75}$) respectively, which although slightly higher than the 62.9 g/kg $LW^{0.75}$ obtained for the H in this experiment, were within the range quoted in the literature for feeding hay *ad libitum* (Croizer *et al.,* 1977; Martin-Rosset and Dulphy, 1987). Unfortunately, there are no literature values with which to compare the DMI of the BB or HY forages in horses or ponies.

The DMI of CS was significantly lower at 38.8 g/kg$W^{0.75}$ than the values obtained for the other three foods, but similar to the 31.7 g/kg $W^{0.75}$ recorded by McLean *et al.*, (1995) when feeding 'Ecosyl' inoculated *Lolium perenne* clamp silage *ad libitum* to horses and steers. Martin-Rosset and Dulphy (1987) also obtained comparable figures of 40.6 g/kg $W^{0.75}$ when feeding maize silage to 10-month old colts. Thus it would seem from previous studies and the results obtained here that the DMI of clamp silage is lower than for most hays. In the study of McLean *et al.* (1995), the ponies also received 1kg barley, 0.4 kg of grass nuts and a small amount of sugar beet, therefore the lower intake of the silage could be attributed to the ponies meeting DE requirements on the concentrate and silage diet. This could also be the case in this experiment when comparing the DMI of the CS with H where the GE content of the CS (18.6 MJ/kg) was only 1.2 MJ/kg DM higher than H (17.4 MJ/kg), but the DE of CS at 11.98 MJ/kg DM

was twice that for H (5.75 MJ/kg). However, this argument is unlikely to be valid when comparing DMI of CS with HY and BB where the DE of CS was only 2 MJ/kg DM higher than for HY and BB. Additionally, the ponies consumed 1.7 and 1.66 times their DE requirement when eating BB and HY respectively, so energy intake was clearly not an influential factor for controlling the VFI of CS. Figure 3.1.3.1. shows the LW changes for each pony when eating each of the four diets. Two ponies lost a significant amount of LW when consuming the CS diet. Although this loss was significantly lower ($P<0.05$) than the LW recorded on the HY and BB diets for pony 1 this was more a function of the LW gain seen on these two diets rather than a significant drop caused by the lower intake of CS. The drop in LW of pony 3 was due to a lower DMI of CS, which was *ca.* 3.4 kg/d less than he ate when offered the other three diets.

Figure 3.1.3.1. Live weight (LW) changes for the four ponies when consuming hay (H), haylage (HY), big bale silage (BB) and clamp silage (CS) at *ca.* 1.65 kg DM/100 kg LW.



The nutrient profile and fermentation characteristics of silage can affect food intake, and evidence exists which indicates that low intakes are associated with high organic acids (Harris *et al.*, 1966; Mc Cullough 1966). However, the pH, NH_3, and organic acid content of the silage fed in this experiment are well within the normal range for well-preserved silages and are unlikely to be the cause of low DMI (McDonald *et al.,* 1991). Moreover, this silage had a higher DM (340 g/kg) than the normal value of 240 g/kg quoted for UK clamp silage (MAFF, 1992), and should not have restricted intake.

No published data exist on the factors influencing the DMI of grass silage in equids. Clearly some characteristic has influenced the intake of CS in this experiment and this needs to be identified if the full potential of silage is to be realized in rations for equids.

3.1.4.2. In vivo apparent digestibility.

The apparent digestibility of DM, OM, CP and GE for H were significantly lower than the values recorded for the other three foods and are marginally lower than the published values for a variety of grass hays fed to horses (Morrow, 1998; Aiken *et al.,* 1989; Vander Noot and Gilbreath, 1970; Pearson and Merritt, 1991). These differences probably reflect the variation in species, edaphic conditions and cutting time of each of the hays.

The three 'fermented' forages, HY, BB, CS, had similar DMD, and OMD values, although the CS was 9% more digestible than the HY. The value for OMD of 0.62 recorded by McLean *et al.* (1995), is similar to the value of 0.62 noted here for BB than the 0.67 recorded for CS, and may suggest that the chemical composition of the BB fed here and the inoculated *Lolium perenne* silage fed by McLean *et al.* (1995) are similar.

Cuddeford *et al.* (1995) and Cymbaluk (1990) have reported a decrease in DMD of fibre food when the NDF content of the food increases. However, the NDF content of the most digestible forage, CS at 557g/kg DM, was only 3% higher than that of the H at

529g/kg DM; but the difference in digestibility was much greater, with CS being twice as digestible as the H. Additionally the BB diet had a higher NDF content than the HY, yet the NDFD of the BB was 11.2 % higher than that recorded for HY. NDF content *per se* was not, therefore the sole factor determining the digestibility of the four forages in this experiment.

Both the NDF and ADF digestibilities (ADFD, NDFD) of the CS were higher than any of the other foods, being significantly greater ($P<0.05$) than those of the HY or the H. The values of NDFD and ADFD for CS recorded here were also higher than that noted by McLean *et al.* (1995) for inoculated clamp silage. This difference in digestibility could be partly due to the lower cellulose content, which was 5% less in the grass conserved in CS than in the material fed by McLean *et al.* (1995). Morrow (1998) found the NDFD coefficient for big bale silage to be 0.62 and that of ADFD to be 0.64, whereas the BB fed in this experiment had an NDFD of 0.58 and an ADFD of 0.57. It is of note that the respective NDF and ADF contents of the silage fed by Morrow (1998) of 82 and 149 g/kg DM were lower than that fed in this experiment and indicates that the digestibility of silage is at least partly influenced by the cell wall content of the forage. The fermentation of the silage in the clamp may also have contributed to the higher digestibility of the fibre fraction of the CS. Lactic acid fermentation can release pentose sugars from acid hydrolysis of the hemicellulose fraction of the cell wall (McDonald *et al.,* 1991). This action may have rendered the hemicelluloses in the CS more accessible to hindgut microbial degradation thus contributing to the higher digestibility noted for this forage

The NSP analysis allows a more detailed examination of the individual cell wall components and helps to explain why plants with similar NDF and ADF values can be digested to different extents within the animal. The NSP fractions of the forages tested in this experiment shows that the monomers that comprised more than 830 g/kg of the TNSP, namely xylose and glucose, were approximately 70% digested from CS but only 40% digested from H. High contents of xylose and glucose together are indicative of

secondary cell wall formation and these cell walls are known to be less well degraded than primary cell walls, mainly due to the protective lingo-cellulose fraction, which is associated with secondary cell walls (Ben Ghedalia and Rubinstein, 1985). CS contained 24 and 37 g/kg DM less xylose and glucose respectively, than H, moreover, CS had a higher uronic acid content than H, indicating that a higher proportion of the fibre in this food was derived from primary cell walls rather than secondary cell walls. The lack of secondary thickening in the CS is undoubtedly due to the earlier stage of growth at harvesting and this, as the NDFD value indicated, would have been the primary reason for the higher digestibility of the CS food.

GED followed the same trend as the other chemical components with the CS being the most digestible of the four forages, followed by BB then HY and lastly H. However, there are no published values on GED of silage for horses with which to compare these experimental results.

3.1.4.3. Energy and protein intake parameters

The differential intakes of the four forages resulted in significantly higher average intakes of the HY and BB forages. This coupled with GED coefficients of HY and BB being greater than 0.5 resulted in higher DE intakes on these two foods. The DE requirements of the ponies were calculated from NRC (1989) recommendations for ponies on maintenance rations, and are calculated in terms of pony LW. This resulted in higher theoretical DE requirements when ponies were consuming the HY and BB diets. These values do not necessarily reflect the DE requirements for the maintenance of optimum LW, and in fact could erroneously encourage the horse owner to offer more food as LW steadily increases, thereby compounding weight increase in an over fat animal. This naturally works in the opposite manner, and occurred in this experiment when the calculated DE requirement decreased to 32 MJ/d as a result of the lower LW of the ponies when consuming CS. DEI expressed as a proportion of DE requirement was significantly lower ($P<0.05$) for H and CS at 0.9 and 1.07 respectively, but as these

values are based upon theoretical DE requirements they do not necessarily indicate that DE intake was deficient. The fact that DE requirements were met on the CS diet, despite the high food refusals, is a reflection of the high GED of the CS diet.

3.1.5. Conclusion

The results from this experiment clearly indicate that both CS and BB are readily digested by ponies and can be used as dust-free forages to replace hay in diets for stabled horses. Additionally, the high gross energy digestibility of these two foods, which have undergone various degrees of fermentation, will increase the DE obtained from the forage portion of the ration and make these ideal forages for horses with high-energy demands. However, for Animals on maintenance rations, the intake of BB and HY may have to be restricted to prevent obesity, whereas restriction of CS may be unnecessary as the DMI was self-limiting. The reason behind the lower intake of CS requires further investigation, if this potentially valuable forage is to be fully exploited as a food for equids.

3.2. Intra-caecal fermentation parameters and *in vivo* apparent digestibility of 4 botanically diverse fibre-based diets in ponies.

3.2.1. Introduction

By-products of the human food industry such as sugar beet pulp, Soya hulls and oat hulls are used extensively in farm animal foods and also have considerable potential as sources of dietary fibre in equine diets. The potentially energy yielding portion of these fibres, the non-starch polysaccharide (NSP) fraction (NSP + lignin = dietary fibre) is predominantly fermented in the large intestine to yield volatile fatty acids (VFA) which are absorbed and utilized by the animal as an energy source. In comparison to hay, the fibre fraction of these foods is highly degradable, thus on a per kg DM basis they can make a more valuable contribution than hay to the over-all energy balance of the animal (Glinsky *et al.*, 1976).

Tradition plays a major role in performance horse management, and so these animals frequently receive minimal amounts of forage because high levels of dietary fibre are known to increase the weight carried by the horse (Duren, 1998) and this is considered undesirable for an animal involved in high intensity exercise. Additionally, in order to meet the high energy requirements of these horses, high levels of starch-based concentrates are fed, which frequently result in behavioural abnormalities (Pagan, 1997), and metabolic disorders such as acidosis, colic and laminitis (Potter *et al.,* 1992b; Yelle, 1986; Carroll *et al.,*1987; Clarke *et al.*, 1990). Feeding a more energy-dense fibre could help to alleviate these problems, as fibre reduces the glycaemic response which is associated with abnormal behaviour in horses (Pagan, 2000), but more importantly hindgut fermentation parameters tend to remain more stable on fibre diets than when starch based concentrates are fed (Willard *et al.*, 1977; Clarke *et al.,* 1990; Radicke *et al.,* 1991), thus the incidence of acidosis and related metabolic problems is greatly reduced. Moreover, replacing a traditional fodder such as hay with an energy-dense fibre like sugar beet or Soya hulls will reduce the bulk-factor of the diet and may

encourage performance horse owners, in the interests of long-term health and well-being (Pagan, 2000) to increase the fibre portion of the diet to the recommended 1% LW per day.

Much literature has been published on the digestibility of a range of traditional grass hays in horses (see Table 3.2.1). However, relatively little information exists on the digestibility of a range of botanically diverse fibre-based foods. The objectives of the experiment reported here were 1) to examine intra-caecal fermentation parameters in ponies offered a range of botanically diverse fibre foods; 2) to determine the *in vivo* apparent digestibility and nutritive value of these foods in ponies and 3) to use the non-starch polysaccharide (NSP) analysis for a detailed examination of the digestibility of the fibre fraction from these foods.

Table 3.2.1. Apparent digestibility coefficients for dry matter (DMD), crude protein (CPD), acid detergent fibre (ADFD) and neutral detergent fibre (NDFD), in a range of forages fed to horses.

Forage	DMD	CPD	ADFD	NDFD	Author
Timothy hay	0.46	0.54	-	-	Vander Noot and Gilbreath (1970)
Alfalfa hay	0.69	0.77	0.47	0.44	Pearson *et al.* (1992)
Alfalfa hay	0.64	0.72	0.42	0.51	Cymbaluk (1990)
Brome grass Hay	0.48	0.51	0.29	0.44	Cymbaluk (1990)
Kentucky Bluegrass hay	0.45	0.59	0.40	0.51	Cymbaluk (1990)
Coastal Bermuda hay	0.43	0.51	0.36	0.46	Aiken *et al.*(1989)
Fescue hay	0.48	0.67	0.37	0.44	Croizer *et al.* (1997)

3.2.2. Materials and Methods

3.2.2.1. Experiment A

Three mature Welsh cross pony geldings (*ca* 250 kg LW) each fitted with a permanent caecal cannula (Cottrell *et al.,* 1998) at the top of the caecum (caecal base) were used in a 3 x 3 Latin square changeover design experiment consisting of three 21 day periods. Ponies were individually loose housed in 8X12 ft pens bedded with rubber matting (Davies and Co. Kettering, Northamptonshire, UK) and wood shavings (adaptation weeks only) and where water was available *ad libitum.* Ponies were offered 4kg dry matter (DM) per day of one of three diets comprising either ground, pelleted hay cubes (HC), a 67:33 mix of oat hulls : naked oats (OH:NO) or sugar beet food (SBF) in a 75:25 unmolassed : molassed mixture. Diets were soaked in 1.5 times their own weight in water to avoid any potential problems from choke or colic, and fed in two equal meals per day at 09:00 and 17:00 hours. 30 g of a mineral and vitamin supplement (Table 3.2.2.1) was added to each meal. The 21-day periods consisted of 16 days adaptation and 5 days collection. During the collection period intra-caecal fermentation parameters and *in vivo* apparent digestibility measurements were made. Pony liveweight (LW) was recorded weekly, using a weigh-tape (Equitape).

3.2.2.1.1. Intra-caecal fermentation parameters

On days 18 - 21 of each period caecal digesta samples were taken five hours after the 09:00-hour meal. Digesta samples were removed from the caecum *via* suction through an indwelling plastic tube (i.d. 13 mm) attached to the cap of the caecal cannula. Three 60 ml syringes were filled with caecal fluid. The third syringe was taken as the sample and the other two returned to the caecum *via* the in-dwelling tube. This procedure was repeated every time a sample of caecal fluid was taken, to ensure a sample from the body of the caecum was obtained. Similarly, on day 20 of each period, caecal digesta samples were taken at 09:00 hours and thereafter on an hourly basis until the 17:00-hour meal. pH was immediately determined upon withdrawal of each caecal sample, using a

Mettler Toledo 320 pH meter (Mettler – Toledo Ltd, 64 Boston Road Beaumont Leys, Leicester LE4 1AW, UK). 9ml of the sample was then preserved with the addition of 1ml of 1.8 molar H_2SO_4 and stored at –20°C until thawed for VFA and lactate analysis.

Acetate, propionate and butyrate concentrations (mmol/l) in the caecal digesta samples were subsequently determined by Gas Chromatography using the method of Merry *et al.* (1995) detailed below. Once thawed, the samples were centrifuged for 2 mins at 1500 g. 1ml of supernatant was pipetted into a 2ml vial and 0.1ml of internal standard (composed of 0.18 g of 2-methyl valeric acid, Aldrich Chemical Co. Gillingham, Dorset, UK in 100 ml of distilled water plus 760 µl of concentrated orthophosphoric acid) was added. The vial was then placed in a chromopack 9000 chromatograph with an automatic sampler and linked to an IBM computer with chromopack integration soft ware. 0.5 µl was injected into the fused silica capillary column (25 x 32 mm diameter). The column, after warming-up for 8 minutes, operated at 200°C while the injector and detector temperatures were 240 and 260°C respectively, with Helium as the carrier gas. The integrator was programmed to quantify the VFA contents of the samples in mmol /litre.

3.2.2.1.2. In vivo apparent digestibility and nutritive value

During the 5-day collection phase *in vivo* apparent digestibilities of DM (DMD), organic matter (OMD), crude protein (CPD), ADF (ADFD), NDF (NDFD), gross energy (GED) together with total and individual NSP constituents were determined by total faecal collection. Daily faecal samples were collected as described for Experiment 1 (section 3.1.2.1.) and dried in a forced-draught oven at 60°C until a constant weight was obtained. Faecal samples for the collection period were collated from the 5-day collections, according to the proportion of the daily faecal DM output for each pony (Cochran and Galyean, 1994). Composite samples of the food offered and the bulked samples of faeces, milled to pass a 1mm screen, were subsequently analysed for the proximate constituents, NSP and GE contents listed above according to the methods of

the Association of Official Analytical Chemists (1990) (see section 3.1.2.2.). Digestible energy (DE) and digestible CP (DCP) contents were also calculated for each food.

3.2.2.2. Experiment B

After completion of Experiment A the same three ponies were used in Experiment B when they were offered 4kg DM per day of a 50:50 unmolassed sugar beet : hay cubes mix (SB:HC) plus minerals fed twice daily as in experiment A for a single 21 day period. Food, faecal and caecal samples were taken during the last 5 days of this 21-day period and analysed as described for experiment A. In addition, the *in vivo* apparent digestibility coefficients, DE and DCP contents for SB as a sole food were calculated by difference (equation 3.2.2.1.), using the HC values (basal diet) obtained in experiment A.

$$\text{Digestibility} = \frac{b - (a \times \text{fraction of a in b})}{\text{fraction of test food in b}} \qquad \text{(equation 3.2.2.1)}$$

where:

b = digestibility of basal + test food
a = digestibility of basal food

3.2.2.3. Statistical analysis

Intra-caecal fermentation parameters, *in vivo* apparent digestibility and nutritive values in experiment A were subjected to an analysis of variance using Genstat 5 (Laws Agricultural Trust, 1993) and differences between foods determined using the L.S.D. test (L.S.D.= t error d.f x s.e.d.). In Experiment A one pony on the SBF diet had

particularly low intakes and was treated as a missing value in the statistical analysis. All data in experiment B are expressed as simple arithmetic means +/- their standard errors.

3.2.3. Results

3.2.3.1. Food composition and dry matter intake

The chemical composition of the four experimental diets offered in Experiments A and B are detailed in Table 3.2.3.1. The CP contents of all experimental diets were between 80 – 90 g/kg DM. The NSP content of SBF mainly comprised arabinose, glucose and uronic acids (UAC), which accounted for 870 g/kg of the total NSP. By contrast, HC and OH:NO contained very little arabinose and UAC but contained relatively high amounts of xylose and glucose, these two monomers accounting for 850 -900 g/kg of TNSP in these two foods. Dry matter intakes (DMI) for both experiment A and B are detailed in Table 3.2.3.2. When given SBF as the sole dietary ingredient in experiment A, ponies consumed less than half the 4 kg DM per day offered. Conversely, when given SB:HC in a 50:50 mix in experiment B the ponies consumed all of their ration.

Table 3.2.3.1. Chemical composition of the four diets, hay cubes (HC), a 67:33 mix of oat hulls:naked oats (OH:NO), sugar beet food (SBF) and 50:50 mix of unmolassed sugar beet:hay cubes (SB:HC) fed to the ponies in Experiments A and B (g/kg DM unless otherwise stated)

Constituent	Experiment A			Experiment B
	HC	**OH:NO**	**SBF**	**SB:HC**
DM (g/kg)	918	863	866	888
OM	932	968	911	913
CP	83	83	87	85
ADF	323	342	242	311
NDF	643	711	516	643
Starch	94	219	89	93
GE (MJ/kg DM)	18.6	19.4	16.5	17.8
Total NSP	370	414	452	446
Rhamnose	0	0	9	4
Arabinose	25	26	130	90
Xylose	99	119	7	43
Mannose	3	2	6	4
Galactose	10	8	33	25
Glucose	215	249	154	204
UAC	18	10	109	75

Composition of mineral and vitamin supplement:- (g/kg) Ca 160, P 117, Mg 67, Na 67; (mg/kg) Cu 683, Zn 2730, Fe 2730, Mn 2730, I 6.7, Co 6.7; (i.u.) Vit. A 136670, Vit. D_3 20500, Vit. E 3417.

3.2.3.2. Intra–caecal fermentation parameters

Table 3.2.3.2. details the intra–caecal fermentation parameters measured at 5 hours following the 09:00 meal on days 18 – 21 of each period in both experiments. No statistically significant differences in the pH values were seen between the diets in Experiment A, with mean values ranging from 6.50 for HC to 6.65 for OH:NO. In Experiment B, the pH measured 5 hours after the 09:00-hour meal averaged 6.58. In experiment A, total VFA (TVFA) levels were significantly lower ($P<0.05$) for SBF compared with the HC but not with the OH:NO diet, reflecting the lower DMI when SBF was offered as the sole diet. Lactate levels were significantly lower when ponies were fed HC and SBF at 0.3 and 0.89 mmol/l respectively, than when the OH:NO diet was fed, which produced 4 times the amount (3.73 mmol/l) of lactate than the other two diets. The SB:HC diet given in Experiment B resulted in the highest TVFA levels of 66.4 mmol/l and in common with the SBF and HC diets fed in experiment A, produced only low levels of lactate, 0.31 mmol/l, in the caecal chyme. The acetate molar proportion was significantly higher ($P<0.05$) for SBF compared with OH:NO but not with HC, when measured 5 hours after the 09:00 meal. In contrast, propionate levels were significantly higher ($P<0.01$) for OH:NO compared with SBF or HC in Experiment A. Butyrate molar proportions were significantly lower ($P<0.05$) when OH:NO was fed compared with the other diets fed in Experiment A.

Table 3.2.3.2. Pony live weight (LW) dry matter intake (DMI) and intra–caecal fermentation parameters in ponies fed hay cubes (HC), a 67:33 mix of oat hulls:naked oats (OH:NO), sugar beet food (SBF) in Experiment A and a 50:50 mix of unmolassed sugar beet:hay cubes (SB:HC) diet in Experiment B.

Parameter	Experiment A					Experiment B	
	HC	**OH:NO**	**SBF**	**s.e.d**	**Sig**	**SB:HC**	**s.e**
LW (kg)	265	265	267	3.60	NS	272	4.95
DMI (kg/d)	3.75[a]	4.03[a]	1.72[b]	0.26	**	4.03	0.03
Intra–caecal fermentation parameters (measured 5 hours following the 09:00 meal)							
PH	6.50	6.65	6.61	0.09	NS	6.58	0.06
Lactate (mmol/l)	0.30[a]	3.73[b]	0.89[a]	1.09	*	0.31	0.25
TVFA (mmol/l)	55.7[a]	45.3[ab]	35.2[b]	7.28	*	66.4	8.44
VFA molar proportions							
Acetate (mmol/mol.)	764[ab]	739[a]	785[b]	19.9	*	801	36.7
Propionate (mmol/mol)	172[a]	228[b]	164[a]	19.2	**	151	21.0
Butyrate (mmol/mol)	64[a]	33[b]	51[a]	6.6	*	48	17.2

[ab] Values in the same row not sharing common superscripts differ significantly ($P<0.05$).
TVFA = Total volatile fatty acids.

Figures 3.2.3.1 to 3.2.3.6 show the average hourly changes in pH, TVFA (mmol/l), acetate, propionate and butyrate molar proportions (mmol/mol) and lactate (mmol/l), respectively, measured between the 09:00 and 17:00 hour meals for all the experimental diets. Throughout the 8-hour sampling period in both experiments, pH was maintained above 6.5 and acetate molar proportions above 700 mmol/mol regardless of the botanical source of the fibre. The OH:NO diet in Experiment A resulted in pH values above 7 and TVFA levels below 20 mmol/l for the first 3 hours after the 09:00 meal whilst values for the remaining diets were comparable throughout the sampling period. In addition, propionate levels were higher throughout the 8 hour sampling period when OH:NO were fed compared with the other two diets.

Figure 3.2.3.1. Intra-caecal changes in pH following the 09:00 hour meal in ponies offered hay cubes (--■--), oat hulls:naked oats (---◆--), or sugar beet food (--◆--) in experiment A or an unmolassed sugar beet:hay cubes mix (--●--) in Experiment B. s.e.d's for each experiment were as follows:- Experiment A: 0.137; Experiment B: 0.052.

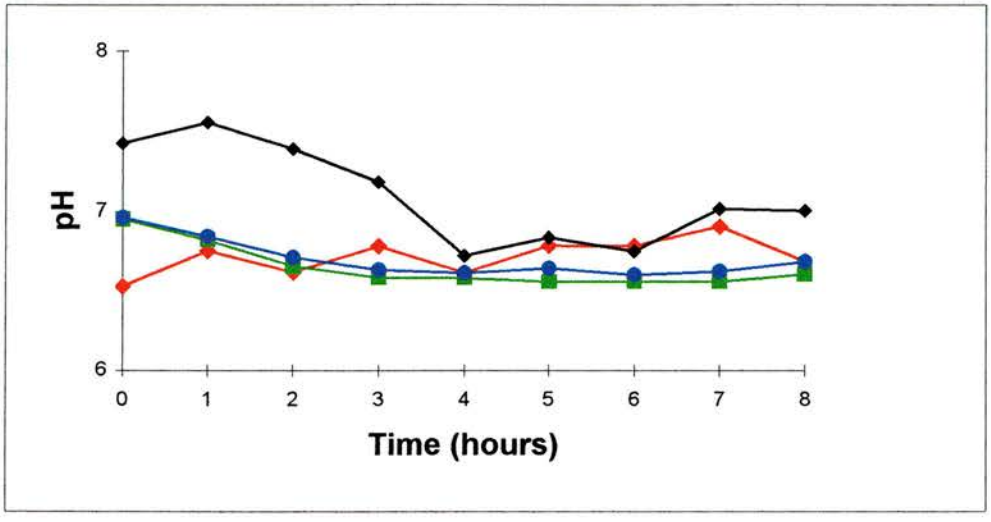


Figure 3.2.3.2. Intra-caecal changes in TVFA concentration (mmol/l) following the 09:00-hour meal in ponies offered hay cubes (--■--), oat hulls:naked oats (-◆), or sugar beet food (--◆--) in experiment A or an unmolassed sugar beet:hay cubes mix (--●--) in experiment B. s.e.d:- Experiment A: 10.14; Experiment B: 12.95.

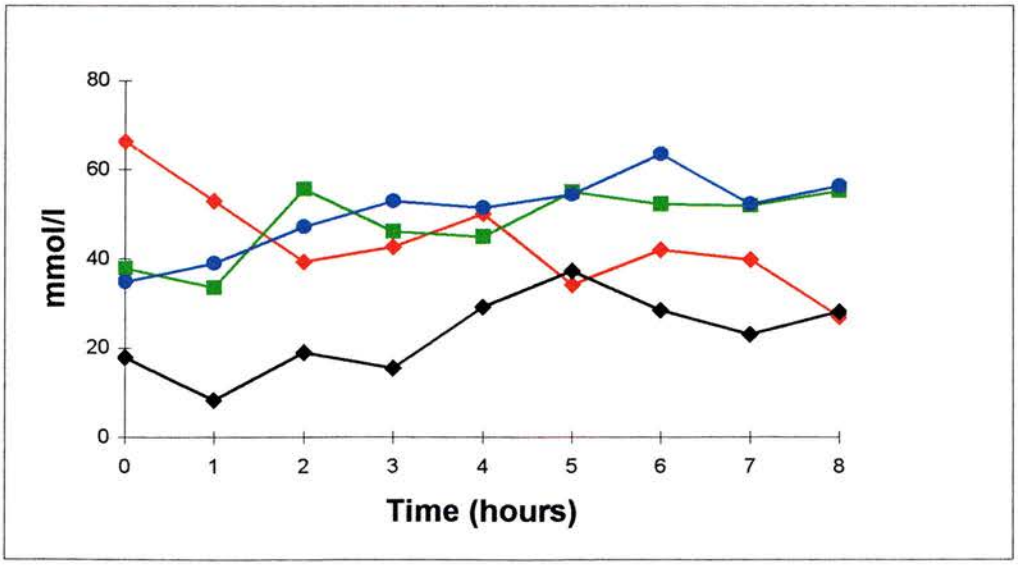


Figure 3.2.3.3. Intra-caecal changes in acetate molar proportion (mmol/mol) following the 09:00-hour meal in ponies offered hay cubes (--■--), oat hulls:naked oats (◆ --), or sugar beet food (--◆--) in experiment A or an unmolassed sugar beet:hay cubes mix (--●--) in experiment B. s.e.d:- Experiment A: 26.9; Experiment B: 41.4.

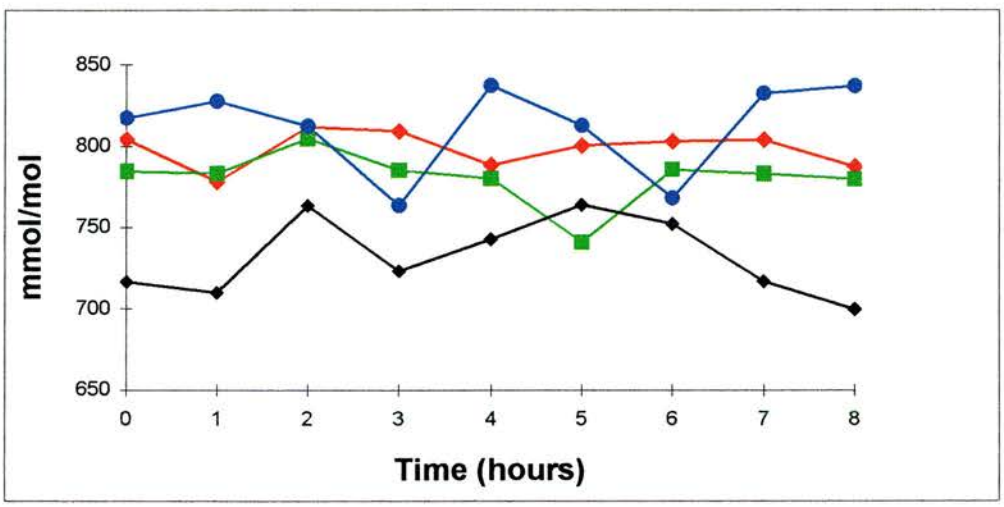


Figure 3.2.3.4. Intra-caecal changes in propionate molar proportion (mmol/mol) following the 09:00 hour meal in ponies offered hay cubes (--■--), oat hulls:naked oats (-◆--), or sugar beet food (--◆--) in experiment A or an unmolassed sugar beet:hay cubes mix (--●--) in experiment B. s.e.d:- Experiment A: 21.1; Experiment B: 22.8.

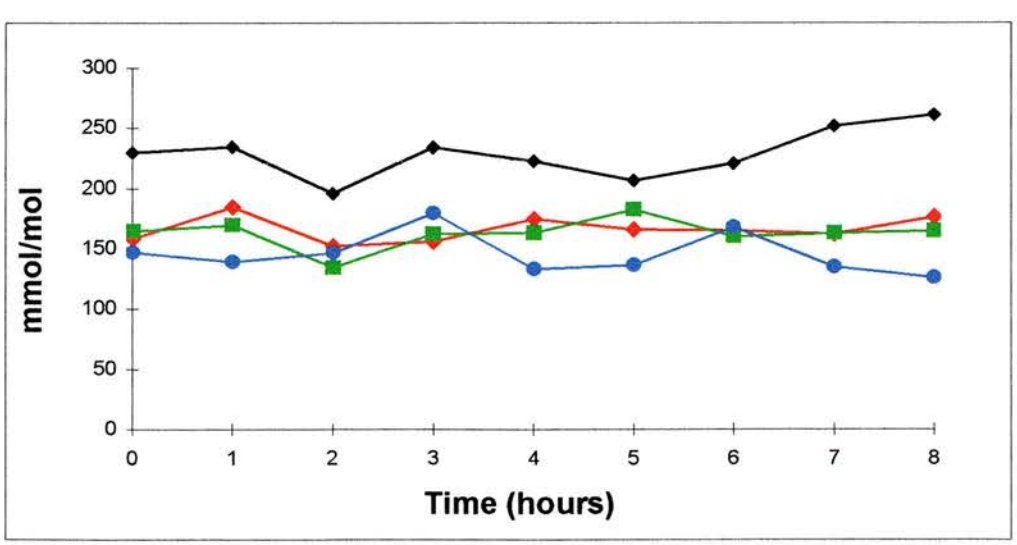


Figure 3.2.3.5 Intra-caecal changes in butyrate molar proportion (mmol/mol) following the 09:00 hour meal in ponies offered hay cubes (--■--), oat hulls:naked oats (-◆--), or sugar beet food (--◆--) in experiment A or an unmolassed sugar beet:hay cubes mix (--●--) in experiment B. s.e.d:- Experiment A: 10.7; Experiment B: 20.8.

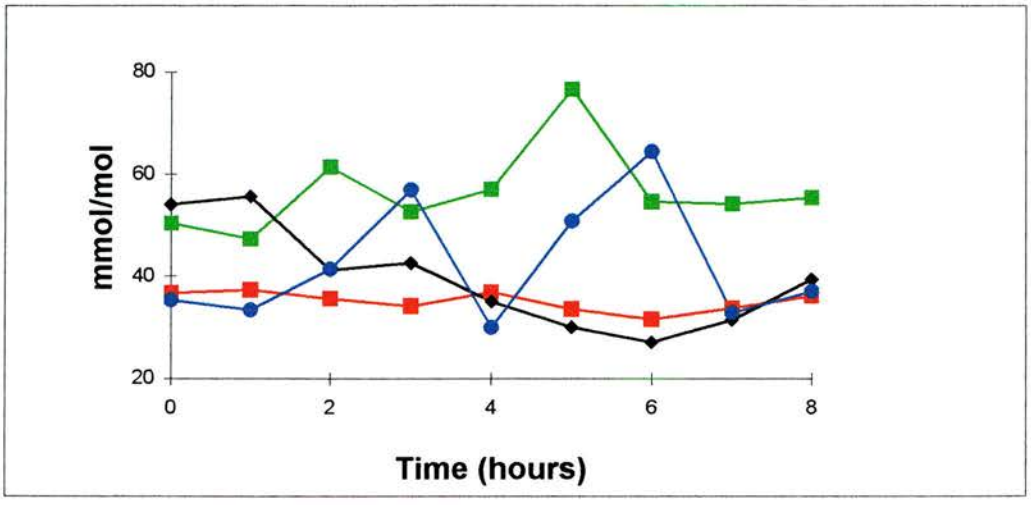


Figure 3.2.3.6. Intra-caecal changes in lactate molar proportion (mmol/mol) following the 09:00 hour meal in ponies offered hay cubes (--■--), oat hulls:naked oats (-◆ --), or sugar beet food (--◆--) in experiment A or an unmolassed sugar beet:hay cubes mix (--●--) in experiment B. s.e.d:- Experiment A: 10.7; Experiment B: 20.8.

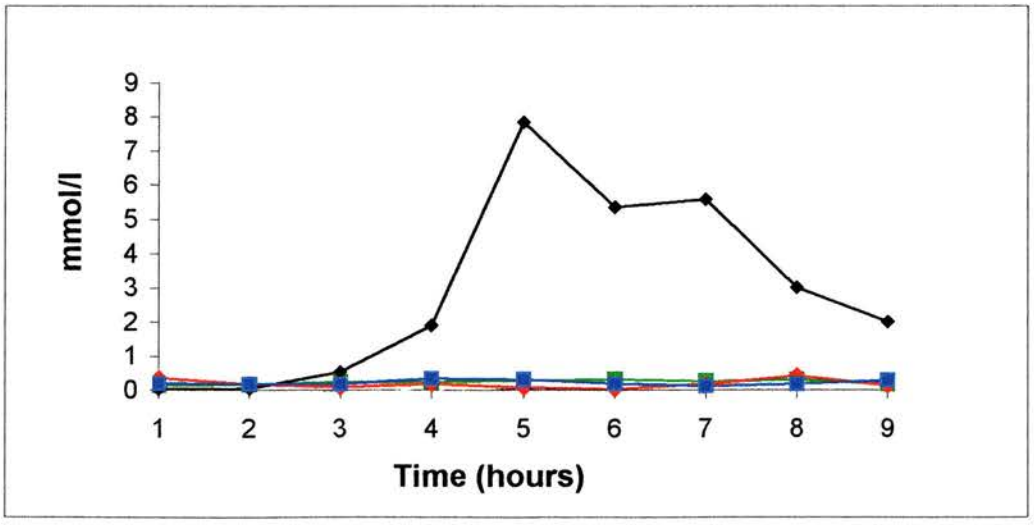


Table 3.2.3.3. details the intra-caecal fermentation parameters measured throughout the day, summarised as average values for 0-3 and 4-8 hours following the 09:00 meal for both Experiments A and B. Average pH was significantly higher ($P<0.05$) at 7.37 during the 0-3 hour period when OH:NO was fed, compared with the 6.86 obtained during the 4-8 hour period. Likewise, higher levels of lactate were present in samples obtained 4-8 hours after feeding compared with those collected between 0-3 hours. By contrast, there were no significant differences in TVFA concentrations or individual VFA molar proportions between the 0-3 and 4-8 hour time periods when the OH:NO diet was fed. Similarly, values for pH, TVFA and individual VFA molar proportions did not differ significantly between the 0-3 and 4-8 hour time periods when either HC or SBF were fed. In general, however, TVFA concentrations and acetate molar proportions were lower whilst propionate molar proportions were higher when OH:NO was fed compared to feeding either HC or SBF in Experiment A.

In Experiment B, TVFA concentration 4-8 hours after the 09:00 meal was 43% higher than that recorded during the previous 3 hours.

Table 3.2.3.3. Average intra–caecal fermentation parameters in ponies fed hay cubes (HC), a 67:33 mix of oat hulls:naked oats (OH:NO) and sugar beet food (SBF) in Experiment A and a 50:50 mix of unmolassed sugar beet:hay cubes (SB:HC) fed in Experiment B as measured 0-3 hours and 4-8 hours following a 09:00 hour meal.

Parameter	Experiment A					Experiment B	
	HC	**OH:NO**	**SBF**	**s.e.d**	**Sig**	**SB:HC**	**s.e**
PH							
0-3 h	6.75^{bc}	7.37^{a}	6.65^{bc}	0.105	*	6.79	0.055
4-8 h	6.57^{c}	6.86^{bc}	6.74^{bc}			6.64	0.050
Lactate (mmol/l)							
0-3 h	0.20^{a}	0.73^{a}	0.13^{a}	0.40	**	0.21	0.02
4-8 h	0.25^{a}	4.76^{b}	0.06^{a}			0.20	0.02
TVFA (mmol/l)							
0-3 h	43.3^{ab}	16.3^{c}	50.4^{a}	7.01	*	43.4	4.68
4-8 h	51.8^{a}	29.6^{bc}	39.4^{ab}			62.0	11.94
Acetate (mmol/mol)							
0-3 h	795^{a}	732^{b}	810^{a}	23.8	*	796	28.3
4-8 h	771^{ab}	736^{b}	798^{a}			825	22.7
Propionate (mmol/mol)							
0-3 h	150^{a}	222^{b}	154^{a}	21.1	*	159	18.7
4-8 h	168^{a}	231^{b}	167^{a}			132	12.9
Butyrate (mmol/mol)							
0-3 h	55^{ab}	47^{abc}	36^{bc}	8.7	*	45	9.8
4-8 h	61^{a}	33^{c}	35^{bc}			42	9.8

^{abc} Values in the same section not sharing common superscripts differ significantly ($P<0.05$).

3.2.3.3. In vivo apparent digestibility and nutritive value

Table 3.2.3.4. details the *in vivo* apparent digestibility coefficients and nutritive values for the three diets in experiment A and the SB:HC diet fed in Experiment B. DMD, OMD, NDFD, GED, TNSPD, rhamnose digestibility (RD), arabinose digestibility (AbD), galactose digestibility (GaD) and uronic acid digestibility (UACD) were all significantly higher ($P<0.05$) for SBF compared with HC and OH:NO. The high TNSPD value for SBF of 0.87 reflects the high apparent digestibility values for AbD, glucose digestibility (GD) and UACD, which together comprise approximately 870 g/kg of the TNSP present in SBF. The DE content (MJ/kg DM) for SBF of 11.9 was significantly higher ($P<0.05$) than the values of 8.3 and 9.8 noted for HC and OH:NO respectively. Conversely, CPD coefficients and the DCP contents (g/kg DM) of 0.50, 0.68, 43 and 56 g/kg DM for SBF and OH:NO respectively were significantly higher ($P<0.05$) compared with the HC value of 0.22 and 18 g/kg DM. No significant differences ($P>0.05$) were seen between the three foods for ADFD, xylose digestibility (XD), mannose digestibility (MD) or GD. Negative values were recorded for UACD for both HC and OH:NO, however the UAC content in both these foods was very low at only 10 to 20 g/kg DM of the TNSP present in these foods possibly impairing accurate detection by spectrophotometric analysis.

Table 3.2.3.4. *In vivo* apparent digestibility coefficients and nutritive values of hay cubes (HC), oat hulls:naked oats (OH:NO) and sugar beet food (SBF) fed to ponies in Experiment A and the unmolassed sugar beet:hay cubes (SB:HC) diet fed to ponies in Experiment B.

	Experiment A					Experiment B	
	HC	**OH:NO**	**SBF**	**Sed**	**Sig**	**SB:HC**	**s.e**
DM	0.47^{a}	0.49^{a}	0.72^{b}	0.048	*	0.58	0.031
OM	0.48^{a}	0.51^{a}	0.78^{b}	0.050	*	0.63	0.032
CP	0.22^{a}	0.68^{b}	0.50^{b}	0.058	*	0.28	0.060
ADF	0.33	0.35	0.55	0.119	NS	0.47	0.046
NDF	0.43^{a}	0.39^{a}	0.74^{b}	0.081	*	0.65	0.041
Starch	0.75^{a}	0.88^{b}	0.90^{b}	0.038	*	-	-
GE	0.45^{a}	0.51^{a}	0.73^{b}	0.040	*	0.59	0.035
TNSP	0.48^{a}	0.48^{a}	0.87^{b}	0.079	*	0.72	0.028
Rhamnose	0^{a}	0^{a}	988^{b}	6.87	***	1000	0
Arabinose	0.63^{a}	0.53^{a}	0.96^{b}	0.081	*	0.94	0.008
Xylose	0.64	0.58	0.29	0.131	NS	0.56	0.037
Mannose	0.78	0.281	0.56	0.327	NS	0.51	0.129
Galactose	0.52^{a}	0.37^{a}	0.90^{b}	0.109	*	0.87	0.020
Glucose	0.41	0.45	0.81	0.124	NS	0.59	0.036
Uronic acid	-0.11^{a}	-0.93^{b}	0.64^{c}	0.205	*	0.82	0.062
Nutritive values							
DE (MJ/kgDM)	8.3^{a}	9.8^{a}	11.9^{b}	0.66	*	10.5	0.62
DCP (g/kg DM)	18^{a}	56^{b}	43^{b}	4.72	*	23	4.95

^{abc} Values in the same row not sharing common superscripts differ significantly ($P<0.05$). See text for abbreviations.

The *in vivo* apparent digestibility coefficients and nutritive values of SB calculated by difference from the total diet values obtained in Experiment B and the HC values obtained in Experiment A are given in Table 3.2.3.5. Calculated values for all parameters except CP are higher than those obtained for SBF in Experiment 1A. DCP content at 29 g/kg DM was markedly lower than the SBF values recorded in Experiment 1A.

Table 3.2.3.5. *In vivo* apparent digestibility coefficients and nutritive value of unmolassed sugar beet calculated by difference from the unmolassed sugar beet:hay cubes (SB:HC) diet fed in Experiment B.

Parameter	**SB:HC**	**s.e**
DM	0.70	0.063
OM	0.78	0.065
CP	0.35	0.122
ADF	0.64	0.106
NDF	0.88	0.085
GE	0.75	0.075
NSP constituents		
TNSP	0.96	0.049
Rhamnose	1	0
Arabinose	1.25	0.016
Xylose	0.49	0.074
Mannose	0.23	0.260
Galactose	1.23	0.040
Glucose	0.78	0.074
Uronic acids	1.75	0.124
Nutritive values		
DE (MJ/kg DM)	12.4	1.24
DCP (g/kg DM)	29	9.97

3.2.4. Discussion

3.2.4.1 Dry matter intake

Despite an attempt to increase the palatability of unmolassed sugar beet pulp by offering a mixture of 75:25 unmolassed to molassed food, the consumption of SBF resulted in very low intakes by all of the ponies. One pony in particular ate so little SBF that virtually no data was collected, thus a missing value was returned for SBF for one of the three experimental periods. Overall, the average daily intake of SBF was only 1.72 kg DM per day compared with an average DMI of 3.94 kg DM per day on the other three diets across both experiments. A similar finding was reported by Smolders *et al.* (1990) who recorded high levels of food refusals when horses were fed a 70:30 sugar beet:hay diet. Additionally, Hyslop *et al.* (1998b) indicated that sugar beet products might suppress voluntary food intake (VFI) in ponies when included at 550 - 700 g/kg DM of the diet. It has also been observed that VFI was lower when sugar beet products were included in the diet of dry sows compared with that of other fibrous foodstuffs (Brouns *et al.,* 1995). These authors suggested that the lower intakes were due to pre-caecal gut distension, caused by the hydroscopic nature of the sugar beet pulp. As the digestive anatomy of equids is similar to that of pigs it is possible that the restricted VFI of the ponies when consuming SBF in this experiment was also due to gut distension. However, when SB was offered in a 50:50 mixture with HC in experiment B, VFI increased significantly to the extent that ponies actually consumed more SB when eating it in a 50:50 mix with HC, than when SBF was offered as the sole diet. Gut distension alone would thus seem an unlikely explanation for the lower VFI of SBF. The results presented on the intra-caecal fermentation parameters for SBF in Experiment A are thus obtained from Animals with a very low VFI . This may possibly confound interpretation of the results and the comparison with the HC and OH:NO diets which had significantly ($P<0.05$) higher DMI. However, despite this reservation the results presented show VFA and lactate levels within the normal range for a fibre food and thus have been used to relate measured parameters to the botanical nature of the diets under study.

3.2.4.2. Intra-caecal fermentation parameters

Each of the fibre-based diets examined here, maintained pony caecal pH above 6.5 at all time points measured, which is similar to the findings of Goodson *et al.* (1988) for ponies fed forage-based diets. However, the average intra-caecal pH of ponies fed the OH:NO diet, which contained more than 200g starch/kg diet,(equivalent to 1.75 g of starch/kg LW per meal) declined significantly from 7.37 to 6.86 measured 0-3 and 4-8 hours respectively, after the morning food. Declines in intra-caecal pH, 4 hours post feeding, have been reported when starch-based cereals have replaced some of the basal forage in equine rations (Willard *et al.,* 1977; Goodson *et al.,* 1988), and although the drop down to 6.86 is not at the critical level of 6.0, noted by Radicke *et al.* (1991) to produce sub-clinical acidosis in equids, it does demonstrate how levels below the upper limit fed per meal of 2g starch/kg LW (Kienzle *et al.,* 1992), or 4g starch/kg LW (Potter *et al.,* 1992b), can significantly affect caecal pH. The intra-caecal pH remained relatively stable in ponies offered the HC and SBF diets which contained a maximum of 20-30 g of starch/kg DM. These findings are in agreement with the values reported by Hsu *et al.* (1987), Argenzio *et al.* (1974) and Goodson *et al.* (1988) and indicate that fibre-based diets maintain a slightly acidic to neutral caecal pH.

The mean intra-caecal lactate levels measured more than 4 hours after the morning food were significantly higher in ponies fed the OH:NO diet than in those fed either the SBF or HC diets. Rapid fermentation of cercal starch in the hindgut of horses causes an increase in intra-caecal lactate production (Pagan, 1998). The 4.76 mmol/l reported here when ponies were consuming the OH:NO diet may be attributed to the rapid fermentation of any naked oat starch which had escaped enzymatic digestion in the small intestine. Pagan (1997 & 2000) suggested that fibre in equid diets stimulates peristalsis causing a high rate of passage of digesta through the small intestine with a corresponding reduction in enzymatic digestion of starch and protein. This could have occurred here with the oat hulls initiating a high rate of passage, causing some of the naked oat starch to pass into the caecum. Alternatively the amount of starch fed per

meal was only 0.3 g/kg LW less than the maximum recommended by Kienzle *et al.* (1992), of 2 g/kg LW per meal, thus the capacity of the small intestine to digest all the starch presented, may have been exceeded resulting in undigested starch passing into the caecum. Kienzle *et al.* (1994) measured the activity of amylase in the gastrointestinal tract of the horse and found pancreatic amylase and jejunal chyme activities to be highly variable between ponies, ranging from 85 to 909 U/g and 1 to 70 U/g wet tissue respectively. Such differences in the ability to digest starch may explain why some ponies are susceptible to laminitis, whilst their stable companions consuming a similar diet remain healthy.

Although the overall TVFA production in caecal samples from ponies fed the HC diet was significantly higher than that produced on the SBF diet, as a proportion of intake, levels of intra-caecal TVFA were higher at 20.46 mmol/l/kg for SBF compared with 14.85 mmol/l/kg HC DMI. This reflects a higher degradability of SBF compared to HC and is supported by the intra-caecal lactate levels, which were similar for these two diets. Furthermore, despite low intakes, all of the ponies maintained their body weight when fed SBF suggesting that their energy requirements were largely being met. These results are in accordance with the findings of Hyslop *et al.* (1998a) and Hyslop *et al.* (1998b) who reported a higher DMD coefficient of sugar beet food at 0.72 compared with that of 0.3 for mature grass hay. Low levels of TVFA were found in the caecal digesta of ponies fed the OH:NO diet during the 09:00 to 12:00 hour collection period. This could reflect the comparatively low degradability of the oat hull fraction of the diet, which would have comprised the digesta remaining in the caecum from the previous evening meal.

At all time points measured, the intra-caecal VFA molar proportions for ponies fed the HC and SBF diets were similar, being maintained at *ca.* 80:15:5 for acetate:propionate:butyrate respectively, and these VFA ratios are similar to those found in the caecal digesta of ponies fed fibrous diets by Hintz *et al.* (1971); Argenzio *et al.* (1974) and Willard *et al.* (1977). By contrast, the corresponding ratios for OH:NO were

73:23:4, and are similar to the 73:21:6 recorded by Glinsky *et al.* (1976) from ponies fed a 3:1 forage:grain diet. The higher levels of propionate recorded in the OH:NO fed ponies are consistent with starch fermentation in the caecum (McLean *et al.,* 1998), and are compatible with the corresponding lactate levels.

3.2.4.3. Nutritive value and in vivo apparent digestibility of energy and protein

The higher ($P<0.05$) GED and DE content of the SBF compared with the HC and OH:NO diets can be attributed to the higher digestibility of the SBF cell walls. The DE values of 11.9 and 12.4 MJ/kg DM determined for the SBF and SB foods respectively are higher than the value of 10.7 MJ/kg DM published by NRC, (1989) but lower than the value of 14.2 MJ/kg DM given for pressed sugar beet pulp silages by Coenen, (1986). *In vivo* apparent digestibilities and DE contents for HC are similar to values for grass hays given in the literature and reflect the chemical composition and stage of growth of this forage at harvest (Fonnesbeck, 1968; Harbers *et al.,* 1981; Cymbaluk, 1990; Pearson and Merritt, 1991; Hyslop *et al.,* 1998a). Apparent digestibilities and the DE value for the OH:NO diet are likely to reflect the high digestibility of the NO cereal grain combined with the poorer digestibility of the OH fraction. Cuddeford, Khan and Muirhead (1992b) found *in vivo* DE contents of 15.5 to 16 MJ/kg DM for winter and spring harvested naked oats while the NRC (1989) propose a DE value of 6.47 MJ/kg DM for oat hulls. Applying these values to the DE of 9.8 MJ/kg DM for the 67:33 mix of OH:NO fed in this experiment suggest that the DE of the NO and OH are similar to those found by Cuddeford *et al.* (1992b) and the NRC (1989).

Both the SBF and OH:NO diets contained significantly higher ($P<0.05$) DCP contents than the HC diet reflecting their higher ($P<0.05$) CPD. It can be postulated that the NO fraction of the OH:NO diet was the main supply of DCP from this diet since the CP content of OH is reported to be low at 41 g/kg DM (NRC, 1989). Low *in vivo* apparent digestibility of CP in HC probably reflects a high output of microbial nitrogen in equine faeces (Uden and Van Soest, 1982a).

3.2.4.4. Fibre analysis methods and in vivo apparent digestibilities

The amount of total cell wall material present in each of the three foods is indicated by the ADF, NDF and TNSP contents given in Table 3.2.1. Although the TNSP value for SBF was similar to the other three diets at 452 g/kg DM, the amount is considerably lower than the 635 g/kg DM noted for this food by Longland and Low (1995). These differences can be partly attributed to the 75:25 unmolassed to molassed mixture of the SBF fed in this experiment. The NDF values for the SBF and the SB offered in this study were considerably higher than the reported values of 410 and 440 g/kg DM respectively (Zhu, 1998; Longland and Low, 1989; MAFF, 1990). The variance between these values could be a factor of the inherent variability of the NDF analysis itself. Longland and Low (1995b) have shown that substantial amounts of the NSP fraction, namely uronic acid and arabinose fractions from both molassed and unmolassed sugar beet pulp were soluble in the neutral detergent solution and so were lost during the analysis. Accurate measurement of both these monomers is particularly important when analysing dicotyledonous foods, since the pectic fraction can comprise a substantial proportion of the cell wall in these plants (Longland and Low, 1995b). Thus the variability of the NDF analysis could readily account for the reported differences in the NDF contents of similar foods.

The inconsistency of the NDF analysis could also partly account for the notably lower NDF digestibility (NDFD) of SBF compared with the value recorded for TNSP digestibility (TNSPD), although it must be remembered that the NDF value does include lignin, whereas the NSP value does not. By measuring the discrete chemical entities through quantification of the individual cell wall monomers, TNSP content represents all the potentially degradable fraction of the fibre (Englyst and Cummings, 1984), and thus, enables a closer examination of degradation of the different parts of plant cell walls.

High levels of glucose, arabinose and UAC and low levels of xylose indicate the presence of primary cell walls (Birch and Parker, 1983). SBF contains near equal

proportions of glucose, arabinose and UAC and results presented here confirm that these monomers were all highly digested in ponies. In contrast, HC and OH:NO contain high levels of xylose and glucose which together indicate the presence of secondary cell walls which may be lignified or contain other phenolics which inhibit cell wall degradation (Åman and Graham, 1990). Graham *et al.* (1986) and Longland *et al.* (1993) reported similar digestibilities of sugar beet food and cereals in growing pigs, indicating that the highly digestible nature of sugar beet fibre is enjoyed by other monogastrics, with a hindgut fermentation capacity.

An interesting feature noted when ponies were consuming the 50:50 SB:HC ration in experiment B, was the associated effects, whereby an apparent increase in the digestibility of arabinose, galactose and uronic acids (leading to coefficients of greater than 1) of the HC occurred. Such an effect of sugar beet on the NSP of graminaceous species has been noted in piglets by Longland, Carruthers and Low (1994) and in sheep and cattle by Silva *et al.*(1989). Graham *et al.*(1986) suggests that this effect is precipitated by pectin rich, high cation-exchange capacity foods which appear to promote increased rates of microbial activity, and thus stimulate the degradation of fibre within the fermentation chamber. Chesson (1990) agrees that feeding highly digestible fibre foods with poor fibre sources such as cereal straws, increases the degradability of the straw, but he attributes this increased degradation to re-dressing the balance of primary to secondary cell walls to the normal 2:1 ratio found in grass. By doing this, the activity of the microbial population can be enhanced by supplying them with a readily degradable substrate which increases microbial activity causing a subsequent increase in degradation. It is thus possible that in this experiment the SB promoted microbial activity in the hindgut by either supplying readily degradable primary cell walls, or by improving cation exchange capacity, causing a higher degradation of the HC fibre.

3.2.5. Conclusions

Alternative fibre based diets offered to ponies in this study produced intra-caecal fermentation parameters broadly similar to those observed when a grass based forage was offered indicating that ponies can be fed a wide range of botanically diverse fibrous foods. However, results from Experiment A indicate that sugar beet products should not be used as the sole source of fibre in pony diets since VFI may be markedly reduced, which could lead to negative energy balance, compromised gut function and behavioural problems.

In vivo apparent digestibilities for sugar beet pulp were significantly higher ($P<0.05$) than for hay cubes or a 67:33 oat hulls:naked oats mix, primarily due to the higher content of readily degradable cell wall components. The significant drop in intra-caecal pH and accompanying increase in lactate levels seen on the OH:NO food suggest that 1.75 g of starch/kg LW offered per meal is close to the maximum amount of starch which can be safely digested in the small intestine, and agrees with the maximum level of 2 g/kg LW per meal set by Kienzle *et al.* (1992).

The non-starch polysaccharide analytical method provides a more complete description of cell wall content and offers the potential to evaluate the nutritive value of food more precisely. However, NDF determination is of more limited value when measuring the fibre content or the apparent digestibility of dicotyledonous foods.

3.3. Digesta passage rate and mean retention times of four botanically diverse fibre-based diets in ponies.

> 'models of digestion can be used to simulate aspects of the system that we think are important, to aid our understanding of biological processes.....to develop strategies for optimising or controlling the real system.'
>
> (Mertens, 1993)

3.3.1. Introduction

The difference between the extent of degradation of fibre-based foods in equids and ruminants is frequently attributed to the difference in time that digesta remains within the respective fermentation chambers (Janis, 1976). Ruminants have a digesta mean retention time (MRT) of approximately 70-90 hours (Balch and Campling, 1965), whilst that of equids is closer to 48 hours (Haenlein *et al.,* 1966b; Vander Noot *et al.,* 1967). Equids have no particle size restriction mechanism like the reticulo-omasal orifice found in ruminants (Frandson, 1981) thus, relatively large food particles can pass through the tract, thereby maintaining digesta passage rate when consuming low-quality high-fibre diets (Janis, 1976). Accordingly, information on the passage of different foods through the gastrointestinal tract of equids will allow a clearer understanding of the dynamic interactions between food and its degradation by the micro-flora and thus, in time enable improved diet formulation for horses.

Mathematical modelling of faecal excretion data, which describes the passage of marked digesta through the gastrointestinal tract, is a non-invasive method by which digesta passage rate and MRT can be obtained. Such models simulate aspects of the digestive system and so allow a greater understanding of digesta kinetics within the animal. Mathematical modelling has been well documented in ruminants (Uden *et al.* 1982b; Lalles *et al.* 1991; Mertens, 1989; Ellis *et al.,* 1994). However, the process is far from established, even in cattle and sheep, so when selecting a model to describe digestion

kinetics, Moore *et al.* (1992) suggest trying all non-linear models and evaluating the fit in order to determine which model best describes the passage of the digesta through the gut. Models are generally categorised under two headings, ie: time-independent and time-dependent. Those based on time-independent principles describe the flow of digesta through one or more, sequential compartments according to first order kinetics (Lalles *et al.,* 1991), which assumes a constant proportional outflow of particles per unit time. Time-dependent models, which use non-exponential gamma functions to describe digesta passage, (ie. the proportional particle outflow per unit time increases), have been found to be particularly flexible for modelling ruminant digesta passage (Pond *et al.* 1988). Several research groups, Milne *et al.* (1978), Ellis *et al.* (1979) and Uden *et al.* (1982b) have encountered difficulty fitting the time-independent model of Grovum and Williams (1973) to ruminant faecal excretion data, whereas greater success was achieved with the use of multi-compartmental and time-dependent models (Dhanoa, *et al.,* 1985).

Marker studies in equids carried out by Pearson and Merritt (1991), Uden *et al.* (1982b) and Nyberg (1993), have used variations of the Faichney (1975) and Blaxter *et al.* (1956) algebraic equations to calculate digesta MRT, but documented attempts to estimate the rate of passage through the different segments of the equid gastrointestinal tract, using two-compartment time-independent models have been of limited success (Corino *et al.,* 1992). Digesta passing through the fermentation section of the equid gut, which consists of the caecum, and the four distinct regions of the large colon (Jackson, 1998) has to pass through narrow flexures as it flows through right and left ventral to left and right dorsal chambers. Although mixing within each of these chambers may occur quickly, it seems unlikely that the passage of digesta through the entire large intestine will follow exponential or first order kinetics. Exponential time-independent digesta passage models assume constant volume and instantaneous mixing of digesta within the gut compartment, along with equal opportunity for particle escape irrespective of residence time (Ellis *et al.,* 1994). Due to the physiological arrangement of the digestive tract, digesta passage through the equid gastrointestinal tract may be a time-dependent

process, whereby the probability of particle escape is increased with retention time. Consequently, time-dependent models may prove more successful at modelling equid digesta passage rate than time-independent models. Different research groups working with ruminants have proposed a variety of anatomical reasons for supporting the use of both time-dependent and time-independent models for describing digesta passage. Previous attempts at compartmental modelling of horse digesta passage data has been strictly limited and it thus seems appropriate to test the accuracy of both time-dependent and time-independent models on the pony faecal-marker excretion data obtained here.

The objectives of the following experiment were 1) to assess the suitability of chromium and ytterbium as indigestible food markers for measuring digesta passage rate in ponies; 2) to study daily changes in caecal digest outflow 3) to examine the suitability of four time-dependent (Pond *et al.,* 1988) and two time-independent (Grovum and Williams, 1973 and Dhanoa *et al.,* 1985) models for describing the passage of digesta through the hindgut and total tract of ponies, and 4) to determine hind-gut and total tract mean retention time for three fibre-based diets.

3.3.2. Materials and Methods

3.3.2.1. Experiment A.

Data for this experiment were collected during the 3x3 *in vivo* apparent digestibility trial described previously in experiment 3.2; thus management of the ponies was as described in section 3.2.2.

3.3.2.1.1. Marker preparation and administration

3.3.2.1.1.1. Chromium

At 07:00 hours on the first day of each of the 5-day collection periods, (for details see Experiment 3.2) *ca.* 50g of Cr mordanted hay cubes (HC) or an oat hull:naked oats mix

(OH:NO), prepared by the method of Uden *et al.* (1980) (see appendix 5) was put into the caecum of each pony. Before the marker was introduced into the caecum the fistula cap was replaced with a two-way tap and 12-inch plastic tube combination, which was screwed onto the top of the fistula. This arrangement allowed anaerobic conditions within the caecum to be maintained during marker introduction and digesta removal. The 12-inch plastic tube had holes on either side, and was positioned to penetrate into the caecal digesta. This allowed the body of the caecal digesta to be sampled. The marked food was made into a 'slurry' using *ca.* 200 ml of water and squirted into the caecum using a syringe, the point of which was pushed into the two-way tap, so the marker was introduced into the caecum *via* the 12-inch plastic tube. To ensure no marker remained in the tube two 60ml aliquots of water were squirted down the tube to ensure that the entire marker-dose went into the caecum.

3.3.2.1.1.2. Ytterbium

Yb marked fed was prepared using Yb (111) chloride hexahydrate, 99% (Aldrich Chemical Company), by the immersion method of Teeter *et al.* (1984) (see appendix 4). Fifteen minutes prior to the 09:00-hour food the Yb labelled food was mixed with two table-spoons of molasses and offered as a voluntary oral-dose to the ponies.

3.3.2.2. Sample collection.

3.3.2.2.1. Caecal samples

In order to estimate caecal outflow rates, caecal digesta samples were obtained and the concentration of Cr marker subsequently determined from these samples. Approximately 40 minutes after administration of the Cr marker, a sample of digesta was withdrawn from the caecum using a syringe, which was pushed into the two-way cannula tap. To ensure representative sampling from the body of the caecal digesta, three 60ml aliquots of digesta were removed using 3 syringes; the third was retained as

the sample, while the first two aliquots were returned to the caecum. This procedure was repeated every 30 - 50 minutes throughout day until 17:00 hours, thus *ca.* 12 caecal samples were taken.

Each sample of digesta was placed into pre-weighed foil trays, weighed and dried in a force-draft oven at 60°C for 48 hours. The trays were then re-weighed and the samples stored in plastic screw-top tubes at room temperature, until required for chemical analysis.

3.3.2.2.2. Faecal samples

Six hours after administration of the pulse-dose of Yb, faecal collections commenced, and followed the timetable given below in Table 3.3.1.

Table 3.3.2.1. Timetable of faecal collections during the 5-day collection periods.

Time post-dose (hours)	**Frequency of faecal collections**
6 – 30	All defecations
30 – 54	2 hourly collections between 08:00 and 22:00hours
54 – 74	4 hourly collections between 08:00 and 22:00 hours
74 – 106	8 hourly collections between 08:00 and 16:00 hours.

Faecal sample weight and expulsion times were recorded for every sample. A sub-sample from each collection was then placed into a pre-weighed foil tray, weighed and

dried in a force-draught oven at 60°C for 48 hours and stored at room temperature, prior to chemical analysis.

3.3.2.3. Chemical Analysis

3.3.2.3.1. Chromium

Chromium concentration in the dried caecal and faecal samples was determined using atomic absorption spectrophotometry (Mathers, Baber and Archibald, 1989). Faeces were ground through a 1mm screen before being digested by the modified method of Milner (1965). Five grams of sample were placed into large boiling tubes and wet-ashed with 20 ml concentrated sulphuric acid. Fifteen ml of Milner's digest mixture (appendix 5) was added and the tubes were boiled for 30 minutes. After cooling the solution was made-up to 100ml with distilled water and assayed by atomic absorption spectrophotometry.

3.3.2.3.2. Ytterbium

Ytterbium concentration in faecal samples was determined as follows: 0.25g of ground sample (1mm mesh screen) were placed into digestion vessels and 10 ml of nitric acid was added before sealing with a modified pressure release cap. A control vessel, containing the most reactive sample was fitted with a pressure release control cap and closed to position1. Approximately 10 vessels were placed on to a MDS-2000 microwave turntable and the vent tubes connected to the collection vessel and heated for *ca.* 6 minutes 630 watts. After heating the pressure is allowed to fall to 30 p.s.i.. before the turntable is removed to the fume cupboard. The solution was then washed from the vessel into a 50ml volumetric flask and made up to volume with distilled water. This solution was then transferred into a marked universal bottle for atomic emission analysis.

3.3.2.4. Data analysis

3.3.2.4.1. Caecal samples

All caecal Cr data was subjected to curve fitting using Genstat 5 (Lawes Agricultural Trust, 1993), using the following exponential equation:

$$Y = A\, e^{-kt} \qquad \text{(equation 3.3.2.4.1.1)}$$

Where:

A = intercept

e = exponential

k = digesta passage rate per hour

t = time in hours

Caecal mean retention time (MRT) was then calculated from 1/passage rate, and caecal dry matter (DM) volume calculated from:

$$\frac{\text{marker dose (mg)}}{\text{the predicted intercept on the y-axis (A).}}$$

Goodness of fit (R^2) for each exponential equation was also calculated. DM content, passage rate, MRT, and R^2 values were then subjected to a two-way analysis of variance using Genstat 5 (Lawes Agricultural Trust, 1993), and least significant difference test (LSD = t value for the error degrees of freedom x s.e.d.) to determine if there were significant differences between the three diets in Experiment A.

3.3.2.4.2. Faecal samples

3.3.2.4.2.1. Compartmental models

Faecal Cr and Yb excretion data were fitted to the two time-independent models of Grovum and Williams (1973) and Dhanoa *et al.* (1985) using programmes written for

Genstat 5 (Lawes Agricultural Trust, 1993) (Dhanoa pers. Comm.), which solved the following equations on which the models are based:

$$Y = A\, e^{-k1(t-TT)} - A\, e^{-k2(t-TT)}$$ (Grovum and Williams, 1973) (equation 3.3.2.4.2.1)

where:

Y = adjusted marker concentration in faecal DM

A = adjusted marker concentration in faecal DM and is the anti natural log of the calculated mean of (TT x k1) + C and (TT x k2) + C1.

k1 and k2 are rate constants

t = sampling time in hours (taken as the mid point between successive samples)

TT = transit time in non-mixing compartment (time taken until first appearance of marker in the faeces)

$$Y = A\, e^{-c1\, t} \exp\,[-Be^{-c2\, t}]$$ (Dhanoa *et al.* 1985) (equation 3.3.2.4.2.2)

Y= adjusted marker concentration in faecal DM.

A = anti-natural log of faecal marker concentration

c1 = rate parameter equivalent to k1.

B = number of compartments.

c2 = rate parameter (k2 - k1).

t = time post dose, taken as the mid-point between successive samples.

The four time-dependent models of Pond *et al.*(1988), G1, G2, G3 and G4 were fitted to the Cr and Yb faecal excretion data using the following equations, which were solved using a S.A.S. (SAS Inst. 1985) computer programme with the procedure recommended by Moore *et al.*(1992).

$$F = C_2\, k_1\, (e^{-k2TD} - e^{-k1TD}) / (k_1 - k_2)$$ G1 model (Pond *et al.*, 1988)

(equation 3.3.2.4.2.3)

$$F = C_2 [\delta^2 e^{-k_2 TD} - e^{-\lambda_1 TD} (\delta^2 + \delta\lambda_1 TD)]$$ G2 model (Pond *et al.*1988)

(equation 3.3.2.4.2.4)

$$F = C_2 [\delta^3 e^{-k_2 TD} - e^{-\lambda_1 TD} (\delta^3 + \delta^2 \lambda_1 TD + \delta\lambda^2_1 TD^2/2)]$$ G3 model (Pond *et al.* 1988)

(equation 3.3.2.4.2.5)

$$F = C_2 [\delta^4 e^{-k_2 TD} - e^{-\lambda_1 TD} (\delta^4 + \delta^3 + \delta^2 \lambda_1 TD + \delta\lambda^3_1 TD^3/3)$$ G 4 model

(Pond *et al.*, 1988) (equation 3.3.2.4.2.6)

F = Fractional concentration of marker

C_2 = Initial concentration in second compartment (C= D/V, D= dose V= volume at second compartment)

k_1 and k_2 = exponentially distributed rate parameters.

t = time post dose

λ = rate parameter for gamma-distributed residence times.

$\delta = \lambda 1 / (\lambda 1 - k2)$

TD = $(t - \lambda)$ time delay (time post –dose to first appearance of marker in the faeces)

All models were tested for accuracy of fit using linear regression, to determine R^2 values, for each pony when consuming both HC and OH:NO. At this stage in the data analysis procedure the Grovum and Williams (1973) model was rejected on the grounds of poor agreement between fitted and actual data. The R^2 values for the other 5 models were then subjected to a two-way analysis of variance using Genstat 5 (Lawes Agricultural Trust, 1993) and LSD test, to determine if significant differences existed

between the 'goodness of fit' of the models when fitting different foods; and if a difference existed between models when fitting the same food.

The data obtained from the 5 compartmental models were then used to calculate large intestine (LMRT), determined using the Cr data, and in the total tract (TMRT) determined using the Yb data. The MRT of Cr and Yb were obtained from the Dhanoa, *et al.* (1985) (DMC) model as follows:

$$1/k_1 + 1/k_2 + TD = TMRT \qquad \text{(equation 3.3.2.4.2.7)}$$

Whereas the MRT for the time-dependent G1, G2, G3 and G4 models of Pond *et al.* (1988), were obtained from:

$1/\lambda + 1/k2 + TD = TMRT$ (G1) (equation 3.3.2.4.2.8)

$2/\lambda + 1/k2 + TD = TMRT$ (G2) (equation 3.3.2.4.2.9)

$3/\lambda + 1/k2 + TD = TMRT$ (G3) (equation 3.3.2.4.2.10)

$4/\lambda + 1/k2 + TD = TMRT$ (G4) (equation 3.3.2.4.2.11)

3.3.2.4.2.2. Algebraic equations.

Cr and Yb faecal excretion data were used to determine MRT from the following equations:

$$MRT = \Sigma\, ti\, Mi \qquad \text{(Faichney, 1975)} \qquad \text{(equation 3.3.2.4.2.2.1)}$$

Where:

ti = time post dose, taken as the mid-point between successive faecal samples

Mi = amount of marker excreted as a proportion of total marker excreted.

$$MRT = \frac{\Sigma\, ti\, Ci\, \Delta ti}{\Sigma\, Ci\, \Delta ti} \qquad \text{(Thielmans, 1978)} \qquad \text{(equation 3.3.2.4.2.2.2)}$$

ti = time post dose, taken as the mid-point between successive samples.

Ci = concentration of marker in faeces.

Δti = difference in time between successive samples.

The LMRT from the two algebraic and five compartmental models were then subjected to a two-way analysis of variance Genstat 5 (Lawes Agricultural trust, 1993) and a LSD test, to determine if significant differences existed in LMRT between HC and OH:NO and if differences existed between the LMRT obtained from different models within foods. The same procedure was then adopted for the TMRT data, determined from the Yb data.

3.3.2.4.2.3. Compartmental analysis

The model that demonstrated the best fit, based on the R^2 data, for describing digesta passage through the large intestine, determined using Cr, was the Pond *et al.* (1988) G3 model, which was then used for further compartmental analysis of passage rate in the large intestine. The same procedure was done with the Yb total tract data, thus the Pond *et al.* (1988) G4 model was chosen for compartmental analysis of the total tract passage rate data. The mean compartment passage rate and retention time parameters from the three ponies while consuming HC and OH:NO, and their standard errors, for both the G3 and G4 models were then calculated. Thus, values were obtained for a time-dependent compartment λ, a time-independent compartment k_2 and a time delay (TD) factor τ.

On the basis of these models biological interpretation was attempted, whereby the individual compartments were designated as representing certain regions within the equid gastrointestinal tract. However, with only three ponies and two fibre foods, this compartmental interpretation was severely limited and no clear conclusions could be drawn.

3.3.2.5. Experiment B

Following experiment A, a single period experiment was carried out using the same three ponies. The ponies were fed 4 kg DM per day of a 50:50 mix of unmolassed sugar beet pulp and hay cubes (SB:HC) plus minerals fed as detailed in Experiment 3.2., (section 3.2.2.2.). Marker preparation, administration, sample collection and data analysis were performed as for experiment A. However, as this was a single period experiment analysis of variance could not be performed. The data is therefore presented as mean values across ponies plus their s.e.

3.3.3. Results

In both experiment A and B all ponies readily consumed the ytterbium (Yb) marked foodstuffs and no health problems were noted in any of the ponies during the time that marker passed through the gastrointestinal tract. The caecal doses of chromium (Cr) also passed through the hindgut without causing any apparent effects on pony health. After taking into account the recovery of marker from the caecal digesta samples, average recoveries were 94% for Cr and 100.8% for Yb.

3.3.3.1. Caecal outflow data

The mean caecal DM content, MRT, R^2 and passage rate calculated from Cr outflow data collected from the ponies when consuming HC, OH:NO and SBF in experiment A and SB:HC in experiment B are detailed in Table 3.3.3.1.

Table 3.3.3.1. Caecal DM (kg), digesta passage rate, mean retention time (MRT) (hours), and R^2 values determined from caecal outflow data obtained from ponies consuming hay cubes (HC), a 67:33 mix of oat hulls:naked oats (OH:NO) and sugar beet food (SBF) in Experiment A and SB:HC in Experiment B.

	Experiment A					**Experiment B**	
	HC	**OH:NO**	**SBF**	**s.e.d.**	**Sig**	**SB:HC**	**s.e.**
DM content (kg)	0.32	0.09	0.30	0.114	NS	0.44	0.089
Passage-rate per hour	0.322	0.387	0.246	0.0821	NS	0.294	0.0140
MRT (hours)	3.2	2.6	4.76	1.26	NS	3.4	0.17
R^2	0.928	0.856	0.717[a]	0.0484	*	0.885	0.0320

[a] Values in the same row not sharing common superscripts differ significantly ($P<0.05$)

The average DM content in the caecae of the three ponies were not significantly different between diets, although the amount of material present when ponies were fed the OH:NO diet was 3.5 times less than when consuming the HC and SBF diets. The slowest passage rate was recorded when the ponies were eating the SBF diet, resulting in the longest caecal MRT of 4.76 hours. This MRT was nearly twice that recorded for the OH:NO, but this difference was not significant. The SBF data set was from only two ponies, one of which produced faeces on only 5 occasions during the collection period. This undoubtedly contributed to the higher MRT noted for this diet. The exponential

equation used to model the data was however, significantly ($P<0.05$) worse (determined by R^2 values for each pony on each food), at describing the outflow of Cr marked SBF than for either HC or OH:NO (see Figure 3.3.3.1 to 3.3.3.3). Thus, the SBF values derived from the exponential equation used to model the outflow data are of questionable accuracy.

Figure 3.3.3.1. Chromium marker concentration (—) from the exponential equation $Y = A\,e^{-kt}$, and the actual data collected from the caecum of pony 6 (◆) when consuming a 67:33 mix of oat hulls:naked oats (OH:NO) over a 10-hour period

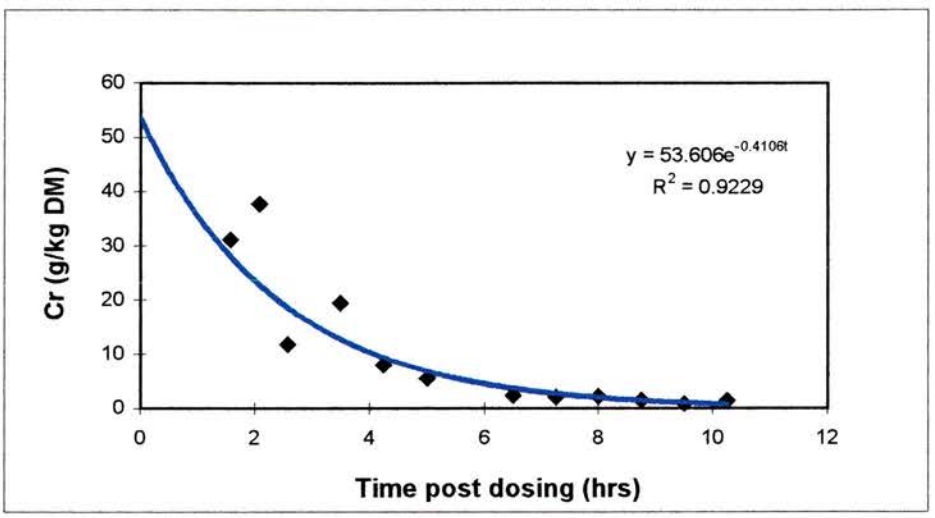


Figure 3.3.3.2 Chromium marker concentration (—) from the exponential equation Y = A e^{-kt}, and the actual data collected (◆)from the caecum of pony 5 when consuming hay cubes over a 10-hour period.

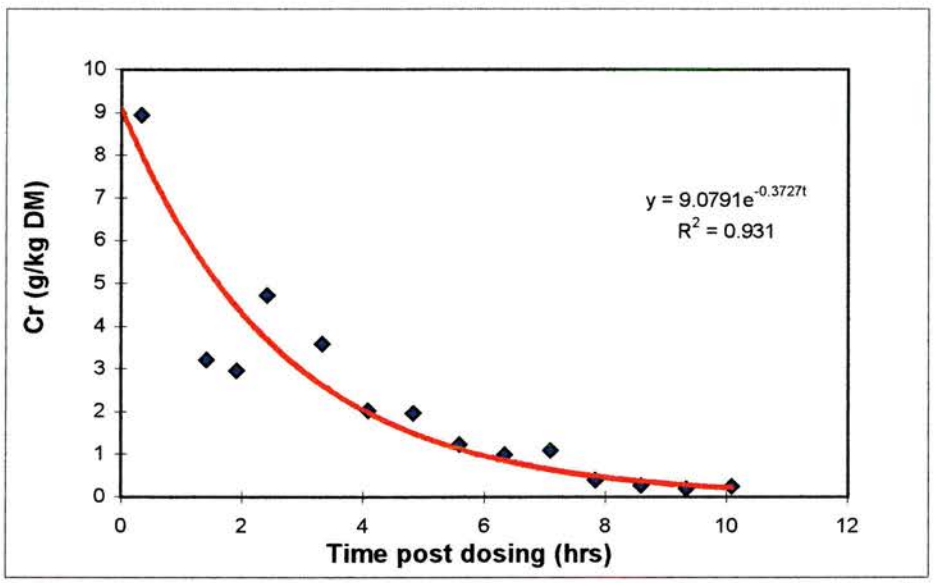
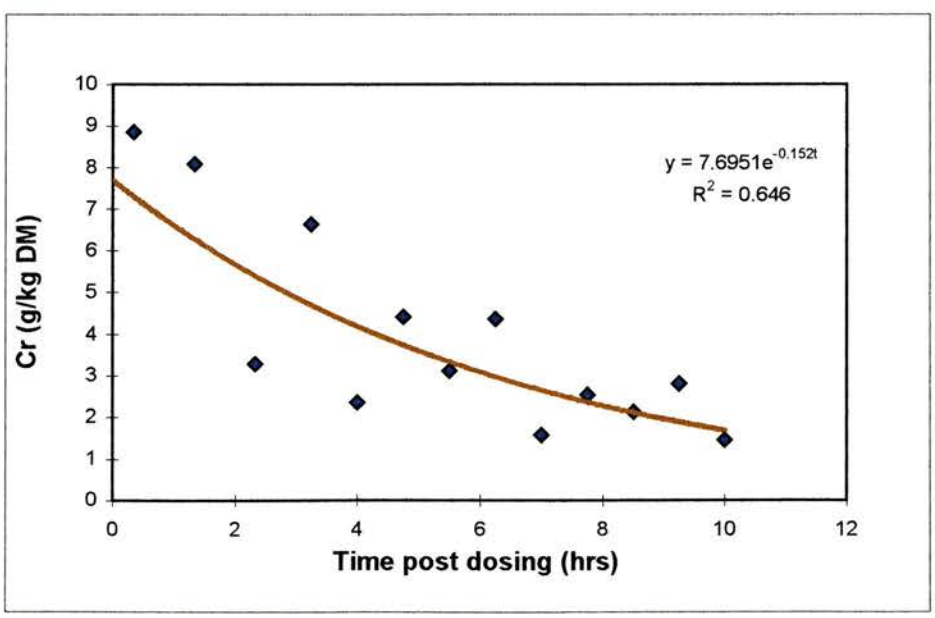


Figure 3.3.3.3 Chromium marker concentration (—) from the exponential equation Y = A e^{-kt}, and the actual data collected (◆)from the caecum of pony 5 over a 10-hour period when consuming sugar beet food.



3.3.3.2. Faecal data.

Initially 8 models, 2 algebraic and 6 compartmental, were applied to both the Cr and Yb faecal excretion data with the objectives of comparing 'goodness of fit' (using R^2 data), LMRT and TMRT between models and between foods. However, the Grovum and Williams (1973) model failed to converge with the observed data in three out of the six data sets obtained from the three ponies in Experiment A. Moreover, the three data sets it did fit, produced comparatively low R^2 values of 0.885 for HC and 0.652 for OH:NO, thus a workable solution with this model was not attained and it was subsequently rejected from further analysis and comparisons in experiment A.

3.3.3.2.1. Modelling of Cr faecal excretion data, using 5 compartmental models .

Table 3.3.3.2. shows the R^2 values for the five models tested on the faecal excretion data obtained from the three ponies, using Cr marked food administered into the caecum. All four Pond *et al.* (1988) time-dependent models (G1 to G4) and the Dhanoa *et al.* (1985) (DMC) multi-compartmental, time-independent model fitted the HC data well, with no significant differences noted between models for this food. However, the best fit was the Pond *et al.* (1988) time-dependent G3 model producing a high R^2 of 0.974. The average G3 fitted-curves across all ponies eating HC, OHNO and SB:HC are shown in Figure 3.3.3.1. The G3 model also fitted the OH:NO data well, although the R^2 was marginally higher at 0.918 for the G4 model. Across all models goodness of fit for OH:NO was lower than noted for HC, although significant differences ($P<0.05$) were only seen between the DMC and all models for HC; and between the OH:NO G1 with the HC G3 and G4 models.

Table 3.3.3.2. Accuracy as determined by R^2 of five compartmental models for describing faecal excretion data using chromium marked food in three ponies consuming hay cubes (HC) and a 67:33 mix of oat hulls:naked oats (OH:NO) in Experiment A and SB:HC in Experiment B.

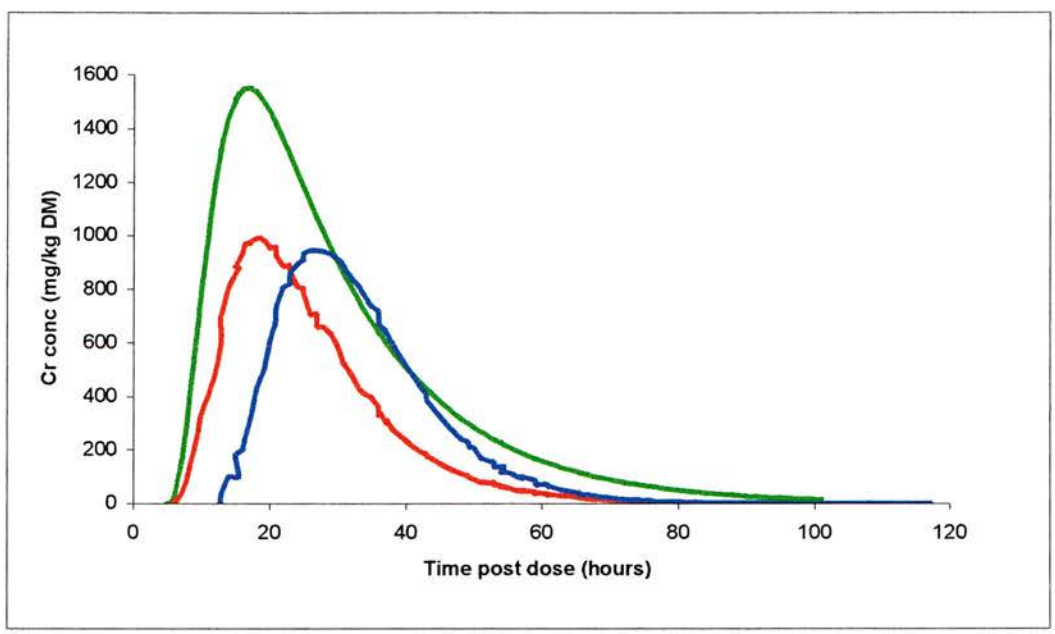
Model	Experiment A		Experiment B
	HC	OH:NO	SB:HC
G1	0.953^{bc}	0.889^{ab}	0.902^{b}
G2	0.941^{bc}	0.906^{ac}	0.827^{a}
G3	0.974^{c}	0.916^{ac}	0.903^{b}
G4	0.973^{c}	0.918^{ac}	0.886^{ab}
GW	^	^	0.859^{ab}
DMC	0.957^{bc}	0.856^{a}	0.887^{ab}
s.e.d.	0.0338		0.0331
Sig	*		*

[ab] Values within the same experiment not sharing common superscripts differ significantly * (P<0.05), **(P<0.01). ^ not included in the statistical analysis. G1 to G4 time dependent models of Pond *et al.* (1988); GW = Grovum and Williams (1973) two compartment model; DMC = Dhanoa *et al.*(1985) multi-compartmental model.

In the single period experiment B, when only SB:HC was fed, reasonable fits were obtained for all models, although the best model for describing the faecal excretion patterns when the ponies were on the SB:HC diet was the G3 model, which gave an average R^2 of 0.903 (see Figure 3.3.3.1. for the average fitted G3 curve). The G2 model (R^2 of 0.827) was the only one to be significantly (P<0.05) worse at describing the data than the G3 and G1 models and although the G4, Grovum and Williams (1973) and

DMC models were lower at 0.886, 0.859 and 0.887 respectively, these differences were not significant.

Figure 3.3.3.4. Average chromium faecal excretion curves obtained from the Pond *et al.*(1988) G3 model for all ponies consuming hay cubes (—), a 67:33 mix of oat hulls:naked oats (_), in Experiment A, and a 50:50 sugar beet :hay cubes mix(_),in Experiment B.



When considering all three diets across both experiments A and B, the G3 model of Pond *et al.* (1988) fitted the chromium faecal excretion data marginally better than the other time-dependent (Pond *et al.*, 1988) and time-independent models (GW and DMC) tested, and was therefore used for further compartmental analysis, see Table 3.3.3.6.

3.3.3.2.2. Modelling of Yb faecal excretion data, using 5 compartmental models.

Table 3.3.3.3. shows the R^2 for the five models applied to the faecal excretion data obtained from three ponies after consuming of a pulse dose of ytterbium (Yb) marked

food. All 5 models fitted the HC data accurately, with R^2 values ranging from 0.895 for the G2 model to 0.965 for the G4 model. When tested on the OH:NO diet, the G2 and DMC models were significantly ($P<0.05$) less accurate, (R^2 of 0.832 and 0.833 respectively), at describing the data than the G3 (0.939) and G4 (0.943) models. As with the Cr marked faecal excretion data, all the models were less accurate at fitting Yb marked OH:NO than HC.

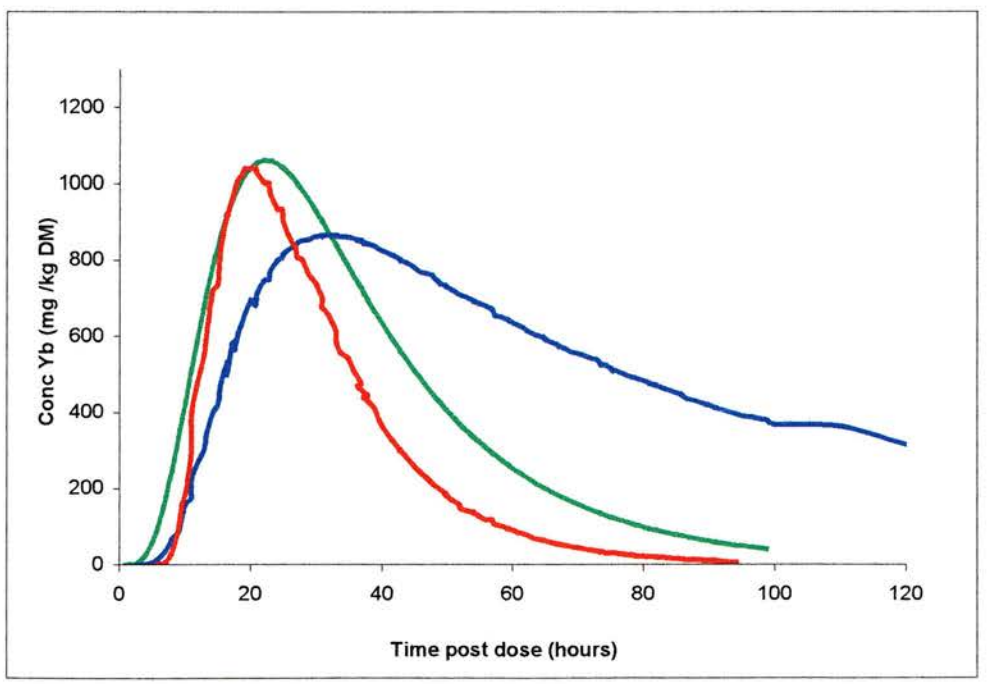
Table 3.3.3.3. Accuracy of fit as determined by R^2 of five mathematical models for describing faecal excretion data using ytterbium marked food in three ponies consuming hay cubes (HC) and a 67:33 mix of oat hulls:naked oats (OH:NO) in Experiment A and SB:HC in Experiment B.

Model	**Experiment A**		**Experiment B**
	HC	**OH:NO**	**SB:HC**
G1	0.952^{b}	0.904^{ab}	0.965
G2	0.895^{ab}	0.832^{a}	0.968
G3	0.963^{b}	0.939^{b}	0.908
G4	0.965^{b}	0.943^{b}	0.967
GW	^	^	0.943
DMC	0.925^{b}	0.833^{a}	0.964
s.e.d	0.0428		0.0340
Sig	*		NS

^{abc} Values within the same experiment not sharing common superscripts differ significantly * ($P<0.01$). ^ values not included in the statistical analysis.
G1 to G4 time dependent models of Pond *et al.* (1988); GW = Grovum and Williams (1973) two compartment model; DMC = Dhanoa *et al.*(1985) multicompartmental model.

By contrast, faecal excretion data obtained in experiment B when ponies were fed Yb marked SB:HC, produced high R^2 values ranging from 0.908 to 0.968 with no significant differences noted between any of the models. Taken across both experiments the model that produced the best fit when using Yb marked food to describe faecal excretion data was the time dependent G4 model of Pond *et al.* (1988), and this model was subsequently used for compartmental analysis. Average faecal excretion curves determined for digesta passage through the total tract of all the ponies on HC, OH:NO and SB:HC are shown in Figure 3.3.3.5.

Figure 3.3.3.5. Average ytterbium faecal excretion curves determined from the Pond *et al.*(1988) G4 model for all ponies consuming hay cubes (—), a 67:33 mix of oat hulls :naked oats (—), in Experiment A, and a 50:50 mix of sugar beet:hay cubes (—),in Experiment B.



3.3.3.2.3. Mean retention time of digesta in the LI, determined using Cr-marked foods.

Table 3.3.3.4. shows the average large intestine mean retention times (LMRT) calculated from five compartmental and two algebraic models, using Cr marked food placed into the caecum of each of the three ponies when consuming HC and OH:NO in Experiment A and SB:HC in Experiment B. The only significant ($P<0.05$) difference between LMRT was between the G2 and G3 models, with LMRT ranging from 26.2 hours (G3) to 30.3 hours (G2). The LMRT of OH:NO was significantly ($P<0.05$) longer (*ca.* 10 hours) than that of HC, thus the OH:NO LMRT ranged from 36.2 obtained using the G4 model, up to 40.2 hours calculated using the DMC model.

LMRT for SB:HC fed in experiment B ranged from 31.5 hours (Thielmans and G3) up to 34.6 hours for the G2 model, the latter being significantly longer than the LMRT obtained from the G1, G3, G4, Faichney and Thielmans methods. The model that produced an MRT closest to the value of 31.79 hours obtained from the accepted standard method of Faichney (1975), was the G1 model at 31.7, followed closely by the Thielmans, G3 and G4 models which produced MRT of 31.5, 31.5 and 31.9 hours respectively.

Table 3.3.3.4. Large intestine mean retention time (LMRT) in hours, of digesta calculated from six models and two algebraic equations using faecal excretion data obtained from ponies given a pulse-dose of chromium marked food into the caecum while consuming hay cubes (HC) and a 67:33 mix of oat hulls:naked oats (OH:NO) in Experiment A and SB:HC in Experiment B.

Model	**Experiment A**		**Experiment B**
	HC	**OH:NO**	**SB:HC**
G1	26.8^{ab}	38.7^{c}	31.7^{a}
G2	30.3^{ab}	38.4^{c}	34.6^{b}
G3	26.2^{a}	36.2^{c}	31.5^{a}
G4	26.3^{ab}	36.2^{c}	31.91^{a}
GW	-	-	33.4^{ab}
DMC	29.4^{ab}	40.2^{c}	32.9^{ab}
Faichney	27.7^{ab}	37.7^{c}	31.79^{a}
Thielmans	27.8^{ab}	38.8^{c}	31.5^{a}
s.e.d.	1.98		0.964
Sig	*		*

[ab] Values within the same experiment not sharing common superscripts differ significantly ($P<0.05$). G1 to G4 = time dependent models of Pond *et al.* (1988); GW = two compartment model of Grovum and Williams (1973); DMC= multicompartment model of Dhanoa *et al.* (1985); Faichney = equation of Faichney (1975); Thielmans = equation of Theilmans *et al.* (1978).

3.3.3.2.4. Mean retention time of digesta within the total tract, determined using ytterbium marked foods.

Table 3.3.3.5. details the TMRT obtained for the five compartmental and two algebraic models from the faecal excretion data obtained from ponies given an oral pulse-dose of Yb marked HC and OH:NO in Experiment A and SB:HC in Experiment B. No significant differences in TMRT were seen between models within foods. The HC TMRT ranged from 29.9 hours for G1 up to 35.1 hours for the DMC model. The models to produce TMRT closest to the standard Faichney (1975) method of 30 hours were, G1 (29.9), G3 (29.4), and G4 (30.5). These were significantly shorter, by *ca* 10 hours, than the MRT obtained from all models for OH:NO. The standard Faichney (1975) TMRT for OH:NO was 40.8 hours which agreed closely with the 41.1, 39.7 and 39.8 hours obtained from the Thielmans, G3 and G4 models respectively. The longer TMRT obtained for the HC using the DMC model was not significantly different from the shorter TMRT obtained for the OH:NO using the G3 and G4 models.

In Experiment B, the MRT of 35.4, 36.1, 36.6 and 36.9 for Thielmans, Faichney, G2 and G1 models respectively, were all significantly shorter than the 41.6 hours obtained from the G3 model.

Table 3.3.3.5. Total tract mean retention time (TMRT) in hours, of digesta calculated from six models and two algebraic equations using faecal excretion data from ponies given an oral pulse-dose of ytterbium marked food while consuming hay cubes (HC) and a 67:33 mix of oat hulls:naked oats (OH:NO) in Experiment A and SB:HC in Experiment B.

Model	Experiment A		Experiment B
	HC	OH:NO	SB:HC
G1	29.9[a]	42.2[cd]	36.9[a]
G2	37.2[abc]	47.2[d]	36.6[a]
G3	29.4[a]	39.7[bcd]	41.6[b]
G4	30.5[a]	39.8[bcd]	38.4[ab]
GW	-	-	37.7[ab]
DMC	35.1[abc]	46.7[d]	38.7[ab]
Faichney	30.0[a]	40.8[cd]	36.1[a]
Thielmans	31.1[ab]	41.1[cd]	35.4[a]
s.e.d.	4.36		1.86
Sig	*		*

[ab] Values within the same experiment not sharing common superscripts differ significantly ($P<0.05$). G1 to G4 = time dependent models of Pond *et al.* (1988); GW = two compartment model of Grovum and Williams (1973); DMC= multi-compartment model of Dhanoa *et al.* (1985); Faichney = equation of Faichney (1975); Thielmans = equation of Theilmans *et al.* (1978).

3.3.3.2.5. Compartmental analysis.

3.3.3.2.5.1. Compartmental analysis of digesta passage from Cr data.

The G3 model of Pond *et al.* (1988) best described the faecal excretion patterns obtained when Cr marker was placed directly into the caecae of the three ponies. This model describes the data in terms of digesta passage thorough a time-dependent lambda (λ) compartment (LC), a time-independent k- compartment (kC) and a tubular compartment called the time delay (TD), or tau (τ). Table 3.3.3.6. details the mean passage rate and retention time parameters in Experiments A and B, estimated using the G3 time-dependent compartmental model of Pond *et al.* (1988).

Table 3.3.3.6. Rate parameters λ and k_2 and calculated MRT for the lambda compartment (LC), k-compartment (kC), time delay (TD) and large intestine MRT (LMRT), obtained from the Pond *et al.* (1988) G3 model applied to faecal excretion data collected from ponies given *ca.* 50g of Cr marked food into the caecum while consuming hay cubes (HC) and a 67:33 mix of oat hulls:naked oats (OH:NO) in Experiment A and SB:HC in Experiment B.

	Experiment A				**Experiment B**	
	HC	**s.e**	**OH:NO**	**s.e**	**SB:HC**	**s.e**
λ	0.311	0.0076	0.22	0.047	0.415	0.1528
LC	9.57	0.230	14.94	3.120	9.07	2.529
K_2	0.093	0.0145	0.122	0.0415	0.058	0.0062
kC	11.28	1.718	9.9	2.56	17.59	2.087
TD	5.29	1.371	11.34	3.248	4.88	1.034
LMRT	26.2	3.06	36.2	3.47	31.54	0.942

In both HC (Experiment A) and SB:HC (Experiment B) λ appears to represent the fast compartment, with MRT's of *ca.* 9 hours, while k_2 giving an MRT of 2 to 8 hours longer, indicates that this is the slow compartment. TD for both foods are fairly similar

at *ca.*5.5 hours for HC and *ca.*5 hours for SB:HC. The difference in LMRT of *ca.* 5 hours is due to the longer retention time of SB:HC in the slow compartment of the gastrointestinal tract estimated at *ca.*11 and 18 hours for HC and SB:HC respectively.

The general pattern of MRT was different in the OH:NO food, than from that noted for the HC and SB:HC diets. Thus the λ and k compartments could have either the longest or shortest digesta residence time.

3.3.3.2.5.2. Compartmental analysis of digesta passage from Yb data.

The time dependent G4 model of Pond *et al.* (1988) best described the faecal excretion data through the total tract determined using Yb-marked food, so this model was used for further compartmental analysis (see Table 3.3.3.7). The HC diet showed only 4 hours difference in the residence times noted between the LC and kC, whereas in Experiment B the SB:HC diet remained in the kC for 9 hours longer than in the LC. As with the Cr marked data, the OH:NO diet behaved differently from the HC and SB:HC diets, producing a MRT of 17.2 calculated from 4/λ for the LC whereas a value of 12.5 hours MRT was obtained for the kC.

Table 3.3.3.7. Rate parameters λ and k_2 and calculated MRT for lambda compartment (LC), k-compartment (kC), time delay (TD) and total tract MRT (TMRT), obtained from the Pond *et al.* (1988) G4 model applied to faecal excretion data collected from ponies given an oral pulse-dose of Yb marked food while consuming hay cubes (HC) and a 67:33 mix of oat hulls:naked oats (OH:NO) in Experiment A and SB:HC in Experiment B.

	Experiment A				Experiment B	
	HC	**s.e**	**OH:NO**	**s.e**	**SB:HC**	**s.e.**
λ	0.40	0.024	0.25	0.053	0.27	0.015
LC	10.00	0.580	17.20	3.689	14.71	0.855
K_2	0.07	0.006	0.08	0.014	0.047	0.010
KC	13.96	1.236	12.58	1.810	23.07	4.500
TD	5.49	1.474	10.06	2.226	0.59	0.387
TMRT	29.4	2.237	39.84	5.346	38.37	4.169

3.3.4. Discussion.

Although no health problems were noted in any of the ponies during the times marker was passing through the gut, the 3, five-day collection periods were not without problems. The design of Experiment A, a 3X3 Latin square, did not materialise into 9 sets of data as originally anticipated. Two of the ponies failed to consume sufficient SBF to produce normal faecal excretion patterns. Accordingly, it was subsequently decided not to analyse the results as a 3X3, by entering two missing values out of a possible three for SBF, but to process the data as an un-balanced 3x2, ie. data from three ponies for HC and OH:NO. This severely limited the conclusions that could be drawn from the results, particularly in relation to the biological interpretation of the two compartment models. It was also not possible to match passage rates with specific physiological segments of the gut, or indeed to clearly establish whether λ or k_2 passage

rate parameters from the G3 and G4 time-dependent models of Pond *et al.* (1988), always related to the mixing compartments having the shortest or longest residence times respectively. However, the data collected was sufficient to allow comparisons to be made between the ability of different models to fit faecal excretion data, and the accuracy with which each model estimated MRT as calculated by the reference algebraic method of Faichney (1975).

In light of the poor acceptance of SBF in Experiment A, the single period Experiment B was carried out when all three ponies were simultaneously offered a 50:50 mix of SB:HC. This diet was readily accepted and a full set of caecal and faecal data were collected for each pony. As this was a single period experiment, statistical analysis (analysis of variance) was confined to a comparison between the 'goodness of fit' and MRT of the eight models. However, the data from this experiment did offer another valuable opportunity to test the use of Cr and Yb as digesta passage rate markers in ponies, and to test the ability of 8 models to describe faecal excretion data and to determine MRT values. Thus, in the light of the limitations mentioned, the following discussion will focus upon the modelling of marker data rather than a comparison of digesta kinetics between diets.

3.3.4.1. Caecal data.

Sufficient caecal data were collected from all three ponies for HC, OH:NO and SBF, to allow a time-independent simple exponential model to be fitted to the 12 to 14 marker concentration samples obtained from each pony. This equation provided a rate parameter k, which represented the rate at which the marker concentration declined within the caecum. The fitted line describes the decrease in concentration of Cr marker in the caecum over a 10-hour period and represents two possible phenomena. The first is the flow of digesta out of the caecum, resulting in a declining marker concentration; whilst the second represents in-flow of digesta into the caecum causing a dilution of the marker present, again resulting in a decreasing marker concentration. The marker was

put into the caecum at approximately 14 hours after the evening food, thus it is likely that when the Cr was introduced, the caecum was relatively empty. Two hours after introduction of the Cr, the ponies were fed, and, as digesta has been noted by Frape (1986) to arrive in the caecum as little as 45 minutes post-prandium, it seems likely that digesta in-flow was as significant a factor in decreasing marker concentration as digesta out-flow. The fact that the simple exponential model fitted the HC and OH:NO data well ($R^2 > 0.9$) suggests that the decline in Cr concentration (albeit from marker outflow or digesta inflow, or a combination of both) is a time-independent process. One of the assumptions of time-independent models is that compartmental mass or volume remains constant ie. steady-state conditions prevail (Ellis *et al.,* 1994). However, in this experiment the meal-feeding regime is likely to have prevented the maintenance of a constant caecal DM volume. Assumptions of steady state conditions generally accompany time-independent modelling of rumen out-flow data. However, such assumptions have been noted to be flawed by Reid *et al.* (1979), who suggested that the flow of digesta into and out-from the reticulo-rumen varies with feeding activity, thus in meal-fed Animals 'steady-state conditions' are unlikely to exist. Furthermore, considerable variations in caecal volume have been noted by Goodson *et al.* (1988) who reported a range of 0.6 to 8.6 litres in a caecally fistulated pony meal-fed a corn and Soya bean diet, and these values closely agree with the 0.5 to 3 litres recorded from meal-fed ponies by Argenzio *et al.* (1974). This demonstrates an anomaly in the modelling process and demonstrates the necessity for careful consideration of all the anatomical and physical conditions impinging upon the animal before any conclusions can be drawn regarding digesta passage rate based on mathematical modelling. Moreover, the caecal sampling was only performed for a single 10-hour period in this study, thus this data could only provide a 'snap-shot' of caecal volume and as such is unlikely, particularly in a meal-fed animal, to be representative of caecal MRT for the entire 5-day collection period. The caecal data therefore cannot be used to assist identification of fast or slow compartments from the five compartmental models fitted to the faecal excretion data in this study.

3.3.4.2. Mathematical Modelling of faecal excretion data.

The compartmental models that most successfully described the flow of digesta (based on R^2 values) through the large intestine, hereafter described as the Cr data, and the flow through the total tract, hereafter described as the Yb data, were the time-dependent models of Pond *et al.* (1988). These models which incorporate time-dependency through using the family of gamma distributions, signified by lambda (λ), offer greater flexibility in modelling passage through a tubular gut, because the initial rate function is zero and increases with time (Pond *et al.,* 1988). Although reporting no significant difference between the fit of time-dependent or time-independent models to faecal excretion data collected from early-weaned calves fed hay or concentrate diets, Lalles *et al.* (1991) also suggested that stochastic (time-dependent) models represent a more general and flexible approach than time-independent models when describing ruminant digesta passage. This flexibility is attributed to the ability of the stochastic models to deal with passage of marked particles through mixing compartments caudal to the rumen, eg. the caecum (Cruickshank *et al.,* 1989), and to deal with variable time-delays associated with rectum emptying (Pond *et al.,* 1988). Moore *et al.* (1992) further suggested that the time-dependent models gave flexibility to modelling the ascending phase of the faecal excretion data obtained from yearling rams fed hay or concentrates. Moreover, the range of gamma functions resulted in an ability to describe rapidly ascending data, such as that collected from dairy cows with high food intakes, to slow ascending phases recorded from animals with low food intakes. Moore *et al.* (1992) found the best fits for ram faecal excretion data were achieved using the G3 and G4 models, with poorer fits obtained from the G1 and G2 models. The best-fit models in the present experiment proved to be the higher gamma orders of G3 and G4, the former fitting the Cr data best, while the latter fitted the Yb data most accurately. The flexibility of these time-dependent models may have given the G3 and G4 models the advantage over the time-independent models, but with only three sets of data tested per food offered, it is not possible to say why one model was better at describing pony faecal excretion data than another.

The deterministic two compartment model of Grovum and Williams (1973), which applies first order kinetics to both k_1 and k_2 compartments, did not fit the Cr or the Yb faecal excretion data in 4 out of the 6 data sets collected in Experiment A. Some of the data showed the k_1 and k_2 rate parameters to be equal, which could have accounted for the difficulty in fitting this time-independent model. Mertens (1989), suggested that equal rate parameters indicate that digesta passage is in fact a time dependent process, and this seemed to be the case in Experiment A, where observed data, which could not be fitted using the time-independent model of GW, was successfully described by the time-dependent models. Cruickshank *et al.* (1989) also found that data collected from early-weaned lambs fed high quality herbage diets were more accurately described by time-dependent or multi-compartmental models, than by the two compartment time-independent model of Grovum and Williams (GW) (1973). The GW model was deemed inappropriate, as it required initial estimates for k_1 from the descending part of the faecal excretion curve, which if treated as a single compartment (ie. treating the rumen as a single pool), ignores the influence of the other mixing compartments eg. the caecum and thus leads to errors in estimating rumen fractional outflow from faecal excretion data (Cruickshank *et al.,* 1989).

Corino *et al.* (1992 and 1993) attempted to fit the Grovum and Williams (1973) model to horse faecal excretion data and in accordance with the present study, failed to achieve convergence between fitted and actual data. Why these authors had difficulty fitting this model is not clear from either of the publications. As with the present experiments poor fit could be the result of trying to describe an essentially time-dependent process with an exponential model, or it could possibly be a function of data collection.

Failure to collect sufficient numbers of samples during the early stages of marker excretion is frequently blamed for poor agreement between predicted and observed data (Dhanoa, *et al.,* 1985; Matis, 1989). In the current experiment faecal collections started six hours post-dosing which resulted in *ca.* 7 data points compiling the ascending phase of the graphs. Although the best fits were achieved in Experiment A using the G3 and

G4 stochastic models, which require good quality data to model the early part of the excretion curve (Pond *et al.*, 1988), the poorer fits achieved for the G2 model may indicate that in some cases insufficient data was collected to accurately model the early phases of faecal excretion. Alternatively, the out-flow rate from the LC was slower than the G2 model ideally fits (Moore *et al.,* 1992). Dhanoa *et al.* (1985) found the G2 model to be particularly sensitive to initial data points and found deficiencies in the data resulted in poor agreement between predicted and actual excretion patterns.

Clearly the majority of data collected in Experiment A was sufficiently good to successfully model the faecal excretion curves using the higher order of gamma functions. However, in Experiment B one of the data sets yielded a value for τ of zero, indicating that the initial data collection was insufficient to reliably partition passage rate between τ and λ. Such anomalies while appearing not to adversely affect goodness of fit measurements, such as R^2, could confound the process of compartmentalising digesta flow rate through the equid gastrointestinal tract. The Cr data indicated that when ponies consumed the SB:HC diet, digesta flow followed the 'classic' pattern of rate of passage, ie. an initial fast rate (LC 0.415) followed by a slower rate (k_2 0.058), thus allowing a reasonable fit to be achieved with the G1 and DMC time-independent models. The number of sample collections were similar to that in Experiment A, so the availability of only 5 to 7 points in the ascending phase appeared not to adversely affect model fitting of this diet.

The DMC model achieved better fits than the Grovum and Williams (1973) model for the HC. One of the possible reasons why this model fitted the data is that the DMC equation utilises a distribution of multiple sequential compartments each having a small difference in magnitude of its age-independent fractional turn-over rates (Ellis *et al.,* 1994). Thus the DMC model describes a time-dependent process using a different mathematical approach to the time-dependent models [where λ for G2 and above is a sum of two or more exponentials (Matis, 1987)]. However, good fits were not achieved with the DMC for the OH:NO diet which could be a function of this diet as overall

modelling of the OH:NO diet was less accurate than for either HC or SB:HC. When modelling all the data, using the G1, DMC and GW models, the fitted line frequently failed to converge with the peak marker concentrations, although early and late data points were described reasonably well (see appendix 5). Pond *et al.* (1988) suggested that the use of time-dependent models produced better agreement between actual and fitted data in the early stages of marker excretion. However, in this experiment the time-dependent models did not improve the fit of the early phase of marker excretion when ponies were eating the OH:NO diet. On this diet the time-dependent models overestimated the time it took before actual appearance of marker in the faeces when modelling Yb excretion from all the ponies, and in data collected from one pony when Cr was modelled. This did not occur on either of the other two diets, and is therefore likely to be a feature of the OH:NO diet. The most appropriate models for describing digesta flow through the large intestine and total tract of ponies were the time-dependent G3 and G4 models of Pond *et al.* (1988) respectively. Consequently these models were used to provide a more detailed compartmentalisation of digesta passage through the gastrointestinal tract of each pony.

3.3.4.3. Mean retention time of digesta in the LI and total tract.

TMRT can be derived algebraically using the standard Faichney (1975) equation, which because it uses actual data collected rather than assuming a continuous rate of faecal output, is regarded as the most accurate method to achieve total tract residence time (Warner, 1981). Uden *et al.* (1982), Pearson and Merritt, (1991), and Morrow (1998) have all used variations of this equation to establish TMRT in equids consuming hay. These workers have reported TMRT of 23, 29 and 24-27 hours respectively, which are similar to the 26-30 hours noted for HC and 32 hours recorded for SB:HC, but are slightly less than the 37 hours recorded in Experiment A when OH:NO were fed. Pearson and Merritt (1991) reported a longer TMRT of 5 hours when ponies were fed a poorly degraded straw diet than when they consumed a more degradable hay diet. However, in the present experiment the digestibility of OH:NO and HC were similar,

(see table 3.2.3.4) thus the extent of degradation of the two diets was not a major influence on the passage rate of digesta through the total tract of the ponies.

The Thielmans *et al.* (1978) equation for calculating TMRT, uses faecal marker concentration data and therefore dispenses with the need for total faecal collection, proved to be very reliable for both the Cr and Yb data, producing good agreement with the Faichney (1975) method for determining TMRT. Moreover, across both markers and experiments, good agreement was seen for TMRT between Thielmans (1978), G3, G4 and Faichney (1975) models, whereas, the DMC and G2 models consistently over-estimated TMRT. Lalles *et al.* (1991) reported good agreement from both algebraic and compartmental models in early-weaned dairy calves fed hay or concentrate labelled diets. Additionally, Gomez *et al.* (1992) reported that the MRT for sheep fed a roughage-pelleted diet could reliably be calculated using the Faichney (1975), Thielmans (1978) or GW models.

When calculating MRT within different compartments of the digestive tract, different researchers have found different models to be more or less reliable. Moore *et al.* (1992) found good agreement for all models when used to determine TMRT, but when calculating MRT in the three compartments, ie. TD, LC and kC compartments, MRT decreased in the TD but increased in the LC with increasing orders of gamma function. This lack of differentiation between TD and LC was also reported by Pond *et al.* (1988) and noted in the present experiment, and can in part be attributed to lack of data in the early stages of marker excretion. Lalles *et al.* (1991) reported similar MRT for the slow-turn-over k_1 compartment, across the GW, time-dependent and DMC models, but the MRT in the kC was 50% lower when calculated from the DMC than from all other models.

Reports from the above experiments with ruminants on the accuracy of TMRT obtained from G3, G4, Faichney (1975) and Thielmans (1978) models suggest that these can be reliably used to calculate digesta TMRT, and from the data collected in this experiment,

these models also produce accurate estimates of TMRT in ponies. However, no one model is as yet recommended for estimating compartmental MRT in ruminants, and with the limited data collected in this experiment particularly in the early phases of marker excretion, no conclusive recommendations can be made for equids. To obtain reliable estimates for the LC, Pond *et al.* (1988) recommended that sampling closer to the rumen eg. at the duodenum should be carried out, as faecal sampling can be confounded by later mixing compartments and so does not always yield accurate values for rumen out-flow rate.

3.3.4.4. Compartmental analysis

The general assumption when dealing with compartmental models is that the model consists of compartments where particle mixing may occur eg. rumen and abomasum, while the TD phase represents a minimum fixed time that is required for particle passage and where mixing is non-existent (Matis *et al.,* 1989). Most ruminant researchers believe that the slower time-independent (k_1) compartment is the rumen, the faster time-independent (k_2) compartment is the caecum and proximal colon (Moore *et al.,* 1992; Gomez *et al.,* 1992; Lalles *et al.,* 1991; Grovum and Williams, 1973) while the TD represents the passage of digesta through the omasum and the intestines, and is closely correlated with the first appearance of marker in the faeces (Grovum and Williams, 1973). In the time-dependent models lambda (λ) replaces k_2 and represents the reticulo rumen, while k_1 and TD are similar to the time-independent models (Pond *et al.,* 1988).

From the limited data collected and presented in Tables 3.3.3.6 and 3.3.3.7, apportioning the LC and kC into sections of the gut where digesta resides for a short or long time cannot be achieved conclusively, although in two out of the three diets tested across Experiments A and B, the LC appears to be the fast compartment (short MRT) and the kC the slow compartment (long MRT). Time-dependency is consistently associated with the faster turnover compartment in ruminants (Matis, 1972; Ellis *et al.,* 1984); however, when considering the digestive anatomy of the equid, the size, positioning and

sac-like structure of the caecum could result in it being a time-independent, fast compartment, while the tubular colon with its narrow flexures and large capacity could be the time-dependent, slow compartment. Faichney and Boston (1983) found that in ruminants under certain dietary circumstances outflow from the caecum could be slower than from the rumen. This suggests that the dietary ingredients, or the manner in which the food is presented to the animal, can influence the rate at which digesta passes through the different chambers of the gut. In the present experiment all the diets were ground to pass a 1mm screen and presented to the ponies twice daily, thus the differences noted between the MRT of the LC and kC for the OH:NO and HC could not be due to differences in meal presentation. The longer MRT for the λ compartment and shorter MRT for the k_2 compartment noted for the OH:NO compared with the HC diet is thus likely to be a function of diet type. However, before any firm conclusions can be made on digesta passage rate through the different compartments of the equid gastrointestinal tract, a more detailed study of passage rate in ponies consuming a variety of diets is required.

The TD compartment in the gamma models is defined as the time post-dose until first appearance of the marker in the faeces (Pond *et al.,* 1988). This compartment is thought to represent the narrow tubular sections of the gut, where food is drier and has little opportunity to mix, thus a time delay factor is introduced to account for this (Ellis *et al.,* 1984; Matis, 1989). As a TD compartment was clearly identified in the caecally introduced Cr marked food, it may be that this compartment relates to the small colon, where faeces collects before excretion. Additionally the TD compartment is comparable between the two markers, whereas the LC and kC are longer for the Yb data. This suggests that the time digesta remains within the stomach and small intestine are included in the LC and kC retention times and not in the TD section. A comparison of the MRT obtained from the Cr and Yb marked food shows the TMRT to be 3.24 hours less when Cr labelled foods were infused into the caecum compared with when Yb labelled foods were pulse-dosed orally. This observation may infer that the additional

3.24 hours is the time it takes the digesta to pass through the stomach and small intestine.

Which sections of the gut the time-dependent and time-independent rate parameters are referring to is still unclear. The size, positioning and movement of the caecum would suggest that digesta remains within this organ for a short time (ie. the fast compartment) but it's sac-like anatomy suggests that instantaneous mixing may occur and that outflow could be independent of residence time, ie. a time-independent compartment. In contrast, the anatomy of the large colon, with its four sacculated sections divided by narrow flexures (Frandson, 1981), would pre-dispose this part of the gut to be the time-dependent section. Moreover, it is possible that particle mixing within each of the distinct regions of the colon occurs rapidly; implying time-independency, but that passage through the complete organ is time-dependent. The rate constant for the time-dependent section of the faecal excretion curve, λ is a sum of 2 or more time-independent rates, thus passage of digesta through the large colon could easily fit such a mathematical equation. Argenzio *et al.* (1974) found the passage of digesta was particularly slow from the ventral to the dorsal colon (through the pelvic flexure) and from the dorsal to the small colon. Additionally, fluid marker was retained for prolonged periods in the large colon, having passed from the stomach through the caecum into the proximal colon relatively quickly, thus passage through the large intestine could be a time-dependent process. Results from this experiment are again too few to come to any informed conclusion as to caecal or colonic digesta passage rates, particularly as the HC data suggests one scenario, whilst the OH:NO suggests another.

The lack of a definite conclusion on compartmentalising the gut is not a problem unique to this experiment. Having developed a multi-compartment model, which accurately described 80 sets of ruminant faecal excretion data, Dhanoa *et al.* (1985) could not say with certainty which of the two main rate constants represented the rumen and which represented the caecum. Results from their first study indicated that k_1 was the rumen, while k_2 was the caecum. However, their second study did not produce similar

conclusions. It seems that clear biological interpretation of modelled faecal excretion data is extremely difficult. In the present study, the lack of clear compartment identification could be a result of the use of ruminant models for fitting pony faecal excretion data. However, as the R^2 values indicate, the models did accurately describe the marked faecal excretion patterns of the ponies. Alternatively, the modelling process itself could lack sufficient sensitivity to allow the prediction of digesta flow through the different compartments of the gut to be achieved from end-point excretion data. Moreover, if faecal excretion data can be successfully modelled using a wide variety of models (time-independent and time-dependent two compartment models) that make different assumptions regarding digesta flow, the ability of the modelling procedure to partition flow through the various segments of the equid digestive tract may be limited. Considerably more work is required in this area before definitive conclusions can be drawn regarding digesta passage rate through the different compartments of the equid gastrointestinal tract. Moreover, the limitations in the quantity of data produced from this experiment means that the conclusions that can be drawn regarding differences between the diets fed are restricted to the following. 1) The more digestible HC had a shorter large intestine and total tract MRT than OH:NO and these MRT are reflected in the shape of the average faecal excretion curves in Figures 3.3.3.4. and 3.3.3.5. 2) The difference between the HC and SB:HC with the OH:NO diet is also seen in the compartmental analysis with HC and SB:HC producing a shorter MRT associated with the time-dependent LC compartment and the longer MRT associated with the kC compartment, while the OH:NO produced the opposite for LC and kC MRT.

3.3.5. Conclusions

Chromium and ytterbium were successful external markers for determining total tract mean retention time of fibre-based diets in ponies. Additionally, marker excretion patterns allowed existing compartmental mathematical models to be successfully applied to faecal excretion data, with time-dependent compartmental models describing the flow of digesta more accurately than time independent models. Total tract mean retention

time was successfully calculated from model rate parameters, and although compartmental retention times could be obtained, at present there was insufficient data to produce a conclusive biological interpretation on the compartmentalisation of the different segments of the equid gastrointestinal tract.

3.4. Degradation of four botanically diverse fibrous foodstuffs in the small intestine and total tract of ponies, as measured by the mobile bag technique.

3.4.1. Introduction

Although horses evolved principally as hind-gut fermenters, studies by Gibbs *et al.* (1988), Peloso *et al.* (1994) and Coleman *et al.* (1998) have revealed that equids also digest of a high proportion of dietary soluble carbohydrates, starch, proteins and fats *via* enzymatic activity in the small intestine. Whilst a number of workers have examined the total tract digestibility of different foods in horses (Todd, 1995 a and b; Cuddeford *et al.*, 1995), little information exists on the site and extent of digestion in the horse. Currently most horse and pony diets in the UK are formulated on NRC (1989) recommendations, which assume certain digestibility coefficients for energy and protein. However, lack of information on the site of digestion sheds some doubt as to the accuracy of these formulations.

To know the proportion of food that is potentially digestible in the small intestine is particularly useful, as the production of ATP from glucose metabolism is markedly more efficient than the production of ATP from VFA metabolism. Moreover, knowledge of the site of digestion is particularly important when measuring protein availability, as it is only the protein digested in the small intestine, which is utilised by the horse (Hintz, *et al.,* 1971; Hintz and Cymbaluk, 1994). Thus, studies that determine the amount of energy and protein derived from the diet can be used to manipulate rations and more closely match individual animal requirements.

The mobile bag technique has proved to be a fast, reliable method for determining AD in ruminants (Kirkpatrick and Kenelly, 1984; Varvikko and Vanhatalo, 1990) and pigs (Sauer *et al.,* 1983; Leibholz, 1991) and if used in conjunction with fistulated animals, offers an opportunity to measure both the rate and extent of food degradation within

different segments of the gastrointestinal tract. Such a technique would be particularly useful for determining the nutrient value of foods for horses, and, if combined with effective degradability calculations (Ørskov and McDonald, 1979) could provide essential data on food degradation kinetics within the different regions of the equid gut.

The objectives of this study were 1) to determine the extent of degradation of four botanically diverse fibrous foods in the small intestine and total tract of ponies and, 2) to determine the degree of fermentation of protein and individual NSP components, with a view to establishing detailed information on the potential nutrient value of four fibrous foods which could potentially be used as the basal roughage in the diet of stabled horses

3.4.2. Materials and Methods

3.4.2.1. Experimental Design

The experimental design was a 3X4 incomplete Latin square with three animals and four fibrous foods. The foods offered were unmolassed sugar beet pulp (SBP), hay cubes (HC), soya hulls (SH), and a 67:33 mix of oat hulls : naked oats (OH:NO) (supplied by Dalgety Agriculture Ltd. Milton Keynes, UK). Disappearances of dry matter (DM), organic matter (OM), acid detergent fibre (ADF), neutral detergent fibre (NDF), crude protein (CP), and non-starch polysaccharides (NSP), were recorded from 6 X 1 cm polyester monofilament mesh bags. Digestibility (D) values were subsequently calculated according to the formula:

$$D = \frac{I - F}{I}$$

I = the amount (mg) of food component in the bag

F = the amount (mg) of the food component in the faeces

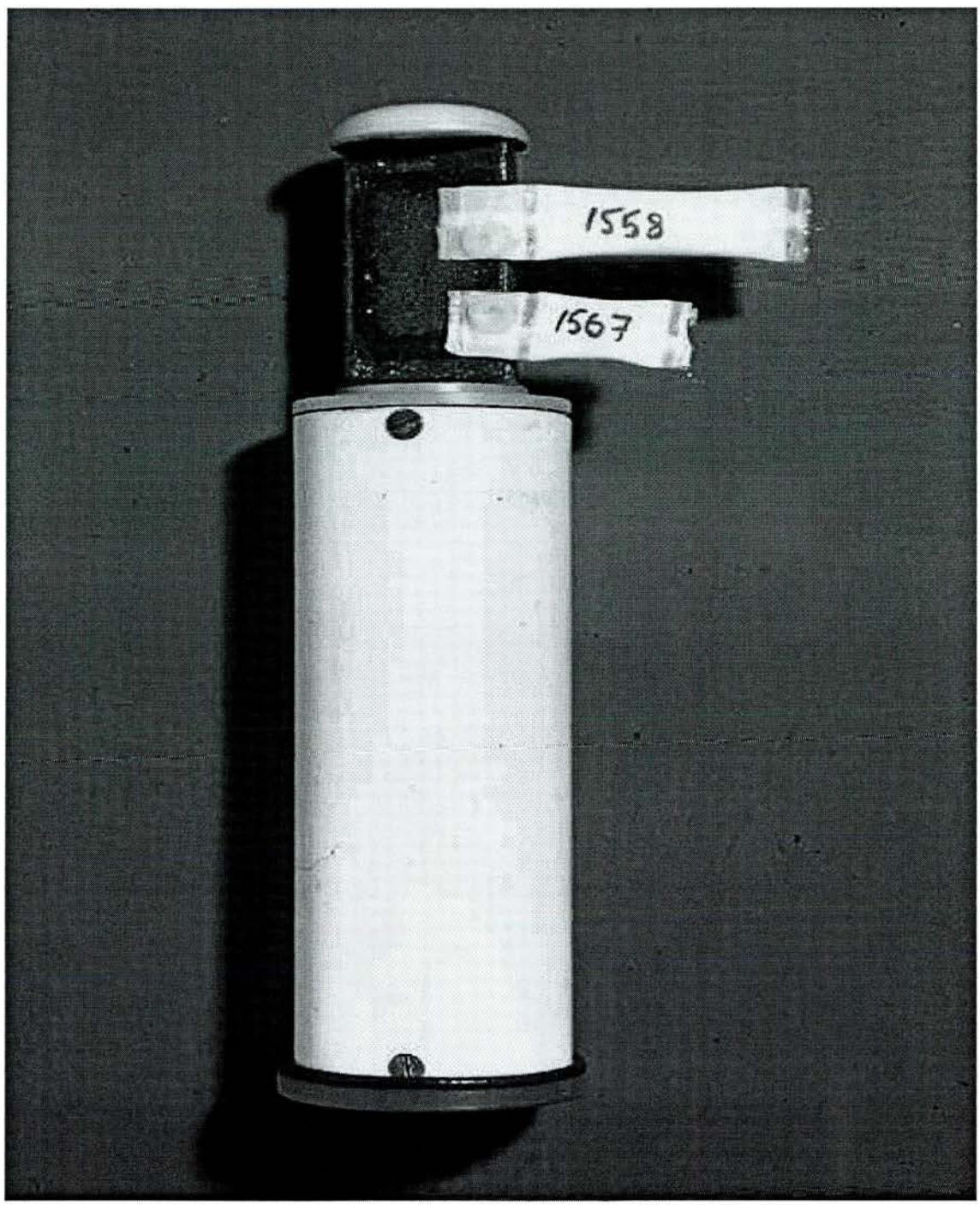
3.4.2.2. Animal Management.

Three mature Welsh cross pony geldings (*ca.*250 kg LW) each fitted with a permanent indwelling caecal cannula at the caecal base (Cottrell *et al.,* 1998) were maintained on a basal diet of 4 kg of soaked grass nuts (Dalgety Agriculture Ltd. Milto Keynes, UK), *ad libitum* hay (Table 3.4.3.1) and 30g of a mineral and vitamin supplement (appendix 2). Ponies were fed twice daily at 08:00 hours and 18:00 hours and had free access to water from automatic water troughs. The ponies were housed in 8 x12 ft pens with rubber matting (Davies & Co. Kettering, UK) covering the floors. During the adaptation periods the floor was also covered with shavings, which were removed during collection periods to facilitate the gathering of faeces.

3.4.2.3. Preparation of mobile bags.

350 mg of each food, ground to pass through a 1mm steel mesh (Christy and Norris eight inch laboratory mill) were heat-sealed using a Packer heat-sealer, (model 15 / 300 H Viking Direct, Leicester, UK) inside 6 X 1 cm monofilament polyester mesh bags (pore size 41 μm) (Seriol Ltd. Broadstairs, Kent, UK). The bags were designed according to the recommendations of Wuensche (1988), Hyslop and Cuddeford (1996), and Cherian, *et al.* (1989), which involved cutting around a 6 x 2 cm wood block, folding the edges together and closing the side and end with the heat sealer. In addition, two steel washers were sealed into one end of each bag. The bags were then numbered with indelible pen, filled with 350mg of food and the top sealed with the heat sealer. The steel washers in the base of the bag allowed the bags to be captured by a specially designed removable electro-magnet (Dept. Physics Edinburgh University), (see plate 3.4.1) which was placed inside the caecal cannula and positioned to lie across the ileo-caecal junction.

Plate 3.4.1. The electro-magnet used to capture the mobile bags (shown attached to the magnetic strip) at the ileo-caecal junction.



3.4.2.4. Administration of mobile bags

On two consecutive mornings of each week, 20 bags containing one of the four diets (pre-soaked in water for 30 minutes) were administered to each pony *via* a naso-gastric tube. The tube was pre-filled with bags before insertion into the nasal passage. Approximately 750 – 1000ml of water were then pumped, using a hand-operated pump, into the tube to flush the bags into the stomach. Immediately post- dosing, the magnet was placed inside the caecal cannula to capture bags passing through the ileo-caecal junction. On capture of one or several bags, the circuit inside the magnet was completed which caused a red light to flash on the external end of the magnet. This indicated to those in attendance that a bag had been caught on the magnet. The cannula top was then opened and the bag(s) removed before re-positioning of the magnet inside the cannula. The capture time of each bag was noted and the procedure repeated until *ca.* 15 bags had been captured on the magnet. Since these bags had passed through the small intestine only, they were designated small intestine bags (SIB). The magnet was then removed to allow the remainder of the bags to continue through the digestive tract to be recovered in the faeces, and these were termed faecal bags (FB). As soon as the bags were collected (both SIB and FB) they were hand rinsed in ice-cold water and kept at 4^0C for a maximum of 24 hours, whereupon they were washed using the cold rinse programme of a domestic washing machine (Indesit model 824, programme 5), according to the recommendations of Macheboef *et al.* (1996) and Van Straalen *et al.* (1993). After drying in a forced draught oven at 60°C for 48 hours, individual SIB and FB bags were weighed to determine DM losses. The bags were then opened and the contents bulked (into SIB or FB residues) to obtain sufficient material for chemical analysis.

3.4.2.5. Chemical Analysis

Bulked residues were lightly ground using a pestle and mortar and analysed for DM, OM, ADF, NDF, total and individual NSP, starch and CP by the methods detailed in appendix 1. To assess the affect of the cold-water rinse in the washing machine alone,

three replicates of the food samples were placed in similar sized bags and were cold-water washed in the washing machine; the residue was subsequently treated to a similar drying process and chemical analysis as the residue from the SIB and FB.

3.4.2.6. Dry matter degradation curves.

DM disappearance data from weighing the individual bags, were grouped according to recovery time (SIB grouped on *ca.* 40 minute time intervals and FB on 5-10 hour intervals), and averaged across all ponies for each food. This data was then subjected to the Ørskov and McDonald (1979) equation (3.4.2.1.) in order to describe the degradation profiles of the foodstuffs.

$$P = a + b\,(1 - e^{-ct}) \qquad \text{(equation 3.4.2.1)}$$

P = degradation after time t.
b = potential degradation of component which will in time be degraded.
c = rate constant for degradation of b
a = intercept of degradation curve at time zero.
e = exponential.

The equation allowed the line of best fit to be plotted through the average degradations from the three ponies for both SIB and FB for each food (see Figures 3.4.1.to 3.4.4). The extent of degradation [effective degradability (ED)] at chosen outflow rates of 0.1, 0.05, 0.025 and 0.016 were then obtained using equation 3.4.2.2. to obtain dry matter loss at the assumed digesta mean retention times of 10, 20, 40 and 60 hours.

$$P = a + \frac{bc}{c + k} \qquad \text{(equation 3.4.2.2.)}$$

where:

c = the degradation rate ie. the rate at which the potentially degradable substance is broken down.

a + b = potential degradation.

k = chosen outflow value.

The degradation at time zero (a), the potential degradation (b), the rate constant (c), a + b, and all of the calculated ED values for FB were subjected to a two way analysis of variance using Genstat 5 (Lawes Agricultural trust, 1993). The SIB were too few in number and too close together in recovery times to allow a sensible analysis of variance to be carried out on individual pony data, thus the SIB data has been presented as the average across all ponies for each food.

3.4.2.7. Particle size Determination

The particle size of each of the foods was determined in a 3 x 4 design where 15 g of food was carefully weighed and placed into the first of a tier of 12 sieves, which ranged in size from 40mm at the top of the stack down to 45μm at the bottom. The cascade of sieves was then placed on an Elf 2000 shaker, which was set to run for 20 minutes. On completion of the shaking, each sieve was weighed and the following calculation carried out.

Weight of sieve + sample - sieve wt = Sample weight.

The data obtained was then subjected to the standard procedure for calculating geometric mean particle size (American Dairy Science Association, 1970), (appendix 3).

3.4.2.8. Water Holding Capacity.

The water holding capacity (WHC) of the three foods was determined by the method

of Robertson *et al.* (1980). 500mg of each food was weighed into 30ml bottles. Twenty ml of distilled water was then added to each bottle. The bottles were then capped and the samples left to soak for 48 hours. The bottles were then centrifuged at 6000g for 15 minutes after which the supernatant was discarded and the tubes laid flat with the ends slightly elevated to assist draining, for 30 minutes. The wet food was then carefully transferred onto pre-weighed foil trays, weighed and placed in a forced-draught oven at 60 °C for 24 hours, whereupon samples were re-weighed and water holding capacity (WHC) calculated as g water/ g food. This procedure was then repeated so that three replicates were obtained for each food.

3.4.2.9. Statistical Analysis

All data were subjected to a two-way analysis of variance using Genstat 5 (Lawes Agricultural Trust, 1993). Differences between foods for all parameters measured were determined using the L.S.D. tests, where L.S.D. = t (error degrees of freedom) x s.e.d. obtained from the analysis of variance.

3.4.3. Results

3.4.3.1. Food Composition

The composition of the experimental foods is given in Table 3.4.3.1. The DM and OM of the four foods were similar, but the CP content ranged from 78g/kg DM (SBP) to 128g/kg DM in SH. This pattern was paralleled by the ADF contents, but the NDF contents were highest in HC and OH:NO at 623 and 610g/kg DM respectively, and lowest in SBP at 547g/kg DM. The total NSP content of SBP and SH was *ca.* 490g/kg DM, whereas the mean value for HC and OH:NO was 100g/kg lower at 380g/kg DM. The proportions of the NSP monomers in HC and OH:NO were similar with 550g glucose and 270-300g xylose/kg NSP for both foods. However, the proportion of the

NSP monomers in the remaining foods differed. Thus, 870 g/ kg of SBP NSP consisted of near equal proportions of arabinose, glucose and UAC, the levels of arabinose being 3.8 - 6.4 times higher than those detected in the remaining foods. The proportions of UAC found in SBP and SH at 276 and 180g UAC/kg NSP respectively, were considerably greater than those of the OH:NO and HC. Indeed most (700g/kg NSP) of the SH NSP consisted of UAC and glucose in the ratio of *ca.*1:3, and contained comparatively little xylose or arabinose.

Table 3.4.3.1. Chemical composition of the four experimental foods, unmolassed sugar beet pulp (SBP), hay cubes (HC), soya hulls (SH) and a 67:33 mix of oat hulls : naked oats (OH:NO) and the basal diet of hay and grass pellets (g/kg DM).

Parameter	HC	OH:NO	SBP	SH	Hay	Grass nuts
DM	933	900	913	903	827	940
OM	920	969	903	924	938	907
CP	82	96	78	120	75	146
ADF	354	331	271	438	383	365
NDF	623	610	547	591	752	623
Total NSP	378	377	488	492	-	-
Rhamnose	1	0	7	4	-	-
Arabinose	29	22	141	37	-	-
Xylose	102	130	11	45	-	-
Mannose	4	2	7	35	-	-
Galactose	13	10	36	20	-	-
Glucose	209	204	150	261	-	-
Uronic acids	20	9	135	89	-	-

3.4.3.2. Washing machine losses

The losses of nutrients (expressed as coefficients, as the treatment was measuring digestibility in a washing machine) from the four foods contained in polyester mesh bags after a cold-water wash in an automatic washing machine are shown in Table 3.4.3.2.

HC and OH:NO lost *ca.* 0.24 of the DM and OM, whereas the corresponding losses for SBP and SH were substantially lower at 0.10 DM and 0.07 OM. CP losses followed a similar pattern, the losses from OH:NO and HC being 0.59 and 0.18 respectively, and these losses were higher than the 0.03 recorded for SH. An apparent gain in CP was noted for SBP of 0.52. There was no significant difference in losses of ADF from SBP, HC and SH, which ranged from 0.02-0.08, but the losses from OH: NO were greater than those from SBP and SH. There were apparent gains in NDF, in the order of 0.15 for all foods except SBP, which lost 0.02. NSP losses from SH and SBP were similar averaging 0.04 and from OH:NO and HC at *ca.* 0.15.

Table 3.4.3.2. Nutrient disappearance coefficients from dried ground samples of hay cubes (HC), a 67:33 mix of oat hulls : naked oats (OH:NO), unmolassed sugar beet (SBP) and soya hulls (SH), contained in 6x1cm monofilament polyester mesh bags, after cold water washing in an automatic washing machine.

Parameter	HC	OH:NO	SBP	SH	s.e.d	Sig
DMD	0.27^{a}	0.24^{a}	0.09^{a}	0.10^{b}	0.017	***
OMD	0.23^{a}	0.23^{a}	0.08^{b}	0.07^{b}	0.019	**
CPD	0.19^{a}	0.59^{b}	-0.05^{c}	0.03^{c}	0.037	**
ADFD	0.08	0.16	0.02	0.03	0.058	NS
NDFD	-0.18^{a}	-0.11^{a}	0.02^{b}	-0.15^{a}	0.027	**
TNSPD	0.15^{a}	0.15^{a}	0.04^{b}	0.04^{b}	0.024	**
Rhamnose D	0.70^{a}	0^{b}	-0.09^{b}	0.32^{ab}	0.215	*
Arabinose D	0.26^{a}	0.20^{b}	0.20^{b}	0.14^{c}	0.014	**
Xylose D	0.18^{a}	0.06^{ab}	0.16^{ab}	0.04^{b}	0.055	*
Mannose D	0.68^{a}	0.84^{a}	0.23^{b}	0.06^{b}	0.118	**
Galactose D	0.41^{a}	0.39^{a}	0.15^{b}	0.15^{b}	0.022	***
Glucose D	0.10^{a}	0.16^{b}	0.03^{c}	0.03^{c}	0.022	*
Uronic acid D	0.10^{a}	0.32^{b}	-0.10^{c}	-0.02^{ac}	0.064	*

^{abc} Values in the same row not sharing common superscripts differ significantly ($P<0.05$)

3.4.3.3. Transit time, particle size and water-holding capacity

The transit times of the mobile bags from stomach to caecum averaged 3.3 hours, and were not significantly different between foods (Table 3.4.3.3). The mean transit times of the FB were very similar for SBP, HC and OH:NO at 65h whereas the SH FB transit time was significantly lower at 55h. The particle size (PS) of HC was significantly lower than for any of the other three foods.

Table 3.4.3.3. Mobile bag transit time through the small intestine (STT) and through the total tract (TTT) in hours, water holding capacity (WHC) and particle size (PS) of hay cubes (HC), a 67:33 mix of oat hulls : naked oats (OH:NO), unmolassed sugar beet food (SBP) and soya hulls (SH).

Parameter	**HC**	**OH:NO**	**SBP**	**SH**	**s.e.d**	**Sig**
STT	3.27	2.95	4.22	2.95	0.778	NS
TTT	65.3^{a}	64.0^{ab}	65.5^{a}	54.8^{b}	3.84	*
WHC	5.10^{a}	2.78^{b}	7.31^{c}	5.54^{d}	0.160	*
PS (gmps)	247^{a}	369^{b}	430^{b}	411^{b}	29.0	*

^{abc} Values in the same row not sharing common superscripts differ significantly ($P<0.05$). gmps = geometric mean particle size (appendix 3).

3.4.3.4. Losses from food in bags passing through the small intestines of ponies

The coefficients of losses of nutrients from the bags collected at the caecum, (SIB) and those that had travelled through the entire tract, (FB) are shown in Table 3.4.3.4. DM and OM losses from OH:NO and HC SIB were significantly higher than from SH which in turn were greater than from SBP. CP losses of 0.77 from OH:NO SIB were significantly higher than the average 0.55 lost from SH and HC SIB. Losses of CP from

SBP SIB at 0.30 were significantly lower than from the other foods. By contrast, losses of ADF and NDF from SIB tended to be greatest from SBP, followed by HC, SH and lastly OH:NO, but these differences were only statistically significant between the SBP and OH:NO with losses of 0.14 and 0.008 DM NDF respectively. Total NSP losses from SIB were not significantly different between the four foods though there was a tendency for losses from SBP to be greater than those from HC and OH:NO. Of the major individual NSP components, losses of glucose from OH:NO (0.12) and xylose from HC (0.08) were significantly greater than from the other foods which showed apparent gains in these constituents. Losses of uronic acids from SBP and SH of 0.48 and 0.66 respectively were significantly higher than from HC and OH:NO, there being an apparent gain of 0.35 of uronic acids from the latter. Losses of the minor NSP constituents mannose and galactose from the SIB were greatest from OH:NO and HC > SH > SBP. There were apparent gains of rhamnose for SBP and SH but losses of this constituent were observed from HC.

Table 3.4.3.4. Coefficients of disappearances of food constituents contained in polyester mesh bags after passing through the small intestine (SIB) and total tract (FB) of ponies.

Parameter		HC	OH:NO	SBP	SH	Sed	Sig
DM	SIB	0.32^{c}	0.34^{c}	0.19^{a}	0.26^{b}		
	FB	0.56^{a}	0.46^{a}	0.85^{g}	0.68^{f}	0.024	*
OM	SIB	0.29^{c}	0.34^{c}	0.18^{a}	0.24^{b}		
	FB	0.54^{e}	0.46^{d}	0.87^{g}	0.67^{f}	0.025	*
CP	SIB	0.52^{b}	0.77^{e}	0.30^{a}	0.60^{bc}		
	FB	0.62^{bcd}	0.86^{e}	0.76^{de}	0.73^{cde}	0.064	*
ADF	SIB	0.13^{ab}	0.03^{a}	0.11^{ab}	0.08^{a}		
	FB	0.36^{c}	0.21^{b}	0.68^{e}	0.53^{d}	0.051	*
NDF	SIB	0.08^{b}	0.008^{a}	0.14^{bc}	0.08^{ab}		
	FB	0.42^{d}	0.20^{c}	0.77^{f}	0.56^{e}	0.035	*
TNSP	SIB	0.05^{a}	0.06^{a}	0.13^{a}	0.08^{a}		
	FB	0.57^{c}	0.37^{b}	0.90^{d}	0.64^{c}	0.066	**
Rhamnose	SIB	0.66^{b}	0^{a}	-0.34^{a}	-0.12^{a}		
	FB	0.88^{b}	0^{a}	0.94^{b}	0.95^{b}	0.202	**
Arabinose	SIB	0.17^{a}	0.18^{a}	0.12^{a}	0.11^{a}		
	FB	0.67^{c}	0.48^{b}	0.98^{d}	0.77^{c}	0.067	*
Xylose	SIB	0.08^{ab}	-0.08^{a}	-0.12^{a}	-0.10^{a}		
	FB	0.46^{cd}	0.27^{bc}	0.72^{e}	0.53^{de}	0.101	*
Mannose	SIB	0.54^{b}	0.92^{cd}	0.10^{a}	0.12^{a}		
	FB	0.86^{c}	1^{d}	0.88^{cd}	0.93^{cd}	0.064	*
Galactose	SIB	0.34^{b}	0.32^{b}	0.06^{a}	0.19^{ab}		
	FB	0.73^{d}	0.57^{c}	0.97^{e}	0.91^{e}	0.067	*
Glucose	SIB	-0.01^{ab}	0.12^{b}	-0.10^{a}	-0.09^{a}		
	FB	0.55^{c}	0.42^{c}	0.84^{d}	0.51^{c}	0.088	*
Uronic acids	SIB	0.08^{b}	-0.35^{a}	0.48^{cd}	0.66^{ce}		
	FB	0.78^{de}	0.38^{bc}	0.98^{e}	0.88^{e}	0.180	*

^{abc} Values within each constituent not sharing common superscripts differ significantly ($P<0.05$).

3.4.3.5. Losses from food in bags passing through the total tract of ponies

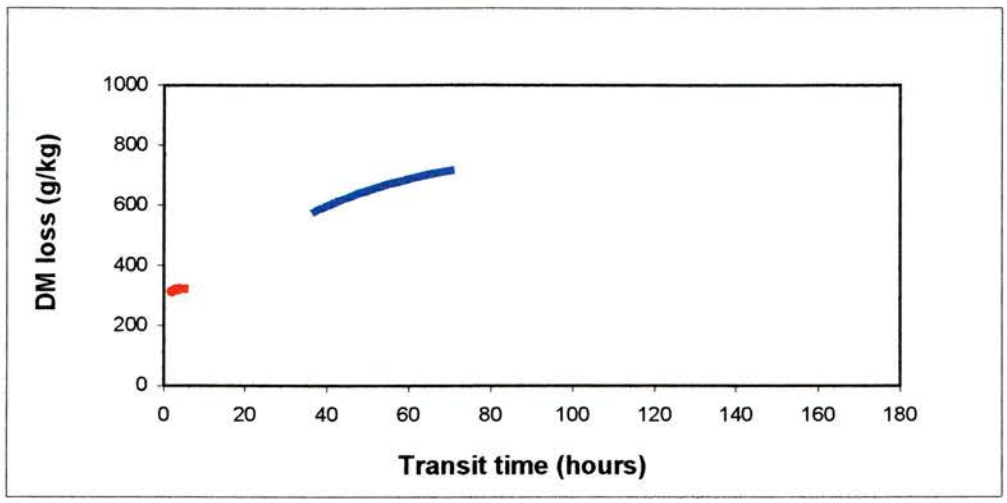
In marked contrast to the losses of nutrients from the SIB, FB showed significant differences between the four foods for most of the nutrients. For all of the parameters

measured, the highest losses were recorded for SBP>SH>HC>OH:NO. More than 0.840 of DM and OM disappeared from SBP FB. Likewise, greater than two-thirds of these constituents were lost from SH FB and *ca.* half of the DM and OM was lost from the HC and OH:NO FB. CP losses from the SBP and SH FB were significantly ($P<0.05$) higher than from their SIB counterparts, this was particularly marked for SBP where CP losses from FB were 2.5-fold that from the corresponding SIB. The increases in CP losses from the HC and OH:NO FB were not significantly greater than from the corresponding SIB. CP losses from HC FB were lower than from the other foods and were significantly ($P<0.05$) lower than CP losses from the SBP and OH:NO FB. Losses of ADF and NDF from the FB for all foods were significantly ($P<0.05$) higher than from the corresponding SIB, and losses of these components were significantly ($P<0.05$) different for each food, with highest losses being recorded from SBP FB of 0.68 and 0.77 ADF and NDF respectively. Lowest losses were recorded from OH:NO, with ADF and NDF disappearances averaging 0.21. TNSP losses from FB of each food were higher than those losses from the SIB. TNSP loss from SBP FB was 0.90 which was significantly ($P<0.01$) higher than that from HC and SH which averaged 0.60: these losses were significantly higher than the 0.37 lost from OH:NO. Losses of the major NSP monomers (glucose, arabinose, uronic acids and xylose) from SBP FB, all exceeded 0.72 and apart from the uronic acids, these losses were significantly ($P<0.05$) greater than from the other foods, whereas losses of these nutrients from OH:NO FB were less than 0.48 which were significantly ($P<0.05$) lower than the corresponding losses from the other foods. Losses of the major NSP monomers from SH and HC FB were not significantly different from each other. The losses of the minor NSP constituents, rhamnose, mannose and galactose from the FB were higher than 0.73 from all foods except for OH:NO from which no net losses of rhamnose occurred and where losses of galactose averaged 0.57.

3.4.3.6. Fitted dry matter degradations

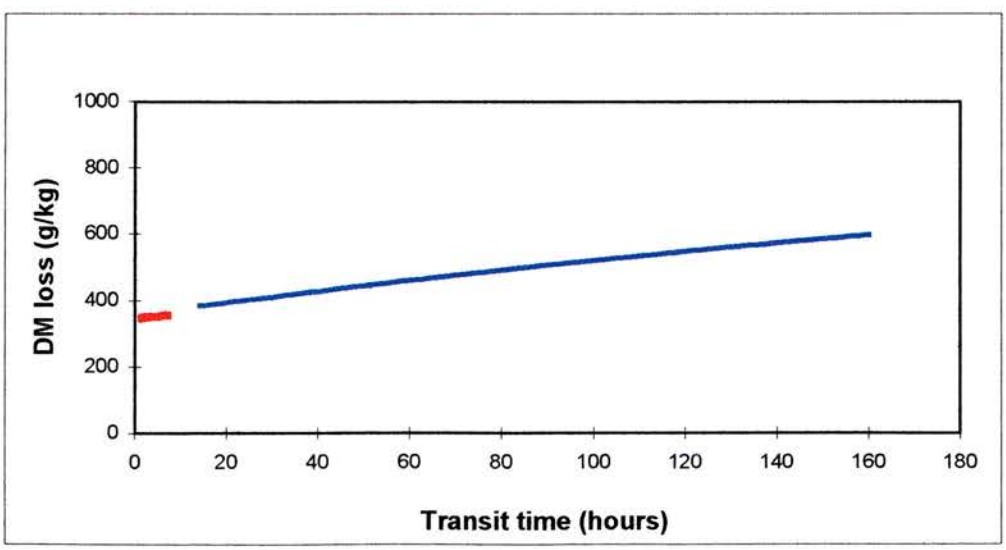
Fitted Ørskov and McDonald (1979) DM degradation curves from the three ponies for each diet are shown in Figures 3.4.3.1 to 3.4.3.4. For all foods tested, bags were retrieved from the caecal cannula in less than 8 hours and only lost 0.15-0.30. Some bags were caught in less than 1 hour post-intubation. Fitted data from the FB suggested that degradation was markedly affected by bag residence time in the large intestine. In excess of 0.65 disappeared from SBP when bags were incubated for 40 hours, whereas SH and HC disappearances only reached this level after 60 hours incubation. OH:NO lost a maximum of 0.58 after 160 hours in the digestive tract. Although both SBP and SH did lose in excess of 90% DM, the speed at which these disappearances occurred was significantly ($P<0.05$) different, with SBP attaining maximum degradation in approximately half the time it took for SH to be fully degraded. The calculated effective degradability (ED) of each food detailed in Table 3.4.3.5. indicate that the longer the incubation time the greater the difference between foods with SBP being significantly more degraded than the other three foods at 40 and 60 hours mean retention time (MRT), whereas the shorter incubation of 10 hours MRT, shows no significant difference between any of the foods.

Figure 3.4.3.1. Dry matter disappearances from hay cubes contained in 6 x 1 cm polyester mesh bags having passed through the stomach and small intestine (SIB) and total tract (FB) of ponies.



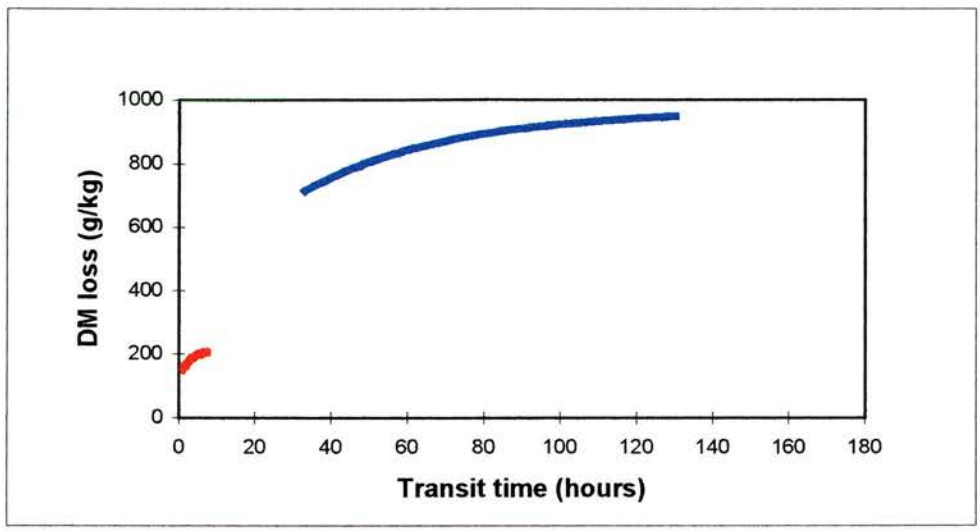
Curves obtained from fitting dry matter loss data to the equation $P = a + b\ (1 - e^{-ct})$ Ørskov and Mc Donald (1979).

Figure 3.4.3.2. Dry matter disappearances from oat hulls : naked oats contained in 6 x 1 cm polyester mesh bags having passed through the stomach and small intestine (SIB) and total tract (FB) of ponies



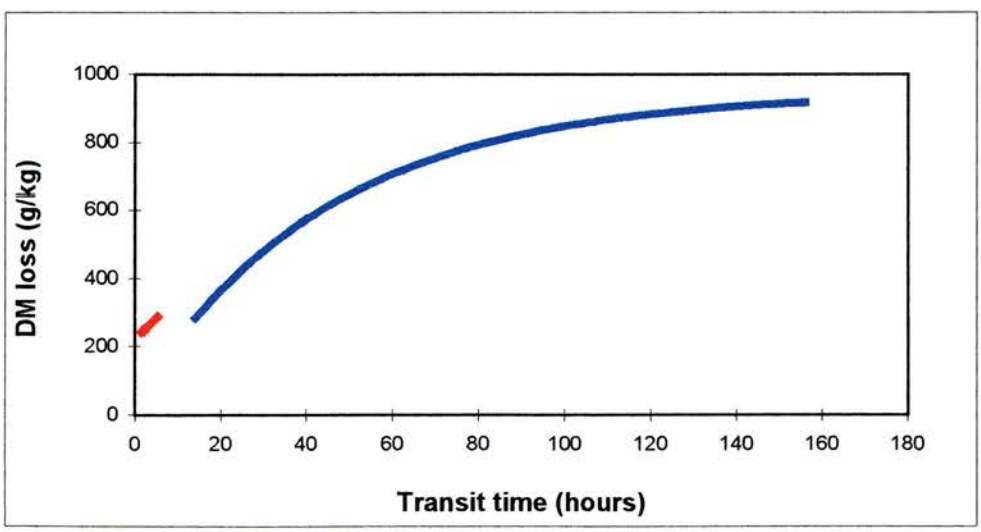
Curves obtained from fitting dry matter loss data to the equation $P = a + b\ (1 - e^{-ct})$ Ørskov and Mc Donald (1979).

Figure 3.4.3.3. Dry matter disappearances from sugar beet food contained in 6 x 1 cm polyester mesh bags having passed through the stomach and small intestine (SIB) and total tract (FB) of ponies.



Curves obtained from fitting dry matter loss data to the equation $P = a + b\ (1 - e^{-ct})$ Ørskov and Mc Donald (1979).

Figure 3.4.3.4. Dry matter disappearances from soya hulls contained in 6 x 1 cm polyester mesh bags having passed through the stomach and small intestine (SIB) and total tract (FB) of ponies.



Curves obtained from fitting dry matter loss data to the equation $P = a + b\ (1 - e^{-ct})$ Ørskov and Mc Donald (1979).

Table 3.4.3.5 Degradation curve parameters and effective degradability (ED)values calculated for 10, 20, 40 and 60 hours mean retention time, for hay cubes (HC), a 67:33 mix of oat hulls:naked oats (OH:NO), unmolassed sugar beet pulp (SBP) and soya hulls (SH) determined from residues in 6x1 cm polyester mesh bags after passing through the small intestine (SIB) and total tract of ponies (FB).

	HC	OHNO	SBP	SH	s.e.d.	Sigf
SIB						
a	29.7	34.7	12.0	22.4	N/C	-
b	2.9	65.3	9.1	77.6	N/C	-
c	0.52	0.002	0.42	0.02	N/C	-
a+b	32.7	100	21.1	100	N/C	-
FB						
a	12.3	35.7	36.8	4.2	25.54	NS
b	66.9	63.1	60.1	90.4	17.98	NS
c	0.031	0.003	0.026	0.022	0.0194	NS
a+b	79.2	98.7	96.8	94.6	11.28	NS
ED10	27.6	37.30	50.6	20.5	14.62	NS
ED20	35.0^{ab}	38.9^{ab}	58.7^{b}	31.7^{a}	10.25	*
ED40	43.1^{a}	41.8^{a}	68.1^{b}	46.1^{a}	6.80	*
ED60	47.9^{a}	44.4^{a}	73.6^{b}	55.0^{a}	5.57	*

^{abc} Values not sharing common superscripts differ significantly ($P<0.05$).
N/C = not calculated.

Table 3.4.3.6. Pre-caecal disappearance coefficients, of hay cubes (HC), a 67:33 mix of oat hulls:naked oats (OH:NO), sugar beet pulp (SBP) and Soya hulls (SH) from 6 x 1 cm polyester mesh bags collected at the ileo-caecal junction (SIB), expressed as a proportion of total tract disappearances determined from corresponding bags that had passed through the total tract of ponies.

	HC	OH:NO	SBP	SH	Sed	Sigf
DM	0.57^{b}	0.75^{d}	0.22^{a}	0.38^{c}	0.033	**
OM	0.54^{b}	0.74^{d}	0.22^{a}	0.36^{c}	0.038	*
CP	0.85^{b}	0.90^{b}	0.39^{a}	0.82^{b}	0.088	**
ADF	0.37	0.22	0.16	0.14	0.122	NS
NDF	0.20	0.02	0.18	0.14	0.140	NS
TNSP	0.09	0.10	0.15	0.14	0.171	NS
Rhamnose	0.85^{b}	0^{ab}	-0.35^{a}	-0.13^{a}	0.348	*
Arabinose	0.26^{ab}	0.36^{b}	0.12^{a}	0.14^{a}	0.080	*
Xylose	0.21	-0.14	-0.16	-0.19	0.380	NS
Mannose	0.63^{b}	0.92^{c}	0.11^{a}	0.13^{a}	0.093	**
Galactose	0.43^{b}	0.56^{b}	0.06^{a}	0.21^{a}	0.091	*
Glucose	-0.03^{ab}	0.27^{b}	-0.14^{a}	-0.17^{a}	0.147	*
Uronic acids	0.10^{ab}	-0.79^{b}	0.49^{a}	0.74^{a}	0.367	*

^{abc} Values in the same row not sharing common superscripts differ significantly ($P<0.05$).

3.4.4. Discussion

3.4.4.1. Food Composition

The NSP content and composition of the foods derived from the graminaceous plants (HC and OH:NO) were similar to those obtained in other studies, but the content of the SBP and SH varied from values quoted elsewhere. Thus, the total NSP content of 380g/kg DM for hay cubes was similar to the average 396g NSP/kg DM reported for grass meal by Bach-Knudsen (1991) and the 377g NSP/kg DM found here for the OH:NO diet was within the range of 310-408g NSP/kg DM calculated from NSP values for naked oats and oat hull meal also reported by Bach-Knudsen (1991) and Longland and Low (1991). Most of the NSP in these two foods were composed of glucose and xylose in almost exactly the same proportions recorded by Bach Knudsen (1991). The SH NSP value of 492g NSP/kg DM was somewhat lower than the range of 621-840 g NSP/kg DM reported by Longland and Low (1995), probably reflecting differences in the efficiency of the de-hulling process, although the ratios of the constituent monomers of the SH NSP observed here were similar to those of 1:1.3:0.9:0.5:6.6:2.4 for arabinose, xylose, mannose, galactose, glucose and uronic acids, reported by Longland and Low (1995). The SBP NSP values found in this study were also lower than previous reported by Bach Knusdsen, (1991), Graham, Hesselman and Åman (1986) and Longland and Low (1991). However, parallel analyses with other 'standard' sugar beet samples yielded NSP values in the region of 600g NSP/kg DM and would suggest that the values obtained here were correct for this batch of material, and possibly reflect an inefficient extraction of sucrose and other nutrients during industrial processing.

3.4.4.2. Transit time, particle size and water holding capacity

The mean transit times of the FB through the ponies for all foods tested in this study are notably longer than the 31 and 32.9 hours reported respectively by Macheboeuf *et al.*

(1996) and Tomlinson (1997). The slower transit noted here could be attributed to the metal washers, which were sealed into one end of the bags. These would have made the bags heavier than those used by Macheboeuf *et al.* (1996), resulting in them sinking to the gut floor, thereby reducing their passage rate through the gut. Although the WHC of SBP>SH>HC>OH:NO, this clearly did not have a major affect on bag passage rate as the SH bags had a significantly faster rate of passage than those containing SBP or HC.

The relative reduction in transit time of the SH FB but not the SH SIB would suggest that the increased speed of transit occurred in the large intestine. The amounts of food in the mobile bags were small, with a maximum of 7g of the experimental food passing through the gut per day, the remainder of the diet being grass nuts and hay. Soya bean products are known to contain a variety of anti-nutritional factors (Liener,1990) and Soya products have been shown to cause post-weaning diarrhoea in piglets (Heppell and Sissons, 1988). It is of note that these authors reported no increase in small intestine passage rate and inferred that an increase in large intestine motility was in some way associated with the onset of diarrhoea in the piglets. It is thus possible that components in the SH fed to the ponies in this study were present in sufficient quantities to elicit a small increase in large intestine motility but that the concentration of these components was insufficient to cause any metabolic disorders.

HC had a significantly smaller geometric mean particle size (gmps) than the other three foods, which may have influenced the DM loss noted after cold-water washing. The HC contained little CP and WSC, so losses recorded after washing machine treatment and transit through the small intestine were unlikely to be due to loss of soluble components. Particle size cannot, however, account for the DM disappearances noted from the FB. SBP had the largest mean particle size, yet the highest DM losses, whereas HC had the smallest particle size but significantly ($P<0.05$) lower DM loss than both SBP and SH. These findings are in contrast with those noted by Weakley *et al.* (1983), who recorded higher DM loss with decreasing particle size when *in situ* bags were incubated in the rumen for 1-4 hour time periods. A common Proceedingseedingsedure when using *in*

sacco techniques is to screen the food prior to filling the bags so that particle size and bag pore size are complimentary. In the study of Weakley *et al.* (1983) a smaller mesh screen than the one used in this study, would have favoured the selection of smaller particles thus the material in the bags could have been skewed towards the selection of more degradable leaf material which tends to shatter into smaller particles than plant stem (Michalet-Doreau and Cereau, 1991). In contrast, the losses recorded from the SBP, SH and OH:NO in this study could be attributed to degradation rather than loss of undegraded particles through the bag pores, since particle size was > 390gmps and significantly ($P<0.05$) higher than the 247 gmps noted for HC.

3.4.4.3. Washing machine losses.

The cold water washing of bags containing the ground food samples resulted in variable losses of some nutrients and apparent gains in others. Although Macheboeuf (1995) and Van Straalen *et al.* (1993) both stress the importance of an intensive wash to remove mucous, enzymes and microbial free nitrogen, it would appear that in this experiment such treatment was too severe. Clearly the fibre matrix was altered in some way, which improved the chemical extraction of the NDF and UAC fractions, resulting in negative values. Certainly, when partially soluble polysaccharides (such as a pectins) are placed in water, the water molecules penetrate the amorphous regions and bind to available sites, greatly reducing inter-polysaccharide associations (Birch and Parker, 1983). Such a phenomenon may have accounted for the apparent gains in NDF and UAC noted here, the washing causing partial dissolution of the polymer rendering the sample more susceptible to the extraction procedure.

The NSP analytical procedure results in the removal of protein, lipids, endogenous N and N of microbial origin, through use of pancreatic enzymes (Longland and Low, 1988). Therefore, if NSP analysis were the method of choice for determining the fibre content of foods, there would be no need for such rigorous post-incubation washing.

3.3.4.4. Losses from food in bags passing through the small intestine and total tract of ponies.

The results of the present study show that fibre composition has an important influence on the degradability of nutrients in both the small intestine and total tract of ponies.

Of the four foods examined here the highest fibre SIB and FB losses were seen from SBP, and the lowest from OH:NO. These losses suggest that the fibre in SBP is not only more degradable, but as the DM degradation curves (Figures 3.4.3.1. to 3.4.3.4.) indicate, it is more quickly degraded than the fibre in the other three foods. These findings are in agreement with the values recorded by Stefánsdóttir (1996), who measured the speed and extent of degradation of four similar fibrous foodstuffs using the *in situ* technique in four caecally-fistulated ponies. The difference in the speed and extent of degradation of SBP can again be more precisely explained by examination of the NSP components in the foods. NSP analysis showed that SBP had a very high proportion of arabinose and uronic acids and both of these monomers are known to be the most degradable fraction of plant cell walls (Van Soest, 1994). By contrast the poor degradation of the OH:NO recorded in both this study (Experiments 2 and 4) and the *in situ* study of Stefánsdóttir (1996), can be attributed to the high levels of xylose and glucose which is indicative of secondary cell wall formation. After SBP, SH was the most readily degradable food, which showed higher losses of NSP and significantly higher losses of ADF and NDF than the values noted for HC. These differences can again be attributed to botanical composition of the foods. Although glucose levels were fairly high in both SH and HC, the HC had a markedly higher xylose content, and thus a higher proportion of secondary cell wall material than SH.

Food composition clearly had a greater influence on disappearance of constituents from the total tract than the time the food remained within the gastro-intestinal tract. SBP and HC remained in the gut for 65 hours, whereas for the SH, total tract transit time (TTT) was 10.5 hours less, at 54.8 hours. Despite this significantly lower TTT for SH, the

recorded disappearances for all monomers except glucose were greater than the disappearances from HC. Thus the cell wall components of SH were more readily degradable than those of HC.

The high level of pre-caecal fibre degradation noted in pigs fed sugar beet pulp-based diets by Longland et al (1989), was not mirrored in this experiment. Foregut losses expressed as a proportion of TNSP disappearance from the FB (Table 3.4.6.) showed a maximum loss from SBP of 15% and a minimum of 9% from HC. Of the individual monomers present in significant quantities, UAC and arabinose readily disappeared, whereas xylose and glucose were poorly degraded and indeed appeared to increase. This increase is likely to be due to ingress of particles into the bags from the basal grass nut diet.

The level of pre-caecal degradation recorded in this experiment could partly be attributed to the fast rate of passage of the foods through the foregut. SBP remained in the foregut for the longest time at 4.2 hours, and although this was not significantly greater than the small intestine transit time (STT) noted for the other foods, disappearances of nutrients from the SBP in the small intestine were higher than from SH, HC and OH:NO. On the other hand the disappearances of NSP could be due to low microbial activity in the foregut, since only those foods that had a relatively high proportion of readily degradable pectin and hemicellulose such as SBP and SH, were susceptible to the limited degradation that occurred in the small intestine.

3.4.4.5. Losses of OM and CP from food in bags passing through the small intestine and total tract of ponies.

The highly significant difference in disappearances of DM and OM from the four foods can in part be accounted for by CP and water-soluble carbohydrate (WSC) losses, as none of the disappearances in terms of total fibre measurements were significantly different. The 0.54 to disappear from HC is similar to the 54% found by Smoulders *et al.*

(1990) for grass hay fed to horses. The 0.87 OM lost from the SBP bags reflects the highly degradable nature of this food, particularly of the NSP fraction, which at 0.90is very close to the 0.85-0.90 noted in pigs by Graham *et al.* (1986), Longland *et al.* (1988) and Yan *et al.* (1995).

The particularly high level of CP disappearance from OH:NO reflects the botanical source of the protein in this food. Cereal CP is known to be well digested in the upper tract of horses (Gibbs *et al.,* 1996), whereas on all roughage diets, a greater proportion of the protein digestion occurs in the large intestine. This is an important consideration when formulating rations for horses with high protein demands. In order to ensure adequate CP absorption, the diet must supply the essential amino acids to the small intestine in a readily digestible form (Hintz and Cymbaluk, 1994). This was confirmed in the present study, as the extent of pre-caecal CP digestion for SPB is minimal, whereas the extent of large intestine degradation of SPB was typical of a fibrous food. It is known that SBP contains a hydroxyproline-rich fraction within the cell wall, which may well be unavailable to proteolytic action in the small intestine, but available in the large intestine when the NSP fraction has been fermented. Although 15% of the NSP did disappear from SBP in the small intestine, clearly this was insufficient to release significant quantities of protein, which only became available after more extensive fermentation in the hindgut.

The CP disappearance of 0.52 from the HC was higher than the pre-caecal values of 0.17 reported by Gibbs *et al.* (1988) for Costal Bermuda grass hay. CP disappearances from bags that had passed through the total tract were also significantly different between this experiment and that of Gibbs *et al.* (1988) who found a DMD coefficient for Coastal Bermuda grass hay of 0.72. These differences probably reflect different types and stage of maturity of the hays used in each study. The Coastal Bermuda hay had a CP content of 117 g/kg DM, and if cut at an earlier stage of growth than the HC, before significant lignification had occurred, more protein would have been available for enzymatic digestion in the foregut.

It is conceivable that graminaceous diets result in a protein-limited environment for the hindgut microbes, resulting in sub-optimal microbial activity. Indeed, it has been noted for pigs by Longland *et al.* (1994) that supplementation of cereal diets with SBP resulted in increased cereal NSP digestibility. The addition of SBP is thought to enhance microbial activity, by supplying a readily degradable substrate, which allows cell wall degrading organisms to proliferate thereby, increasing the overall degradation capacity in the hindgut. If this is the case in the horse, there could be substantial potential for exploiting SBP or other similar foods like citrus pulp, to manipulate the nutritive value of rations for all types of horses.

3.4.5. Conclusion.

Results from this experiment show that the mobile bag technique can be successfully used to determine the AD of botanically diverse fibrous foods in ponies. Use of this technique in fistulated Animals allows digestion to be measured within different segments of the gut and improves knowledge on nutrient availability in the horse. In addition, *in sacco* techniques allow the extent of degradation over time (effective degradability) to be calculated which is very relevant to an animal that has a relatively low digesta mean retention time. Although SBP and SH were both readily degraded after prolonged residence in the gut, normal digesta mean retention times of *ca.* 36 hours indicate that when energy intake is at a premium, SBP should be the preferred food. However, SBP and HC are poor sources of available CP, so diets for animals with high protein demands should include adequate amounts of protein, which can be digested in the small intestine to ensure requirements are met. Clearly a measure of dietary protein content *per se* is insufficient for accurate ration formulation; as such a value cannot yield information on the site of CP digestion nor the availability of that CP to the animal. However, foods, which contain cell wall associated protein that is released in the large intestine, could prove useful, in that a readily fermentable substrate may boost microbial

proliferation and activity and so enable more recalcitrant foodstuffs to be degraded more effectively thereby increasing the energy obtained from the diet.

3.5. *In vitro* degradation of mature grass hay and plain sugar beet pulp using the manual pressure transducer technique.

3.5.1. Introduction

Determination of the nutrient value of animal foods *in vivo* is both costly and time-consuming and unless numerous animals are used, the number of foods that can be examined in one experiment is limited. *In vitro* systems as discussed in section 2.7 offer a rapid, repeatable and relatively inexpensive alternative to *in vivo* experiments, and have the added benefit of allowing large numbers of samples to be measured simultaneously (Lowman, 1998). Applegate and Hershberger (1969) and Trevor-Jones (1991) have successfully used the Tilley and Terry (1963) and Hershberger *et al.* (1959) techniques for determining forage digestibility in horses, reporting an R^2 value of 0.84 between *in vivo* and *in vitro* DM loss from alfalfa, Timothy grass, and oat chaff. However, these techniques are end-point determinations and unless exhaustive serial harvesting of residues is done, they do not allow the dynamics of digestion to be measured. These techniques are therefore of limited use when investigating the rate of food degradation *in vitro*.

In order to examine digestion kinetics, a dynamic system such as the gas production technique of Theodorou *et al.* (1994) can be used. This technique allows the rate and extent of DM disappearance to be determined, by recording the amount of gas produced during the fermentation of food by rumen or caecal microbial inoculum. Lowman (1998) used either caecal or faecal microbial inoculum from hay fed ponies to measure the degradation rate and DM loss, from 16 foods commonly fed to horses. She found that the fitted parameters derived from application of the France *et al.* (1993) model to the corrected cumulative gas volumes, revealed marked differences between degradation rate and DM loss, with rolled naked oats producing the fastest degradation rate, whilst the fermentation of alfalfa produced the most gas. Limited information exists on the

digestibility of many of the foods fed to horses, thus data such as that produced by Lowman (1998) on the rate and extent of food digestibility is an important contribution to the current knowledge on the nutritive value of foods for horses. Furthermore, the manual gas production technique of Theodorou *et al.* (1994) has considerable potential as a tool for deriving predictive equations for calculating the degradability of horse foods. Moreover, the flexibility of this technique, in terms of the type of foods tested and incubation times used, allows interactions and associative effects between foods to be studied and so has the potential to yield valuable information on a poorly researched area of equine nutrition.

Associative effects between plain sugar beet pulp and a conventional cereal diet for growing pigs have been reported by Longland, Carruthers and Low (1994), in 57 day-old piglets, where a digestibility of 1.03 was calculated for the sugar beet portion of the diet when fed with cereal. Additionally, the piglets had higher food conversion efficiency and did not suffer from diarrhoea, which is a common complaint encountered in early weaned piglets. Improved digestibility of hay has also been found in *in vivo* studies with ponies at Aberystwyth (Moore-Colyer and Longland, 1998) when sugar beet products were added to a basal diet of hay at the rate of 30 – 60%. This improved degradation may possibly be due to supplying the hindgut microbial population with nitrogen (only released from the cell walls of the sugar beet on fermentation, see results section 3.4.), which may stimulate microbial proliferation, and thus improve the fermentation capacity of the hindgut. An improved digestion of forage would be particularly beneficial to performance horses, enabling a greater proportion of the daily energy requirements to be derived from the forage portion of the diet and as a consequence, allow the amount of concentrates to be reduced with a concomitant decrease in the debilitating metabolic disorders detailed in chapter 1.

The objectives of this experiment were therefore three fold:

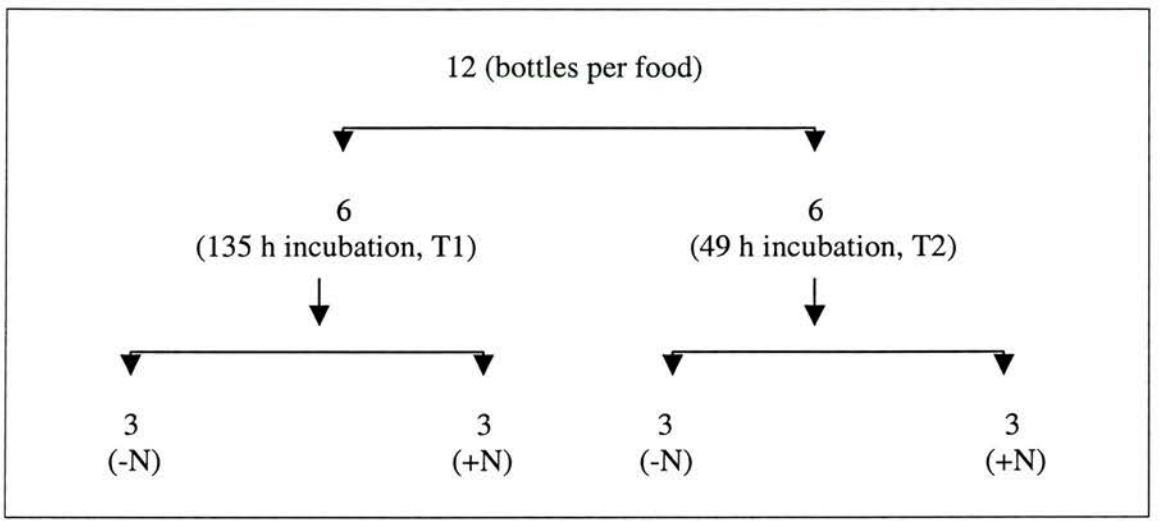
1. To measure the *in vitro* degradation of two types of fibrous foods commonly fed to horses.
2. To determine if there are any associative effects on the digestion of hay when sugar beet is added to the substrate.
3. To measure whether the presence or absence of added N influences degradation rate and DM loss *in vitro.*

3.5.2. Materials and methods

3.5.2.1. *Experimental Design*

The manual pressure transducer technique of Theodorou *et al.* (1994) was used to measure gas produced from two fibrous foods, mature perennial rye-grass hay and plain sugar beet pulp. These foods were mixed in the following ratios, 100% hay (H), 75:25 hay : sugar beet (HSB1), 50:50 hay sugar: beet (HSB2), 25 : 75 hay sugar beet (HSB3) and 100% sugar beet (SB) and fermented with microbial inoculum from pony faeces in 125 ml culture bottles for two time periods, 135 hours (T1) and 49 hours (T2). Twelve replicate bottles of each food were prepared, thus 72 bottles in total were used in the experiment, 60 containing the food samples and 12 control bottles. These bottles were then divided, (Fig. 3.5.2.1.) across two incubation times, T 1 and T 2 and two levels of nitrogen inclusion, -N (where no nitrogen was added to the culture medium) and +N (where trypticase peptone was added, at a concentration of 0.2g N per 900ml of medium).

Figure 3.5.2.1. Experimental design showing the pattern of incubation of replicate bottles between the two incubation times, 135 hours (T1) and 49 hours (T2) and for the two nitrogen treatments, ie. presence (+N) or absence of added nitrogen (-N).



3.5.2.2. *Food preparation.*

The plain sugar beet pulp (SB) used in this experiment was from Badmington Horse Foods, Oakham, Rutland, Leics. UK. The hay was a mature perennial rye grass - Timothy mix, which contained a high proportion of stem to leaf.

50g of each food was hand-chopped with scissors, shaken through a 4mm sieve and screened through a 2mm sieve to produce particles that were between 2 and 4mm in diameter. The 50g of SB was soaked overnight in 800ml of water, to mimic the normal pre-treatment of SB before it is offered to horses. This pre-soaking treatment is necessary to void any problems of choking or colic associated with stomach distension. The following morning any excess water was removed prior to an enzymatic pre-digestion.

3.5.2.2.1. Pre-digestion treatment.

The soaked SB and hay were placed into separate 2-litre glass beakers and the following procedure was performed on each food sample.

1. 20 ml of pepsin HCl solution (2g pepsin / litre 0.075M HCl) was added per g of food DM.
2. The sample mix was then incubated in an oven at 37°C for 2 hours.
3. The pH of each mixture was adjusted to pH 7 by the addition of 2M NaOH.
4. The mix was then filtered through a Buchner funnel fitted with a porosity 3 filter paper and the filtrate was discarded.
5. 1 litre of NaAc buffer was added to the neutralised sample and left to equilibrate for 20 minutes.
6. Pancrex V (Pains and Bryne Ltd, West Byfleet, Surry) was added to the buffered food at the rate of 1 capsule/5g of sample DM. The mixture was then incubated at 37°C, for 2 hours and stirred at 20-minute intervals throughout the incubation.
7. The mixture was then filtered through a Buchner funnel fitted with porosity 3 filter paper, washed with 3 volumes of water and 1 of acetone.
8. Post filtering the samples were spread-out in a thin layer on plastic trays and dried overnight in an oven at 35°C.

3.5.2.3. Preparation of culture media.

Two culture media were prepared A and B as follows:

Medium A (+N), was the modified Van Soest culture medium as described by Theodorou and Brooks (1990) and was prepared in a 2 litre flask at room temperature, by dissolving 0.2g of trypticase peptone (Becton Dickinson Microbiology Systems, BBL, Cockeysville, Maryland, U.S.A.), 0.2ml micro-mineral solution, 400ml buffer,

400ml macro-mineral solution and 2ml of resazurin solution (Difco Ltd., East Molesley, Surrey U.K.) in 1 litre of distilled water.

Medium B (-N) was prepared as described for medium A, except the 0.2g of trypticase peptone was omitted in order to prepare a low N medium.

Both media were freshly prepared before use. A flask containing magnetic followers was placed on a follower stirrer to ensure that each ingredient was fully dissolved prior to addition of the following ingredients (in the order presented). CO_2 was continually passed through the solution until the medium turned pink (*ca.* 5 hours), indicating that it was anaerobic. The composition of the buffer, micro-mineral and macro-mineral solutions are detailed in Table 3.5.2.2. These solutions were prepared in advance and stored in a refrigerator at 4°C until required.

3.5.2.4. Preparation of culture bottles

One gram of each of the foods (for the ratios used, see section 3.5.2.1) was weighed-out and placed into each of twelve replicate 125ml serum culture bottles (Phase Separations Ltd. Clwyd U.K). Each bottle was flushed for *ca.* 4 seconds with CO_2 before the addition of 85ml of either culture medium A or B from an automatic dispenser (Accuramatic 5; Accuramatic, Watlington, King's Lynn, U.K), whereupon the bottle top was immediately sealed with a rubber butyl stopper. Once all of the bottles were filled they were continually gassed with CO_2 in a fume cupboard while 4ml of freshly prepared reducing agent (2.5g Cysteine HCl, 16 ml 1M NaOH, 2.5g sodium sulphate, 380 ml distilled water) was introduced into each bottle *via* a 23-gauge X 1inch syringe (Fisher Scientific UK, Loughborough, UK). The rubber stoppers were then firmly pushed into the neck of the bottles and sealed with aluminium crimp-seals (Phase Separations Ltd. Queensbury, Clwyd, U.K). Bottles were then placed over night into a

timer-programmable refrigerated incubator set at 4°C. The incubator was programmed to increase to 39°C *ca.* 5 hours before inoculation with microbial medium.

Table 3.5.2.1. Chemical composition of the three solutions used in the culture medium.

Solution	Chemical Compound	Formula	g / 1.4 litres of distilled water
Buffer	Ammonium hydrogen carbonate	NH_4HCO_3	5.6
	Sodium hydrogen carbonate	$NaHCO_3$	49
Macro-mineral	Di-sodium hydrogen orthophosphate dodeca-hydrate	$Na_2HPO_4.12H_2O$	13.23
	Potassium di-hydrogen orthophosphate (anhydrous)	KH_2PO_4	8.68
	Magnesium sulphate 7-hydrate	$MgSO_4.7H_2O$	0.84
Micro-mineral	Calcium chloride 2-hydrate	$CaCl_2.2H_2O$	13.2g / 100ml distilled water
	Magnesium chloride 4-hydrate	$MnCl_2.6H_2O$	10g / 100ml of distilled water
	Cobalt chloride 6-hydrate	$CoCl_2.6H_2O$	1g /100ml of distilled water
	Ferric chloride 6-hydrate	$FeCl_3.6H_2O$	8 g /100ml of distilled water

3.5.2.5. Preparation of microbial medium and inoculation of bottles.

Freshly voided faeces from a Welsh section A pony gelding, maintained on a diet of Spillers Meadow-chop hay-replacer (Old Wolverton Road, Milton Keynes, UK), were collected in a pre-warmed wide-necked flask. The faeces were then immediately transported to the laboratory where they were prepared by the following procedure in the fume cupboard whilst being continually gassed with CO_2 as follows:

The faeces were placed into a string-topped plastic bag and 0.75 litres of anaerobic medium was added to the faeces under a stream of CO_2 and thoroughly pounded in a Seward 400 stomacher (Coleworth, Bedford, UK) for 3 minutes. The mixture was then filtered and squeezed through a double layer of muslin. 10 ml of this filtrate, which consisted of the microbial inoculum, was then added to each bottle using a 21-gauge syringe fitted with a 1.5-inch needle. Immediately prior to inoculation the headspace pressure in each bottle was adjusted to ambient pressure (zero reading on the LED display) using the pressure transducer (see section 3.5.2.6). Bottles were inoculated, 10 at a time, thoroughly shaken and placed in a water bath at 39°C. Once all of the bottles were inoculated, the headspace pressure was again adjusted to ambient pressure as above, and the time noted. Thereafter, all readings were taken whilst the bottles remained in the water bath.

The 10 control bottles received exactly the same treatment detailed above except that they did not contain any substrate.

3.5.2.6. Gas accumulation measurements

The readings were made using the manual pressure transducer technique of Theodorou *et al.* (1994). A three-way valve was attached to a pressure transducer containing a light emitting diode (LED) digital read-out meter. A syringe and needle was attached to one

arm of the valve and with the valve set to allow gas to flow from the bottle up the syringe to the transducer, the needle was inserted through the rubber stopper and the pressure reading on the LED recorded. The syringe plunger was then carefully withdrawn until the LED read zero, whereupon the syringe was removed from the bottle and the volume of gas it contained noted. The gas was discarded in a fume cupboard and the process repeated on the next bottle. The size of syringe and needle varied according to the volume of gas produced, so 10, 20 and 60 ml syringes were used. The transducer LED displayed pressure as lbs / square inch (psi), thus readings of 0 – 15 psi were recorded.

Gas volume and pressure readings were made *ca.* every three hours after inoculation for the first 24 hours. Thereafter, the rate of gas accumulation decreased enabling readings to be made less frequently. Readings for T 1 bottles thus took place *ca.* 3, 6, 9, 12, 15, 19, 23, 27, 31, 37, 45, 57, 69, 85, 110, and 135 hours post inoculation, whereas those for T 2 were recorded 3, 6, 9, 12, 15, 19, 23, 27, 31, 37, 45, and 49 hours post inoculation. After each reading the bottles were shaken to ensure good contact between the microbial inoculum and food substrate.

3.5.2.7. Dry matter loss

After completion of the last gas reading the aluminium crimps and butyl stoppers were removed from all of the bottles. The contents of each bottle were then filtered and washed with 20 ml of distilled water, using Buchner funnels, and previously weighed and labelled sintered glass crucibles (porosity 1, Gallenkamp, Fisher Scientific, Loughborough, U.K.). Crucibles were freeze dried until constant weights were reached and the weight of the residue noted. Apparent dry matter loss was then calculated by:

$$\text{DM loss (mg/g)} = \frac{\text{DM into bottle} - \text{DM residue in bottle}}{\text{DM into bottle}}$$

3.5.2.8. *Data Analysis*

An Excel spread sheet (Microsoft Office 2000) was used to process the pressure and volume readings from each bottle. Gas volume readings were corrected for pressure using linear regression (Theodorou *et al.*, 1994). These corrected volumes were then adjusted according to corrected volume readings from the control bottles (bottles containing inoculum and medium but no food substrate) and summed to produce cumulative gas volumes for each bottle. The maximum likelihood programme (MLP; Ross, 1987) was used to fit curves to the cumulative gas profiles using the France *et al.* (1993) model detailed below (equations 3.5.2.1 and 3.5.2.2).

$$Y = A - BQ^t Z^{\sqrt{LT}} \qquad \text{(equation 3.5.2.1.)}$$

$$Y = A - BQ^t \qquad \text{(equation 3.5.2.2.)}$$

Where:

Y = cumulative gas pool in ml

t = time in hours

A = asymptote value for gas pool size (ml)

$B = A\, e^{bT+c\sqrt{t}}$

$Z = e^{-c}$

$Q = e^{-b}$

L_T = lag time in hours

b = rate constant (h^{-1})

c = rate constant ($h^{-0.5}$)

When the initial rate of gas production was quadratic ie. the rate constant c was negative, equation 3.5.2.1 was used, whereas if an exponential rate was noted ie. when c = 0, equation 3.5.2.2 was used.

The fitted France *et al.* (1993) parameters of b, c, L_T, A, B, Q and Z, the time to reach 50% of gas produced (t_{50}), time to reach 95% of the gas produced (t_{95}), % DM loss, extent of DM loss (Ext D) and the calculated fractional rate of gas production (FRGP) obtained from equation 3.5.2.3, were all analysed *via* regression analysis using Genstat 5 (Lawes Agricultural Trust, 1993), so that the effects of nitrogen treatment, -N and +N across both incubation periods could be tested.

$$FRGP = b + (c / \sqrt{t_{50}} \times 2) \qquad \text{(equation 3.5.2.3)}$$

Associated effects on the disappearance of hay when incubated with different levels of SB were calculated from equation 3.5.2.4 (Church and Pond, 1988). These were then subjected to regression analysis using Genstat 5 (Lawes Agricultural Trust, 1993).

$$\text{Disappearance} = \frac{b - (a \times \text{fraction of a in b})}{\text{fraction of test food in b}} \qquad \text{(equation 3.5.2.4)}$$

where:

b = value for basal + test food

a = basal food

Differences between foods, treatments (-N) and (+N), and the food X treatment interaction were compared using the least significant difference test (LSD = t value for the error d.f x s.e.d.).

3.5.3. Results

3.5.3.1. Gas production

Gas production profiles of the observed cumulative gas volumes, together with the France *et al.* (1993) fitted curves from the five foods incubated for 135 hours in the presence (+N) or absence of nitrogen (-N) are shown in Figures 3.5.3.1 a and b respectively. The least gas volume was produced from H which was < amount produced from HSB1 < HSB2 < HSB3 < SB and this pattern of gas production was similar for both –N and +N treatments. Similar gas production profiles were obtained from the foods when they were incubated for 49 hours (figure 3.5.3.2. a and b), although the shape of these curves is slightly different due to the shorter incubation period, which emphasises the early stages of gas production before the asymptote was reached in all cases.

Regression analysis of the fitted parameters derived from the France *et al.* (1993) equation (see Table 3.5.3.1) showed the mean relationships between foods, ie. SB inclusion level, and parameters b, c, L_T, B,Q, Z, FRGP, t_{50} and t_{95} to be quadratic in nature. The responses of the foods, averaged across the two nitrogen treatments, showed highly significant differences ($P<0.001$) between the 4 foods, with H in particular being consistently different from the other three sugar beet : hay mixtures.

Significant differences between N treatments (T) ie. averages for all foods, was only noted for the t_{50} measurement.

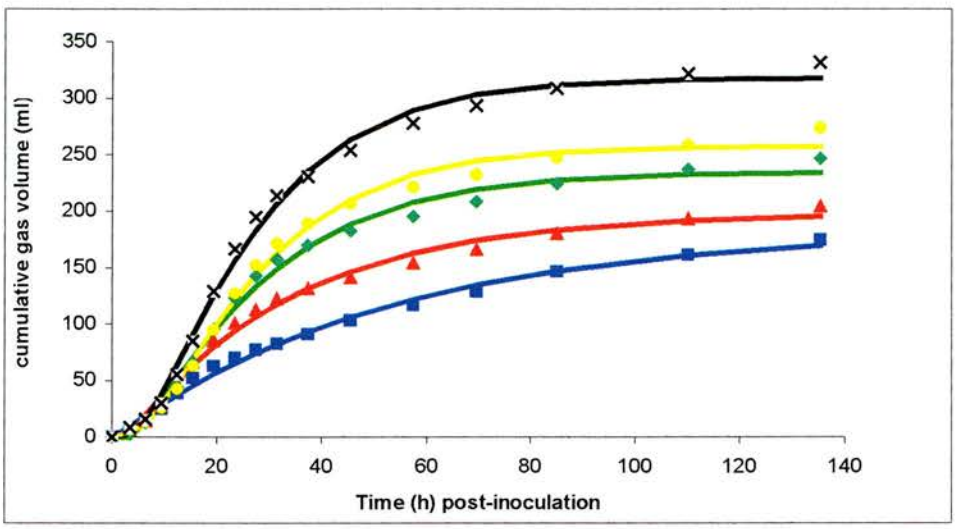
Analysis of the treatment x food interaction showed the rate parameters b (independent of time) and c (influence of which decreases with time) to be lowest for H at 0.024 / h and –0.011 $h^{-0.5}$ respectively, increasing up to 0.102 /h and –0.375 $h^{-0.5}$ respectively for the SB food, these differences were significant ($P<0.05$). Figure 3.5.3.3. a and b shows the amount of gas produced (ml) per hour from both –N and +N treatments respectively during the 135 hour incubation. The H diet showed a faster (exponential) initial rate than any of the other four food combinations, although the asymptote was attained rapidly resulting in a maximum of 3ml of gas produced per hour, which occurred in the first 2 – 5 hours post inoculation. All other food combinations showed a quadratic rate (ie. initial lag followed by a rapid rate) of gas production with 6.8 ml of gas produced per hour at *ca.* 20 hours post inoculation.

The amount of gas produced per hour (ml/h) after incubation for 49 hours at the two levels of N are shown in Figure 3.5.3.4. a and b. The initial amount of gas produced from all five-food combinations is similar up to 3-5 hours post incubation, whereupon the production of gas from the HSB combinations decreases considerably (*ca.* 5-8 hours), whereas the production of gas from the H remains constant. From 8 hours onwards the production of gas from the HSB combinations increases rapidly, reaching a peak of 6 ml at *ca.* 20-25 hours post inoculation.

A significantly ($P<0.05$) shorter lag time (L_T) was noted during the incubation of the H food (mean of 1.44 hours for both –N and +N) than for any of the other food combinations, which produced lag times averaging 3.53 hours. The total gas pool (A) was significantly different between foods but not between treatments (-N or +N) for all foods except H (-N) and HSB, thus SB produced more gas than HSB3 > HSB2 > HSB1 = H. The mean pool of gas across all foods was 217ml $\pm$16.09.

Figure 3.5.3.1. Cumulative gas production profiles from hay (—), 75:25 hay:sugar beet pulp (—), 50:50 hay sugar beet pulp (—), 25:75 hay:sugar beet pulp ( ) and sugar beet pulp (—), incubated for 135 hours with a pony faecal inoculum in the presence (a) or absence (b) of added nitrogen

a)



Each value represents the mean of three bottles, while the line indicates the profile as described by the France *et al.* (1993) model. R^2 between observed and fitted data was at least 0.992.

b)

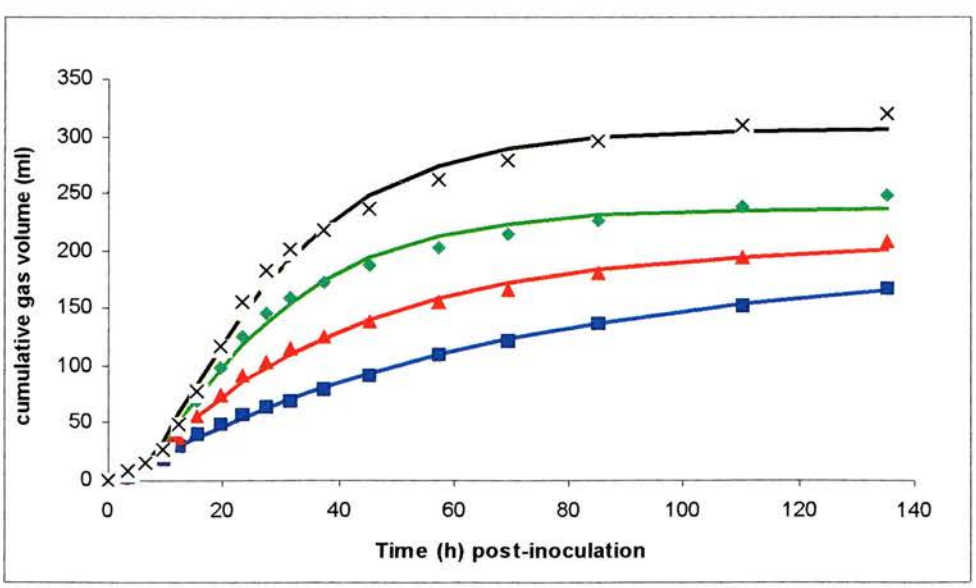
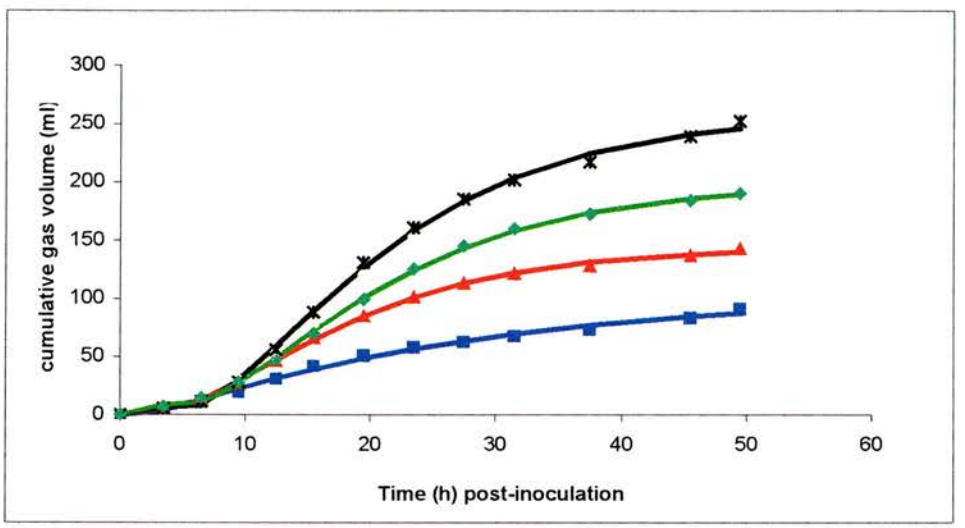


Figure 3.5.3.2. Cumulative gas production profiles from hay (—), 75:25 hay:sugar beet pulp (—), 50:50 hay sugar beet pulp (—), 25:75 hay:sugar beet pulp ( ) and sugar beet pulp (—), incubated for 49 hours with a pony faecal inoculum in the presence (a) or absence (b) of added nitrogen.

a).



Each value represents the mean of three bottles, while the lines indicate the profile as describes by the model of France *et al.* (1993). R^2 between actual and fitted data was at least 0.991.

b).

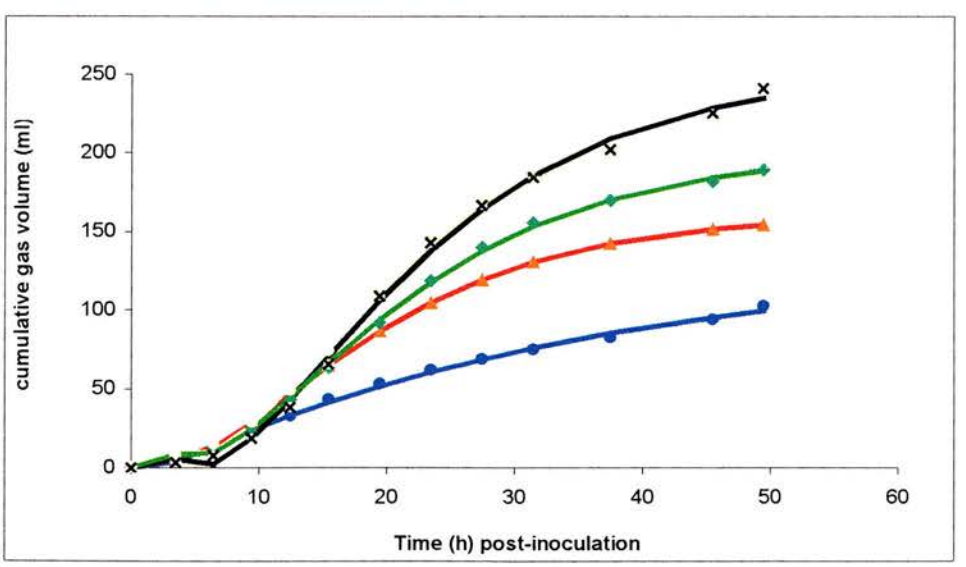
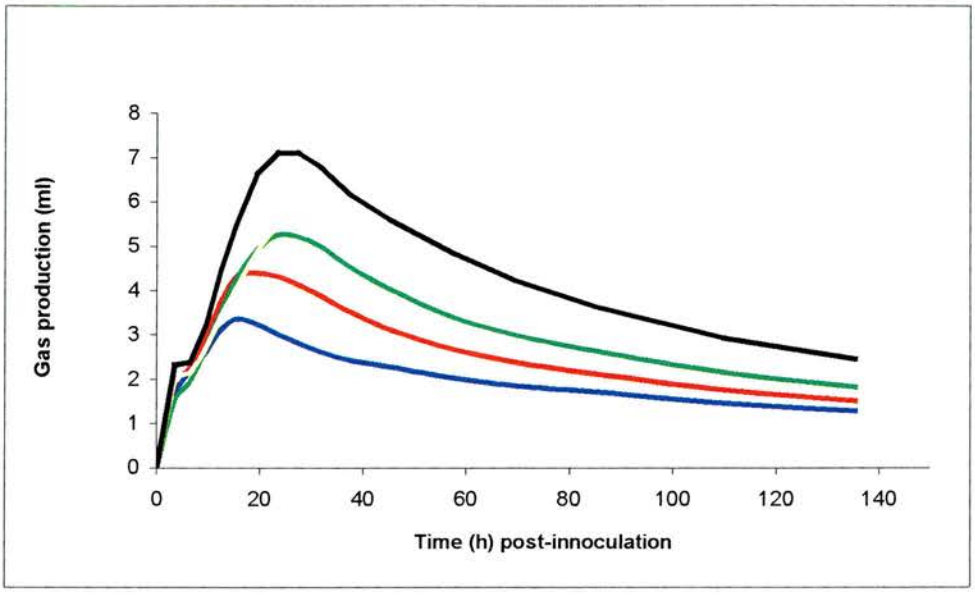


Figure 3.5.3.3. Amount of gas produced per hour (ml) from hay (—), 75:25 hay:sugar beet (—), 50:50 hay:sugar beet (—), 25:75 hay:sugar beet ( ) and sugar beet (—) when incubated for 135 hours, in the presence (a) or absence (b) of added nitrogen.

a)



b)

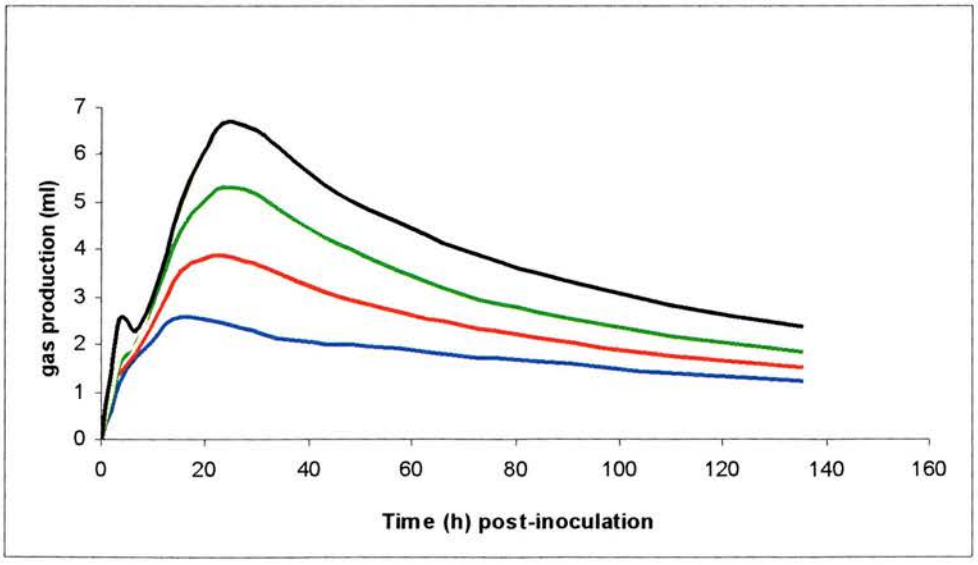
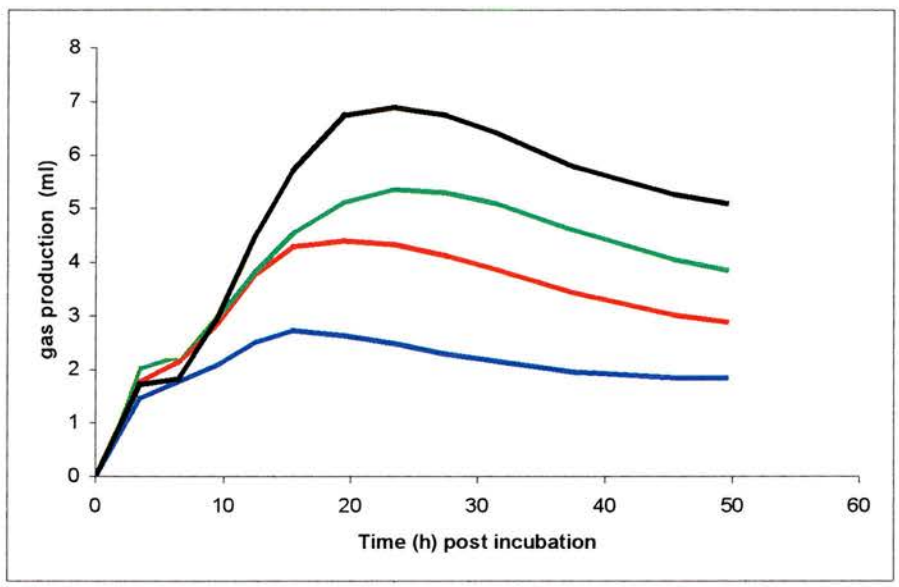
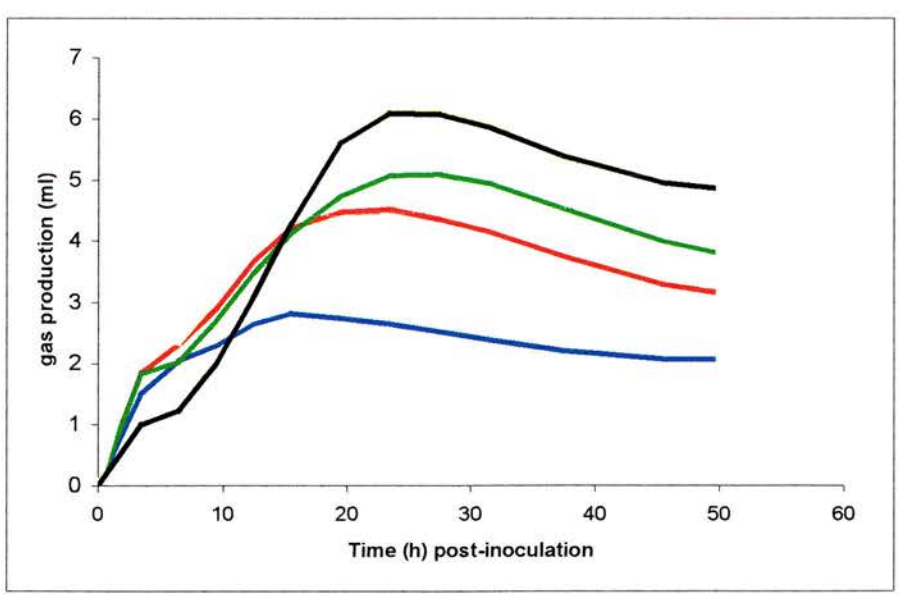


Figure 3.5.3.4. Amount of gas produced per hour (ml) from hay (—), 75:25 hay:sugar beet (—), 50:50 hay:sugar beet (—), 25:75 hay:sugar beet ( ) and sugar beet (—) when incubated for 49 hours, in the presence (a) or absence (b) of added nitrogen.

a)



b)



Fractional rates of gas production (FRGP) followed a similar pattern, with a significantly ($P<0.05$) slower rate for H compared with any of the other foods. The FRGP for H across both nitrogen treatments was 0.026 / h, whereas that of the other four substrates averaged 0.054 / h $\pm$ 0.0008. The time taken to produce 50 or 95% of the total gas, t_{50} and t_{95}, were both significantly ($P<0.05$) longer for the H food than for the other four foods. Additionally, the t_{50} for H (-N) was significantly ($P<0.05$) longer at 35.64 hours than for H (+N) at 28.98 hours. This trend was also noted for the t_{95} value, which was 117 hours for H (+N) and 145 hours for H (-N). Values for t_{50} and t_{95} for the HSB combinations were *ca.* 22 hours and 60 hours respectively.

Table 3.5.3.1. Gas production parameters derived from the France *et al.* (1993) equation, by fitting data collected during the incubation of five foods, hay (H), 75:25 hay:sugar beet (HSB1), 50:50 hay:sugar beet (HSB2), 25:75 hay:sugar beet (HSB3), and sugar beet (SB), in the presence (+N) or absence (-N) of added nitrogen, with a faecal inoculum from ponies fed Spillers meadow chop hay-replacer.
1h

	100 H	**75:25 HSB1**	**50:50 HSB2**	**25:75 HSB3**	**100 SB**	**Treat. Means**	**s.e.d and Sig.**
b (-N)	0.024^{a}	0.076^{b}	0.092^{bc}	0.098^{c}	0.095^{bc}	0.077	T s.e.d. 0.0043 (NS)
b (+N)	0.032^{a}	0.084^{bc}	0.094^{bc}	0.102^{c}	0.102^{c}	0.083	FxT s.e.d 0.0100
Food means	0.028^{a}	0.080^{b}	0.093^{bc}	0.100^{c}	0.098^{c}		(P<0.05)
			Food s.e.d. 0.0068	P< 0.001 (Q)			
c (-N)	-0.011^{a}	-0.240^{b}	-0.361^{cd}	-0.401^{d}	-0.366^{cd}	-0.276	T s.e.d 0.0258 (NS)
c (+N)	-0.023^{a}	-0.262^{bc}	-0.359^{cd}	-0.431^{d}	-0.375^{cd}	-0.290	FxT s.e.d 0.0578
Food means	-0.017^{a}	-0.251^{b}	-0.360^{c}	-0.416^{c}	-0.371^{c}		(P<0.05)
			Food s.e.d. 0.0409	P<0.001 (Q)			
L_T (-N)	1.36^{a}	3.80^{c}	3.19^{bc}	3.75^{c}	3.72^{c}	3.16	T s.e.d 0.220 (NS)
L_T (+N)	1.51^{a}	3.38^{bc}	2.60^{b}	4.10^{c}	3.68^{c}	3.05	FxT s.e.d 0.480
Food means	1.44^{a}	3.59^{bc}	2.89^{b}	3.92^{c}	3.70^{c}		(P<0.05)
			Food s.e.d 0.349	P<0.002 (Q)			
A (-N)	160.3^{ab}	184.0^{c}	218.4^{d}	257.8^{e}	279.0^{f}	219.9	T s.e.d 3.95 (NS)
A (+N)	144.1^{a}	171.2^{bc}	216.8^{d}	249.5^{e}	287.8^{f}	213.9	FxT s.e.d 8.831
Food means	152.2^{a}	177.6^{b}	217.6^{c}	253.7^{d}	283.4^{e}		P< 0.05
			Food s.e.d 6.25	P<0.001 (L)			
B (-N)	164.5^{abc}	163.0^{abc}	157.0^{ab}	175.6^{bcd}	188.4^{cd}	169.7	T s.e.d 5.71 (NS)
B (+N)	147.1^{a}	151.4^{ab}	160.9^{ab}	162.8^{abc}	196.1^{d}	163.6	FxT s.e.d 12.84
Food means	155.8^{a}	157.2^{a}	158.9^{a}	169.2^{a}	192.2^{b}		P<0.05
			Food s.e.d 9.03	P<0.03 (Q)			
Q (-N)	0.976^{c}	0.929^{b}	0.912^{ab}	0.907^{a}	0.910^{a}	0.927	T s.e.d 0.0040 (NS)
Q (+N)	0.969^{c}	0.920^{ab}	0.911^{a}	0.904^{a}	0.904^{a}	0.922	FxT s.e.d 0.0089
Food means	0.972^{c}	0.924^{b}	0.912^{a}	0.905^{a}	0.907^{a}		P<0.05
			Food s.e.d 0.0063	P<0.001 (Q)			
Z (-N)	1.012^{a}	1.305^{b}	1.456^{bc}	1.516^{c}	1.518^{c}	1.361	T s.e.d 0.0359 (NS)
Z (+N)	1.024^{a}	1.345^{b}	1.465^{bc}	1.578^{c}	1.530^{c}	1.388	FxT s.e.d 0.0803
Food means	1.018^{a}	1.325^{b}	1.460^{c}	1.547^{c}	1.524^{c}		P<0.05
			Food s.e.d 0.0568	P<0.001 (Q)			

Table 3.5.3.1. Cont.

FRGP (-N)	0.023[a]	0.048[b]	0.053[bc]	0.055[bc]	0.053[bc]	0.046	T s.e.d 0.0016 (NS)
FRGP (+N)	0.029[a]	0.053[bc]	0.054[bc]	0.055[bc]	0.057[c]	0.050	FxT s.e.d 0.0035
Food means	0.026[a]	0.051[b]	0.054[b]	0.055[b]	0.055[b]		P<0.05
			Food s.e.d 0.0025	P<0.001 (Q)			
t_{50} (-N)	35.64[c]	23.27[a]	22.26[a]	22.41[a]	23.68[a]	25.45[a]	T s.e.d. 0.905 (P<0.04)
t_{50} (+N)	28.98[b]	21.15[a]	21.94[a]	23.52[a]	21.96[a]	23.51[b]	FxT s.e.d 1.999
Food means	32.31[b]	22.21[a]	22.10[a]	22.97[a]	22.82[a]		P<0.05 (L)
			Food s.e.d 1.431	P<0.001 (Q)			
t_{95} (-N)	145.3[d]	76.9[b]	61.1[ab]	60.0[ab]	62.2[ab]	81.1	T s.e.d 3.98 (NS)
T_{95} (+N)	117.4[c]	70.5[ab]	61.7[ab]	63.4[ab]	58.5[a]	74.3	FxT s.e.d 8.91
Food means	131.4[c]	73.7[b]	61.4[ab]	61.7[ab]	60.3[a]		P<0.05 (L)
			Food s.e.d 6.30	P<0.001 (Q)			

Rate parameters (b and c); fractional rate of gas production (FRGP); gas asymptote (A); lag time (L_T); $Q = e^{-b}$; $Z = e^{-c}$; t_{50} and t_{95} time taken to produce 50 and 95% of the total gas pool.

[abc] Values within the same parameters not sharing common superscripts differ significantly (P<0.05);

s.e.d = standard error of difference.

NS = not significant.

T = Treatment; FxT = Food x Treatment interaction;

(L) = significant linear relationship

(Q) = Significant quadratic relationship.

3.5.3.2. Dry matter loss

Table 3.5.3.2. DM loss (mg/g) and extent of degradation (Ext D) obtained from hay and plain sugar beet food when incubated in the presence (+N) or absence (-N) of added nitrogen, with faecal inoculum from ponies fed Spillers meadow chop hay-replacer.

	100 H	**75:25 HSB1**	**50:50 HSB2**	**25:75 HSB3**	**100 SB**	**Treat. Means**	**s.e.d and Sig**
DM loss							
(-N) %	39.8[a]	51.9[b]	61.9[c]	75.8[d]	86.6[e]	63.2	T s.e.d 1.13 (NS)
(+N)%	37.5[a]	50.9[b]	60.9[c]	76.1[d]	85.5[e]	62.2	FxT s.e.d 2.52 P<0.05
Food Means	38.6[a]	51.4[b]	61.4[c]	76.0[d]	86.1[e]		
		Food s.e.d 1.78 P<0.001 (L)					
Ext. D							
(-N)	14.2[a]	23.8[b]	29.2[c]	35.6[d]	39.1[e]	28.4	T s.e.d 0.61 (NS)
(+N)	15.3[a]	24.7[b]	28.7[c]	34.9[d]	40.7[e]	28.9	FxT s.e.d 1.37
Food Means	14.8a	24.3b	28.9c	35.2d	39.9e		P<0.05
		Food s.e.d 0.967 (Q)					

[abc] Values in the same row not sharing common superscripts differ significantly (P<0.05)
T = treatment; FxT = Food x Treatment interaction.
NS = not significant.
(Q) = significant quadratic relationship
(L) = significant linear relationship.

The presence (+N) or absence (-N) of added nitrogen in the culture medium did not affect the % loss of DM from any of the foods. However, the difference in loss between each food was significant (P<0.05) with SB losing *ca.* 10% more DM than HSB3 which in turn lost 10% more than HSB2 which was 10% > HSB1 which was 10% > H. The

extent of degradation (Ext.D), which is the DM loss value adjusted for food passage rate (*ca.* 3% per hour) produced a similar trend when incubated with the two treatments +N and –N, with SB > HSB3 > HSB2 > HSB1 > H.

3.5.3.3. Gas production and dry matter loss from hay when fermented with sugar beet pulp.

The FRGP from hay (see Table 3.5.3.3) when fermented alone was significantly lower ($P<0.05$) than the rate produced when SB was present HSB1 (+N), HSB2 (+N and –N) and HSB3 (+N and –N) in the bottles. The highest FRGP from the hay fraction was recorded from the HSB3 –N combination of 0.061/ h, and this rate was significantly different ($P<0.05$) from H for both (+N) and (–N) treatments. No significant differences were noted between the (–N) or (+N) treatment within foods, thus the addition of SB increased the FRGP from hay, but the presence or absence of added nitrogen had no effect on fermentation rate.

Loss of DM (mg/g) from the H fraction of the HSB food combinations was variable, with no clear pattern emerging as to the influence of the added SB on the degradation of hay. Similarly, the Ext.D of the H fraction when fermented with different levels of SB was variable with significant ($P<0.05$) differences occurring between HSB3 (–N) and HSB3 (+N), and H (+N) and (–N).

Table 3.5.3.3. Fractional rate of gas production (FRGP), % dry matter (DM) loss and Ext.D from hay (calculated by difference) when fermented with three levels of sugar beet pulp in the presence (+N) or absence (-N) of added nitrogen (-N),with faecal inoculum from ponies fed Spillers meadow chop hay-replacer.

	100 H	**75:25 HSB1**	**50:50 HSB2**	**25:75 HSB3**	**Treat. Means**	**s.e.d and Sig.**
FRGP (-N)	0.023^{a}	0.047^{abc}	0.054^{bc}	0.061^{c}	0.046	T s.e.d 0.0062 (NS)
FRGP (+N)	0.029^{ab}	0.052^{bc}	0.052^{bc}	0.051^{bc}	0.046	FxT s.e.d 0.0124
Food means	0.026	0.049	0.053	0.056		P<0.05
			Food s.e.d 0.0087 P<0.06 (Q)			
DM loss (-N) mg/g	39.8^{a}	40.3^{ab}	37.3^{a}	43.6^{ab}	40.2	T s.e.d. 2.03 (NS)
(+N)mg/g	37.5^{a}	39.4^{a}	36.3^{a}	48.0^{b}	40.3	FxT s.e.d 4.07
Food means	38.6^{a}	39.9^{a}	36.8^{a}	45.8^{b}		P<0.05
			Food s.e.d 2.87 P<0.05			
Ext D (-N)	14.21^{a}	18.75^{ab}	19.25^{ab}	25.10^{b}	19.33	T s.e.d 1.641 (NS)
(+N)	15.32^{a}	19.42^{ab}	16.75^{a}	17.50^{a}	17.25	FxT s.e.d 3.282
Food means	14.76^{a}	19.08^{ab}	18.00^{ab}	21.30^{b}		P<0.05
			Food s.e.d 2.321 P<0.05			

^{abc} values in the same section not sharing common superscripts differ significantly (P<0.05 or greater); NS = not significant

T = treatment; FxT = Food x Treatment interaction

(Q) = significant quadratic relationship

3.5.4. Discussion.

3.5.4.1. Gas Production

The gas production profiles of the H and SB (Figs 3.5.3.1. and 3.5.3.2) demonstrate the differences between H and SB which were slowly and rapidly degraded respectively, with the HSB mixtures giving gas volumes between the two extremes, directly reflecting the step-wise increment of the addition of SB to the H. These gas profiles also show that the manual pressure transducer technique of Theodorou *et al.* (1994) was sufficiently sensitive to detect differences in food composition, and these differences were reflected in the rates and quantity of gas produced. Additionally, the faecal inoculum used in this experiment, gave gas production profiles and rate parameter values similar to those recorded by Lowman (1998) thereby further demonstrating the suitability of the *in vitro* system for determining the degradation kinetics of horse foods.

The initial rapid rate of gas production seen for the H diet may be partly attributed to the specificity of the microbial inoculum. The donor animal had been fed a diet of Spillers Hay Replacer, which is a low-dust forage containing similar grass species to those found in hay. Therefore, the micro-organisms voided with the faeces would have been composed of a microbial population adapted to degrade hay. Equine faeces contain a high number of viable microbes (Uden and Van Soest, 1982) and in the present study these would have quickly started the fermentation of the readily degradable fraction in the H food thus initiating a rapid rate of gas production. Such an initial rapid rate of fermentation was not seen when SB was added to the bottles suggesting the microbes needed a period of adaptation to this substrate. The L_T recorded for the HSB food combinations and SB was *ca.* double the L_T seen for the H food indicating that fermentation was initially slower when SB was present. Once the fermentation had started, the rate was rapid, although this rate dropped *ca.* 5 hours post inoculation before rising rapidly again. This rise-drop-rise sequence was also evident in

the cumulative gas production profiles recorded by Lowman (1998) when naked oats were incubated with rumen fluid from hay-fed steers. Lowman attributed the uneven gas production of the first 3 – 4 hours, to be due to the microbes adapting to their new environment. In the present study, the initial rise was probably due to the fermentation of sucrose or other water-soluble carbohydrates from the SB. Once this readily available substrate was depleted, the fermentation rate decreased until the microbial population had adapted to the new substrate, that was rich in pectin and the hemicellulose arabinose, which are minor components of grass hay (Longland *et al.*, 1995).

The b and c rate parameters together with the FRGP were higher for all foods when incubated in the presence of added nitrogen (+N) although none of these differences were significant. In contrast, the linear relationship for the t_{50} and t_{95} values for H were significantly ($P<0.05$) different between +N and –N treatments, with the –N treatments taking longer to attain 50 and 95% of the total gas produced. This together with the slightly higher, although non-significant, FRGP for the (+N), may suggest that the addition of nitrogen, did have a positive effect on fermentation rate. Although the –N treated bottles were not devoid of N as they contained 162 mg NH_3 per litre, and undoubtedly the inoculum contained microbial protein, the addition of 0.2g of N per 900 ml of medium was enough to have a positive effect. The discrepancy between the significant t_{50} and non-significant FRGP may be explained by examining the two-treatment design of the experiment and the relative influence of the c rate parameter at the two incubation times. The regression analysis blocked the two incubation times of 135 and 49 hours and concentrated on the two nitrogen treatments. The results presented in Table 3.5.3.1 are therefore average values for treatment, food x treatment and food means across both incubation times. Rate parameters b and c are determined by fitting the France *et al.* (1993) model to the cumulative gas volumes. The model is constructed so that the influence of the c rate parameter decreases with time. However, when the incubation period is reduced to 49 hours, the relative influence of the c rate is

much greater than when the incubation period runs its normal time course of *ca.* 140 hours. Thus, the rate of gas production for a short incubation period is calculated to be much higher than the gas production profiles indicate, due to the premature cessation of the incubation period. This occurred here, with the c parameter falsely elevating the FRGP for the 49-hour period thereby causing a wide spread of data and consequently making differences in N treatment difficult to detect. Although the t_{50} and t_{95} (+N) and (–N) values are also averages of both incubation times, these values are calculated from direct arithmetic means, without placing undue emphasises on the shorter incubation time, thus the regression analysis was able to detect significant differences between treatments for t_{50}, whereas only the trend is seen for the FRGP.

The FRGP recorded from any of the HSB food combinations, or the SB alone, did not produce a significantly higher rate of gas production with the +N treatment. Even at the relatively low inclusion of SB ie.HSB1, lack of additional N did not affect fermentation rate, suggesting that SB contained sufficient N to allow the rapid degradation of the substrate to be maintained.

Equine faeces contain a high proportion of N (Uden and Van Soest, 1982) so it is possible that the faecal inoculum itself contained sufficient N to maintain initial rates of microbial growth and regeneration without the need for additional N. However, the H only food did benefit from the addition of N, suggesting that degradation of mature, 'stemmy' fibrous foods could be enhanced if additional N were supplied.

3.5.4.2. DM loss

In contrast to the gas production rate parameters, the (+N) or (–N) treatments did not increase the DM loss from any of the foods tested in this study, indicating that added N was not a limiting factor in substrate degradation. DM loss, and Ext. D. between each food was significantly greater ($P<0.05$) with each incremental addition of SB reflecting

the gradual increase of more readily degradable food within the bottles. The degradation of the H and SB at 39% and 87% respectively, agree well with the *in vivo* data recorded in Experiments 1 and 4, and suggest that in these studies *in vitro* determination of DM loss by the pressure transducer technique was an accurate reflection of the observed *in vivo* DMD. Morrow (1998) recorded *in vitro* DM losses from hay incubated with a pony faecal inoculum of between 49 and 51%, these values are considerably higher than those recorded in this experiment and are principally due to the quality of the hay incubated. In the experiment conducted by Morrow (1998) the hay used was a leafy well preserved *Lolium perenne* forage, while in the present study, the hay was very mature and contained a high proportion of stem to leaf. As the fibre content of forages increased Olsson and Rudvere (1955) noted that the OMD by equids became progressively lower, thus the lower DM loss observed in the present experiment may well be attributed to the low nutritive quality of the substrate. The DM losses of the SB food in this experiment of *ca.* 87 % agree well with the 86% reported by Lowman (1998) when SB was incubated with pony caecal fluid for 72 hours.

The results obtained here indicate that using pony faeces as the source of microbial inoculum produces gas production profiles similar to those obtained when caecal fluid is the microbial inoculum source. Additionally, the *in* vitro gas production technique produces DM degradation data consistent with pony *in vivo* values for both readily and less degradable fibrous foods.

The effect of incubation time on DM loss showed notably higher losses occurring from the 135 h compared with 49 h from all the HSB food combinations, but the SB alone had a similar loss *ca.* 85%, when incubated for both time periods. This demonstrates that SB is a rapidly degraded food and these results agree well with the *in vivo* data recorded in the mobile bag experiment (Experiment 4) where *ca.* 80 % of food present in the bags were lost during the 50-hour MRT in the gut. Furthermore, losses of 0.88 from un-molassed sugar beet pulp were recorded by Hyslop *et al.* (1997) after *in situ*

incubation for 48 hours in a pony caecum, and this loss was significantly greater than the 0.67 noted for hay cubes. These results suggest that the *in vitro* gas production technique may be used to measure the speed and extent of degradation of both rapidly and slowly degraded fibrous foods and thus has considerable potential as a method for determining the nutritive value of a variety of equine foods. However, before this technique can be used as a routine tool for horse-food evaluation, the *in vivo* digestibility of a wide variety of foods must accompany *in vitro* experiments so that accurate validation of *in vitro* results can be performed.

3.5.4.3 Degradation of hay when incubated with sugar beet pulp

When the FRGP for the H portion of the food without added nitrogen, was calculated by difference from the HSB combinations, an increase of 2.3 and 2.6 times the amount of gas produced by the H was recorded when 50 and 75% respectively of the food in the bottle was SB, compared with the rate recorded for the H fermented alone. These results suggest that the FRGP cannot be calculated from multiplying the % of food present by the FRGP when that food is fermented alone. This is in contrast to the findings of Lowman (1998) and Newbold (1996) who did not find any associative effects when foods were incubated together, and thus suggested that FRGP could be accurately predicted by multiplying the proportion of food present with the FRGP when the food was fermented alone. The results of the present experiment indicate the importance of measuring fermentation rate when foods are offered together, rather than relying on arithmetically derived data. However, when nitrogen was added to the culture medium (+N) the addition of SB had no effect on the FRGP. This suggests that when N is limited the SB food provides the necessary N to maintain a high rate of fermentation of H, thus SB has a positive associative effect on the fermentation rate of poor quality hay.

Chesson (1990) suggested that additional levels of readily degradable structural carbohydrates such as pectins and hemicelluloses found in sugar beet pulp could maximise the degradability of poor quality forages. He suggests that this is achieved because the SB provides the readily degradable primary cell wall material while the forage (straw or hay) provides the secondary cell wall material, so the microbial population are supplied with a diet which is close to the 'ideal' ratio of 2 : 1 primary to secondary cell walls. Thus the addition of SB to the H in this experiment could have provided the microbes with both additional N and readily fermentable carbohydrates, which in some way increased microbial fermentation and subsequently improved the degradation of the poor quality H forage.

No clear pattern in DM loss from the H fraction of the substrate when incubated with varying levels of SB emerged from this experiment. This could possibly be due to the experimental design discussed earlier, or the nature of the substrate in the bottles. Although considerable care is taken to ensure that similar substrate is placed into each bottle, when dealing with forage particles it is difficult to ensure that the proportion of leaf to stem is uniform between bottles. It is therefore possible that some bottles contained more readily degradable H particles than others, thus contributing to the variation in DM degradation.

When the Ext.D of the hay portion of the diet was analysed for the effect of SB and N the only significant difference was between H (-N) and the H portion when fermented in the HSB3 (-N) combination. This again demonstrates the fact that SB is improving the fermentation of H to an extent that DM loss when corrected for outflow rate, is *ca.* 10 mg/g greater than when H is fermented alone.

3.5.5. Conclusions.

Results from this experiment on the degradation of hay and sugar beet pulp agree well with the *in vivo* data recorded in Experiments 2 and 3 of this thesis and with previously published *in vitro* data and confirm that sugar beet pulp is rapidly degraded by equine hindgut micro-flora. Additionally, when sugar beet or nitrogen is added to an *in vitro* system containing hay an improvement in the degradation of hay is noted. This may be attributed to an increase in microbial activity stimulated by the presence of readily available N. This experiment also shows that the use of pony faecal inoculum produces consistent results on food fermentation kinetics when employed with the manual pressure transducer technique of Theodorou *et al.* (1994) and further indicates that this technique has considerable potential as a routine method for determining the degradation of equine foods.

Chapter 4. General Discussion

The work presented in this thesis investigated the suitability of a range of botanically diverse fibrous foods as high-energy alternatives to hay in rations for horses. The experiments conducted covered a broad area of research, relating the chemical composition of these foods and the influence of digesta mean retention time (MRT) to their degradability *in vivo*. The results highlight some of the problems associated with feeding different dietary fibre sources to ponies, and relate these findings to recommendations for levels of inclusion in rations for stabled horses. In addition, the suitability of several techniques, originally developed for measuring apparent digestibility in ruminants, were tested in ponies. This chapter summarises the factors that influence the degradation of fibre foods, discusses the potential application of *in sacco* and *in vivo* techniques as replacements for total collection trials in ponies, and finally, offers the main conclusions of this thesis.

4.1. Factors influencing the degradation of fibrous foods in ponies.

There are several chemical, physical and biological factors that influence degradation of fibre foods in ponies, all of these must be considered when compiling rations for horses engaged in different activities.

4.1.1.Chemical composition.

Of the ten fibrous foods examined in the experiments presented above, SBP had the highest AD value for all parameters measured, followed by SBF > CS > SB:HC > BB > SH > HY > HC > OH:NO > H. These results indicate that a wide variety of fibrous foods provide a better source of digestible energy, than the traditional forage of hay. Thus all of them were > 9MJ DE/kg DM, whereas hay contained considerably less at only 5.7MJ/kg DM. The AD values obtained can be related to the chemical composition

of the fibre in each food. NSP analysis allows a more detailed examination of the potentially energy yielding fraction of fibre, the cell wall carbohydrates, than gravimetric procedures, and helps to explain why foods with similar NDF contents, such as SBF (516 g/kg DM) and H (529 g/kg DM), are degraded to different extents, so much so, that SBF with an NDFD coefficient of 0.74 is twice as degradable as H which has a NDFD of 0.33. Ben Ghedalia and Rubinstein (1985) suggest that foods, containing high amounts of secondary cell wall material are less degradable than foods largely composed of primary cell walls. This was found to be the case in the present study, where SBF (Experiment 3.2) which contained 7 g xylose/ kg DM and 154 g glucose kg/ DM, had a TNSPD of 0.87, whereas the H (Experiment 3.1) which contained 93 g/kg DM xylose and 261 g/kg DM of glucose had a TNSPD of 0.41.

Detailed examination of the NSP profile of the other fibre foods fed in Experiments 3.1, 3.2 and 3.4 indicate the same trend. Thus CS, BB and SH are all readily degradable due to their high primary cell wall content, whereas the HC and OH:NO NSP was poorly degraded due to their high secondary cell wall content. The quantification of the individual carbohydrate components of the fibre fraction of cell wall material by NSP analysis makes this method of fibre analysis superior to gravimetric techniques and shows potential for inclusion as factors in equations developed to predict the digestibility of fibrous foods by horses and ponies.

Cell wall composition, which Mertens (1973), suggested influences voluntary intake (VFI) of grasses, could not explain why the intake of CS and SBP in Experiments 3.1 and 3.2 respectively were significantly lower than the other foods offered to the ponies in these experiments. Both CS and SBP contained the highest proportion of primary cell wall material out of the ten foods offered, yet their DMI's were significantly lower than the intakes recorded for the other foods. A detailed discussion of the possible reasons behind these lower intakes can be found in section 3.1.4.1 and 3.2.4.1, but clearly gut-fill characteristics and energy intake were not major factors limiting DMI,

since the ponies consumed large amounts of HY, BB and SH. Present results would seem to indicate that although pony LW was not adversely affected by the low intakes of SBP, this food should not be offered as the sole dietary ingredient to stabled horses if normal consumption patterns are to be maintained. Although the DMI of CS was low, and two out of the four ponies did lose weight, the FWI was not significantly different from BB or HY, therefore the time spent eating was not compromised. However, until the reason behind the low DMI is determined it is recommended that CS does not form the sole constituent of the diet.

The total tract values obtained of CPD for SBP would again suggest that this food is a good replacement for hay in pony rations. However, as detailed in section 3.4.4.4, a high proportion of this protein was only released when significant microbial degradation of the cell walls had occurred in the large intestine and thus the CP in this food could not be utilised for growth and production by the animal. However, the CP appears not to be 'wasted' as the microbial population in the hindgut can utilise it for growth and regeneration. Indeed, increased CP supply to the micro-flora may have accounted for the increased digestibility of H, which was seen *in vivo* in Experiment 3.2.B and *in vitro* in Experiment 3.5. In contrast, small intestine digestibility of OH:NO CP was high, confirming the recommendations of Gibbs *et al.,* (1988) that to ensure CP availability to the horse, a cereal source of CP should be fed. Small intestine digestion of SH and HC CP were also quite high, being > 50%, suggesting that these foods were good sources of available CP. Although gut-partition studies were not performed on the silages fed in Experiment 3.1, the NSP profiles of BB and CS in particular, showed that these forages contained a high proportion of readily degradable cell wall material. Since these foods, like the HC, were derived from the *Gramineae*, it seems reasonable to assume that digestibility of their CP in the small intestine would also be high, making these two forages superior foods to hay, in terms of energy and CP availability, for inclusion in horse rations.

4.1.2. Physical factors affecting food degradation.

In their experiment on comparative digestion of Timothy *(Phelum pratensis)* fibre by ruminants, equids and rabbits, Uden and Van Soest (1982) suggested that the lower degradation of hay fibre by horses compared with ruminants may be attributed to the shorter digesta MRT recorded in the horses. Pearson and Merritt (1991) and Cuddeford *et al.* (1995) also emphasised the importance of MRT when they compared the digestion of fibre foods in ponies and donkeys, attributing the greater AD attained by the donkeys to a longer digesta MRT. The time digesta remains in contact with the microbial population clearly influences the extent of degradation of fibre components within the fermentation chamber. In Experiment 3.4. MRT was a significant factor in determining the DMD of the fibre foods studied. The effective degradability (ED) obtained for each food using the Ørskov and McDonald (1979) equation, confirmed the above findings, as the degradation of HC, OH:NO, SBP and SH all increased with increasing residence time.

However, although MRT clearly had an effect on the extent of degradation of the fibre foods studied here, the chemical composition of the foods exerted a greater influence on AD than the residence time within the gut. This was demonstrated by the DM loss from bags containing SH (Experiment 3.4), which showed a significantly greater DM disappearance than bags containing HC, yet the HC bags had a total tract MRT of 10 hours longer.

4.1.3. Microbial factors affecting fibre degradation.

The results from Experiment 3.4. suggested that microbial fermentation of dietary fibre in the foregut of ponies is strictly limited, with maximum TNSPD coefficients recorded

from SBP of 0.13. The most degradable fractions of the fibre were the uronic acids with a digestibility coefficient of 0.48. These losses are only half that noted for foregut NSP degradation in pigs by Longland *et al.*, (1989), so that microbial activity in the equid foregut appears to be significantly less than that found in the pig.

Caecal fermentation parameters measured in Experiment 3.2. when the ponies were consuming HC, OH:NO, SBF and SB:HC were similar to those recorded by Goodson *et al.* (1988) and Willard *et al.* (1977). The acetate:propionate:butyrate profile obtained from the caecal digesta when ponies were consuming the OH:NO diet, altered the ratio of 80:15:5 found in horses fed fibre-based diets (Hintz *et al.,* 1971) to 73:23:4, and this together with the significant rise in intra-caecal lactate and corresponding drop in pH suggests that the 1.75 g of starch/ kg LW/ meal offered in the OH:NO was very close to the maximum of 2 g starch/kg LW/meal recommended by Kienzle *et al.,* (1992) which can safely be fed before metabolic acidosis occurs (Radicke *et al.,* 1991). All of the other fibrous foods, except the SH and SB offered in the experiments reported here were of graminaceous origin, and although caecal fermentation parameters were not measured for all the foods, it is reasonable to suggest that VFA lactate and pH production from these foods would be similar to those recorded when HC was fed.

In all of the *in vivo* studies reported here, the ponies had a two-week adaptation period on each diet followed by a five-day collection period, which should have ensured that the hindgut micro-flora had become adapted to the new diet prior to the nutrient balance studies. However, results from Experiment 3.2B and 3.5 would seem to confirm the comments of Uden and Van Soest (1982) who suggested that the fibrous nature of the substrate arriving in the large intestine will affect substrate-specific microbial proliferation. This may have affected the extent of fibre fermentation, because an increase in degradation of hay fibre was noted *in vivo* and *in vitro* when SBP was fed or fermented with hay, which was presumably due to an increase in microbial activity. This associated effect could be due to an increased delivery to the hindgut of readily

degradable nitrogen or primary cell wall material, both of which are readily utilised by the microbial population for growth and synthesis, which in turn increases the overall fermentative capacity of the micro-flora. Moreover, the initial speed of degradation recorded in Experiment 3.5. when hay and sugar beet pulp were inoculated with equine faeces may well demonstrate the specificity of the hindgut microbial population. The faecal inoculum which came from a pony fed Spillers meadow chop hay-replacer, initiated a fast degradation rate in the hay diet, whereas a 3 to 4 hour lag was clearly evident before degradation of the sugar beet occurred, indicating that time was required for either the production of enzymes capable of degrading SB, or for the proliferation of microbial species which can utilise SB-type substrates.

4.2. Application of ruminant techniques for measuring apparent digestibility in ponies.

The marker, mobile bag and *in vitro* techniques adapted for use in ponies in Experiments 3.3, 3.4, 3.5, produced AD values that were in broad agreement with the standard *in vivo* AD Experiments 3.1 and 3.2, and with values reported by other workers (Lowman, 1998; Morrow, 1998; Pearson and Merritt, 1991).

In the mobile bag studies, the FB containing HC and SBP showed a greater DM loss than the degradation values recorded for these foods in Experiment 3.2, indicating that loss from a bag does not necessarily equate with *in vivo* degradation. Experiments 3.4 and 3.2 were not performed simultaneously, nor, due to unforeseen circumstances, were the same ponies used for both studies. Accordingly, seasonal and individual variation could have accounted for some of the noted discrepancies between the two studies. However, differences are more likely to be due the difference in mean retention time in the two studies, and the manner in which the faecal bag samples were bulked. The FB AD values were determined from bulked samples compiled from all of the bags, which had an average TTT of 64 hours. This meant that the food contained in the bags remained in the tract longer than when the corresponding test food was fed to the ponies

in Experiment. 3.2. when an average transit time of 27 hours for HC and 37 hours for OH:NO was recorded, thus yielding elevated AD values for food in FB compared with *in vivo* values. ED values which were determined for DM loss only were markedly lower at ED 40 (the time chosen in light of the 27-37 MRT determined for the HC and OH:NO in Experiment 3.3) than the value reported for the FB again illustrating that the longer MRT (64 h) of bags, allowed an increased degradation of bag contents. The advantage of *in sacco* studies over the more traditional *in vivo* total collection trial, however, is the opportunity *in sacco* studies offer for determining degradation kinetics. The ED values for HC at 40 hours estimated the degradation to be slightly lower than the actual degradation observed in Experiment 3.2. This ED calculation (equation 3.3.2.2.) derived a large value for parameter c, the rate at which the potentially degradable substance is broken down, suggesting that although material was leaving the bag very quickly, it was not being degraded at a similar rate. These anomalies indicate that although the mobile bag technique appeared to work well, there were still some shortcomings with this the use of this technique in ponies.

Knowledge of the degradation kinetics of a foodstuff is of particular benefit when dealing with animals whose digesta passage rate can alter according to diet (Janis, 1976). In horses such knowledge is particularly useful when used with ileally or caecally fistulated animals, which can yield essential information on the digestibility of CP in the small intestine of the horse.

Despite the difficulties encountered in the rate of passage study, (Experiment 3.3.) (due to poor acceptance of SBF) the results show that Cr and Yb can be successfully used to determine rate of passage of digesta through the gastrointestinal tract of ponies. From the models used it appears that the gamma-dependent models of Pond *et al.* (1988), which incorporate a time-dependent phase, best described the flow of HC and OH:NO thorough the equine gut. Markers, like *in sacco* studies, offer the opportunity to measure degradation kinetics, and potentially have the added advantage of determining the

passage through the different segments of the gut without the need for fistulated animals. Although the models used produced good agreement (high R^2 between observed and fitted data) perhaps the flaw in trying to compartmentalise the gut arose from using a model derived for ruminants to describe flow through a monogastric gut. Moreover, compartmentalising the gastrointestinal tract using faecal excretion data may not be a sufficiently sensitive approach to allow biologically meaningful results to be derived. However, insufficient foods and ponies were used in this study to enable any conclusions to be drawn on the biological interpretation of the flow rate through the different segments of the tract.

The use of equine faeces in the gas production technique of Theodorou *et al.* (1994) produced gas profiles, similar to those obtained by Lowman (1998). DM loss from SBP determined at 49 hours was similar to the loss of SBP from the faecal bags in Experiment 3.4, although slightly higher than the degradation recorded *in vivo* in Experiment 3.2. Rate parameters *in vitro* also indicate that SBP was degraded quickly whereas the more recalcitrant hay was degraded comparatively slowly. The extent of degradation *in vitro* was calculated using a rumen MRT of 50 hours, which, based on results from Experiment 3.3 does not reflect digesta MRT (27-37 hours) in the horse. However, the *in vitro* technique has been developed for ruminants and uses culture media and buffer appropriate for digesta entering the rumen having been chewed with saliva, rather than digesta which has undergone enzymatic digestion in the small intestine, as is the case in the horse.

4.3 Modifications to ruminant in vivo and in vitro techniques for future use in equids.

Although all of these techniques show promise as relatively rapid, reliable and non-invasive methods for determining AD and MRT (the marker method) in ponies, they still require further modification before they can be adopted as routine methods for evaluating the nutrient value of foods for equids.

4.3.1 Modifications to the mobile bag technique

The mobile bag technique requires validation with total collection trials so that losses such as the 133 g /kg TNSP recorded from bags passing through the small intestine of the ponies (Experiment 3.4) can be corrected for any over or under-estimation of disappearances, which can be due to particle loss from the bags (Cherian *et al.,* 1989), or ingress of material from the basal diet (Tomlinson, 1997). To do this effectively digesta samples need to be obtained directly from the gut, necessitating the use of fistulated ponies. Surgical modification of animals is less than ideal, both from an ethical and practical standpoint. On the other hand a full understanding of the availability of protein to the horse, and the contribution of different types of food to the energy balance of the animal, is only likely to be achieved by the perusal of such studies.

Modification of the mobile bag technique for total tract studies, using intact animals, requires manipulating the size and weight of the bags, so that their passage through the tract is closer to actual digesta passage rate. Proper validation of this *in sacco* technique is also required, which involves deriving a correction factor obtained from testing a wide range of fibrous foods *in vivo* and *in sacco,* before it can be used as a routine method for evaluating the AD of fibre foods in ponies. The mobile bag technique has considerable potential as a rapid method for determining the degradation kinetics (Cherian *et al.,* 1989) of food *in vivo*. Before this technique can be widely employed, however, a less invasive method of bag administration, such as placing bags in the back of the mouth using a dosing gun, should be investigated so that many more foods could be tested in a wide range of animals.

4.3.2. Development of digesta rate of passage studies in equids using external markers.

Although the use of Cr and Yb produced clear faecal excretion patterns from the ponies, the passage rate of more foods need to be measured, in order to determine if digesta passage rate through the equid gut is a time-dependent or time-independent process. Additionally, mathematical models considering the anatomy of the equid digestive tract need to be developed, so that a clearer understanding can be gained on the movement of digesta through the different compartments of the equid gastrointestinal tract. Such information would greatly facilitate ration formulation, allowing a greater understanding of the physiological effects of feeding different dietary ingredients in combination so that energy supply can be optimised without compromising digestive health.

4.3.3. Development of the *in vitro* gas production technique for determining the nutritive value of horse foods.

Modifications to the gas production technique of Theodorou *et al.,* (1994) are required if the conditions in the bottles are to mimic the *in vivo* environment in equids. The culture media and buffer, which are presently compiled to mimic ruminant saliva, need to be altered to mirror the pH and chemical composition of the chyme entering the equid caecum. Additionally, the mathematical modelling of the gas profiles should employ mean retention times (*ca.* 27 to 37 hours) reflecting the residence time of digesta within the gastrointestinal tract, thereby enhancing the accuracy of the extent of degradation calculations. Finally, as with the other methods used in this study, the *in vitro* technique requires validation with *in vivo* data so that the nutritive value of foods for horses can be routinely determined using this relatively cheap and rapid technique.

4.4 The potential of in vivo and in vitro techniques for predicting the nutritive value of horse foods.

The results presented here indicate that both the mobile bag and *in vitro* techniques show promise as rapid methods for evaluating the nutritive value of fibrous foods for equids and should be further developed so that accurate rations can be compiled for horses engaged in a wide range of activities. However, a fast, reliable method for predicting the nutritive value of a range of botanically diverse horse-foods is required if ration formulation for horses is to be generally improved. The NSP analysis has been demonstrated here to be more definitive than the gravimetric techniques, so that the application of NSP analysis together with *in vivo* physical parameters such as MRT and effective degradability values, should allow prediction equations to be developed by using food chemical characteristics and *in vitro* gas production parameters. Although the gas production technique is more rapid than *in vivo* trials, it is a time consuming procedure, and ideally an *in vitro* technique such as NIR or FTIR (transformed infra red spectroscopy) to determine the AD of horse foods would be preferable. However, NIR and FTIR require validation with *in* vivo data and the mobile bag technique may offer a rapid alternative to the total collection trial for this purpose. Development of the gas production technique for equids is also worth-while, as to maximise the use of NIR, the calibration data set would require continual up-dating, which could be more rapidly performed using gas production than *in vivo* experiments.

4.5 Conclusions

All of the foods studied in this thesis contained more readily available energy and crude protein than conventional hay fodder, and as such are suitable high-fibre foods for stabled horses. Results indicate however, that neither SBP nor CS should be used as the sole dietary ingredient as DMI may be compromised. However, BB, HY, HC, OH:NO,

HC:SBP, can all be used as complete replacements for hay in horse rations and could provide the answer to many of the metabolic disorders encountered by horses, by increasing the energy density of the fibre portion of the diet, thereby allowing a reduction of starch-based concentrates in the ration.

Ruminant techniques for determining AD can be successfully used in ponies to determine the nutritive value of botanically diverse fibrous foods. The techniques applied yielded novel information on the digestibility of dietary protein from a range of fibrous foods and the contribution these foods make to the energy balance of the animal. Additionally the effect of combining two fibrous foods, sugar beet and hay, produced improved *in vivo* and *in vitro* degradability values, indicating an enhancement in hindgut micro-flora activity. Combining the data presented in this thesis on AD, MRT and associative food effects, should allow for a more accurate matching of dietary ingredients to the consumption times and energy demands of individual horses and should aid the process of ration formulation for horses.

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APPENDIX 1. Chemical analyses.

Acid detergent fibre (ADF)

The ADF analysis involved refluxing 2 g of sample for 1 hour with 100 ml acid-detergent solution containing 2 % Cetyltrimethylammonium bromide (CTAB) in 0.5 M sulphuric acid. Solutions were then filtered through porosity 1 sintered-glass crucibles, and washed twice with hot water and once with acetone, until the filtrate ran clear. Crucibles + sample were oven-dried at 100°C over night, cooled and weighed.

Neutral detergent fibre (NDF)

NDF analysis involved refluxing 0.5 g of sample for 1 hour with neutral detergent solution containing 3% sodium lauryl sulphate, disodium Ethylenediaminetetraacetic acid (EDTA), sodium borate, disodium hydrogen phosphate and 2-ethoxyethanol. The residues were then placed in pre-weighed, numbered sintered glass crucibles (porosity 1) and rinsed successively with hot water and acetone. Crucibles + sample were oven-dried overnight at 100°C, then cooled and weighed.

Crude protein (CP)

CP analysis was performed on 0.1 g samples which were digested using acid as per the method of Faithfull (1969) and subsequently analysed for nitrogen *via* the modified Kjeldahl method described by Faithfull (1971), the resulting value for nitrogen content was multiplied by 6.25 to obtain the value for CP.

Calcium, Phosphorus and Magnesium (Ca, P Mg)

Ca and P were determined using the method of Faithfull (1971). This involved an acid digest followed by successive dilutions with concentrated sulphuric acid and water, followed by atomic absorption spectrophotometry. Mg was similarly determined, but using the appropriate lamp in the atomic absorption spectrophotometer (Faithfull, 1974).

Starch

Starch was determined by the following method: Freeze-dried food samples (0.1 g) (milled to pass 1 mm mesh screen) were extracted in a beaker for 30 minutes with 10 ml 10% v/v ethanol to dissolve sugars, dextrins and tannins. It was centrifuged at 1500 gs for 5 mins and the residue washed into McCartney bottles, graduated at 5 ml, with M HCl and made up to the mark with HCl. The bottles were then capped and heated to 106^{o}C for 40 mins, then cooled and the contents added to 50 ml water. The pH was then adjusted to 3.0 +/- 0.2 and made up to 100ml. 10 ml aliquots were made up to 50 ml with saturated benzoic acid solution prior to analysis by the WSC method [adaptation of the Auto-Analyzer method of Yemm and Willis (1954)]. The starch content of the sample was obtained by multiplying by 5000.

Gross energy (GE)

Gross energy (GE) was determined using a Gallenkamp Autobomb Automatic Bomb Calorimeter CB-110. The energy was determined by burning the sample in an adiabatic bomb, pressurized with oxygen and placed within an enclosed constant weight water reservoir (calorimeter) The temperature increase of the water in the calorimeter was measured and the energy value determined using the following equation:

GE = (T1 – T2) x BF / SWt joules per gram

Where:

Sample weight in g = SWt

Initial temp ^{o}C = T1

Final temp ^{o}C = T2

Bomb Factor *BF

* The bomb factor depended on the quantity of water in the calorimeter and the thermal capacities of the bomb, calorimeter etc.

The bomb factor was determined by using two 0.2g tablets of certified benzoic acid of known energy value (26,454 joules per gram).
Non-starch polysaccharides (NSP)

Determination of non-starch polysaccharide content in food and faeces.

The NSP procedure was performed in four steps, 1) de-starching, 2) acid hydrolysis, 3) preparation of alditol acetate derivatives. 4) Gas chromatography for NSP analysis Each sample was done in triplicate.

1). De starching.
150 mg of sample was placed into 50 ml screw top glass centrifuge tubes and a magnetic stirrer added to each tube. 2ml of dimethyl sulphoxide was added and the mixture boiled for 1 hour in a water-filled beaker. 8 ml of sodium acetate buffer[1] was then added and the mixture allowed to cool to *ca.* 40°C before addition of 50µl of Termanyl (an α-amylase) which was pre-boiled in a test tube for 10 mins. The mixyure was then re-boiled for 1 hour. After cooling to 55°C Pullulanase [2] (Sigma Chemicals Co) and 0.15 ml Pancrex [3] (Pains and Bryne Ltd, West Byfleet, Surry) were added. This was than allowed to incubate overnight at 39°C.

Next morning 40 ml of absolute ethanol (ETOH) was added to each test tube and inverted to ensure good mixing. This was then incubated at 0°C for 1 hour. Tubes were then
centrifuged at 1500 g for 15 mins; the liquid was then aspirated, before adding 50 ml 85% ETOH and spinning at 1500 g for 15 mins. This procedure was repeated three times. For the last wash 50 ml acetone was used instead of ETOH. The test tubes were placed in water bath at 65°C to evaporate remaining acetone.

[1] (0.1 M pH 5) prepared by dissolving 13.6 g Na acetate trihydride in 950 ml distilled water, adjusting pH to 5.2 by addition of 0.1 mol l^{-1} acetic acid and add 4 ml of 1 mol l^{-1} CaCl make up mixture to 1 litre by addition of distilled water.

[2] 1:100 dilution

[3] 2 capsules in 9ml water spin for 10 mins, use supernatant.

2). Acid hydrolysis

2 ml 12 M sulphuric acid was added to each test tube and vortex before incubation at 35°C for 1 hour. 22 ml distilled water was added the test tubes placed in a water bath and boiled for 2 hours using a hot plate with a magnetic stirrer. The mixture was then cool rapidly by placing in cold water. The tubes were then stored over night at 4°C.

3). Preparation of alditol acetate derivatives

An internal standard ie. 5ml Allose (Sigma), (1 mg dissolved in 50% saturated benzoic acid) was added to each test tube and mixed using a Vortex. 1ml from each sample was then transferred into fresh test tubes and 0.2 ml 12 M ammonium hydroxide (Sigma) added. The alkalinity, (if necessary add more ammonium hydroxide) of each mixture in the individual test tubes was checked using pH paper. 0.1 ml freshly prepared ammonium hydroxide / sodium borohydride [4] was then added followed by 1 – 5 µl octan-2-ol antifoaming solution (Sigma) each tube was then given a Vortex before being incubated at 40°C for 1 hour

Post incubation 0.1 ml glacial acetic acid was added and the tubes given a Vortex. The mixture in each test tube was then checked for acidity, before transferring 0.5 ml of each sample into fresh screw-top test tubes. The following 2 chemicals were than added, 0.5 ml 1-methylmidazole, 5 ml acetic anhydride, and the test tubes given a thorough mixing using a Vortex. The test tubes were then left at room temperature for 10 mins. Before

adding 0.9 ml absolute ETOH, mixed using a Vortex, left for a further 5 mins at room temperature before adding 5 ml distilled water. Test tube + the test tube rack were placed into an iced-water bath before adding 5 ml 7.5 M KOH, followed by another 5 mins break before addition of another 5 ml 7.5.M KOH and left in the water bath for 10 mins.

200µl of phase was then drawn off and placed into labelled gas chromatography (GC) vial (Vials Direct Ltd.,Macclesfield, UK) and a cap immediately placed onto each test tube.

[4] Solution of 3 M ammonium hydroxide containing 50mg sodium borohydride per ml.

4).Gas Liquid chromatography for NSP analysis

A varian 3400 chromatograph fitted with an automatic dispenser (Varian 8000) and a flame ionisation detector, linked to a Dell PC fitted with Dionex A1-450 integration software, was used to quantify individual NSP monomers. The Chromatograph was fitted with a glass column (2.1 m x 2mm internal diameter) packed with Supelco (100/120 mesh) coated with 3% SP 2330. 2 µl of sample was automatically injected from each sample. Nitrogen was the carrier gas maintained at a pressure of 30-35 psi. The injector and detector operated at 250°C and the column was maintained at 225°C.

Vials were arranged on the automatic dispenser so that 2 external standards were sampled first, followed by 10 – 15 samples and then another 2 standards etc.
External standards were prepared as follows.
0.25 ml calibration mixture [5] + 0.25 ml allose + 0.5 ml 2 M sulphuric acid. These were neutralised, reduced and derivatised (step 3 above) in the same procedure as the samples.

[5] 100 mg rhamnose + 200 mg arabinose + 500 mg xylose + 100 mg mannose + 100 mg galactose + 700 mg glucose were added to 100 ml of 50% saturated benzoic acid.

Uronic acid determination

The uronic acids were determined by the spectrophotometric method of Scott (1979). 0.3 ml of supernatant was taken from acid hydrolysis stage and 0.3 ml of sodium chloride / boric acid mixture [6] added. 5 ml concentrated sulphuric acid was added and the mixture mixed using a Vortex. Test tubes were then incubated at 70°C for 40 mins. Whereupon they were cooled by placing in a cold water bath. 0.2 ml of dimethylphenol solution (0.1 g of 3,5 dimethylphenol dissolved in 100 ml glacial acetic acid) was added before mixing using a Vortex.

10 – 15 mins later the absorbance at 400nm and 450 nm was read in a spectrophotometer against a water reference. Standard solutions[7] were read before and after sample readings.
The reading at 400nm was subtracted from that at 450nm for each sample. The concentrations were then calculated using the standard curve of absorbance against uronic acid concentration obtained from the standards, through a linear regression procedure on a calculator.

[6] dissolve 2 g of sodium chloride and 3 g boric acid in 100 ml of water.
[7] Glucoronic acid (made up in distilled water) at the following concentrations; 25, 50, 75, 100 and 150µl ml^{-1}. 0.3 ml of each standard was placed in a testube and treated as per samples.

Expression of results.

The amount of each neutral sugar in g kg^{-1} were calculated using the following formula

$$\% \text{ sugar} = \frac{Ws \times At^* \times 100}{As \times Wt}$$

* corrected for response factor

R.F. = Area of internal standard / are of test sugar in calibration mix.

Ws = weight of internal standard.

At and As = the peak areas for the test solution and the internal standard respectively.

Wt is the weight (mg) of the test sample taken for analysis.

Total non-starch polysaccharide = rhamnose x 2 + arabinose + xylose + mannose + galactose + glucose + uronic acids (from acidic sugar determination Scott, 1979)

APPENDIX 2.

Composition of mineral and vitamin supplement:-

Element	amount (g/kg DM)
Ca	160
P	117
Mg	67
Na	67
	amount (mg/kg)
Cu	683
Zn	2730
Fe	2730
Mn	2730
I	6.7
Co	6.7
	amount (i.u.)
Vit. A	136670
Vit. D_3	20500
Vit. E	3417

APPENDIX 3.

Determination of geometric mean particle size.

$$dgw = \log^{-1} \left[\frac{\Sigma Wi \log đi}{\Sigma W} \right]$$

$$Sgw = \log -1 \left[\frac{\Sigma Wi(\log đi - \log dgw)^2}{\Sigma W} \right]^{0.5}$$

dgw = geometric mean diameter

Wi = weight fraction on ith sieve

đi = geometric diameter of particles on ith sieve

ΣW = total sample wt

$đi = (di \times (di + 1))^{0.5}$

(geometric mean particle size is particle size at which 50% of the total is recovered)

di = diameter of screen openings of ith sieve

di + 1 = diameter of screen openings of the next larger to ith sieve

Sgw = geometric standard deviation

APPENDIX 4.

Preparation of ytterbium (Yb) marked food.

Yb solution was prepared by dissolving 10 g Yb (111) chloride hexahydrate, 99% (Aldrich Chemical Company) in 1 litre of distilled water. The pH of the solution was measured and if required, adjusted to 3.8 using 0.1M hydrochloric acid. Approximately 100 g of each foodstuff was weighed out and suspended in 1 litre of the Yb solution for 24 hours. The food was then removed and washed hourly for 6 hours with distilled water. Post-rinsing foods were dried in an oven at 50°C for 48 hours.
15 minutes prior to the 09:00-hour food the Yb labelled food was mixed with a little molasses and offered as a voluntary oral-dose to the ponies.

APPENDIX 5

Preparation of chromium (Cr) marked food.

Food preparation:

125 g of milled foodstuff (ground to pass a 1mm mesh) was suspended in *ca.* 2 litres of 3% sodium lauryl sulphate and boiled for 3 hours. The suspension was then filtered through a nylon stocking and thoroughly washed in tap water. A final wash was done using acetone and then the fibre was spread out on a tray and dried at 65°C.

Mordant preparation:

The dried food was re-suspended in a solution of sodium dichromate, which contained an amount of Cr equivalent to 14% of the fibre weight, eg. HC with *ca.* 70% NDF:-

Wt of fibre = 125 X 0.7 = 88g

Therefore the amount of sodium dichromate used was 88 X 0.14 X 298/100 = 35g

After ensuring thorough mixing the solution was transferred to an enamel dish covered with aluminium foil and baked in an oven at 100°C for 24 hours.

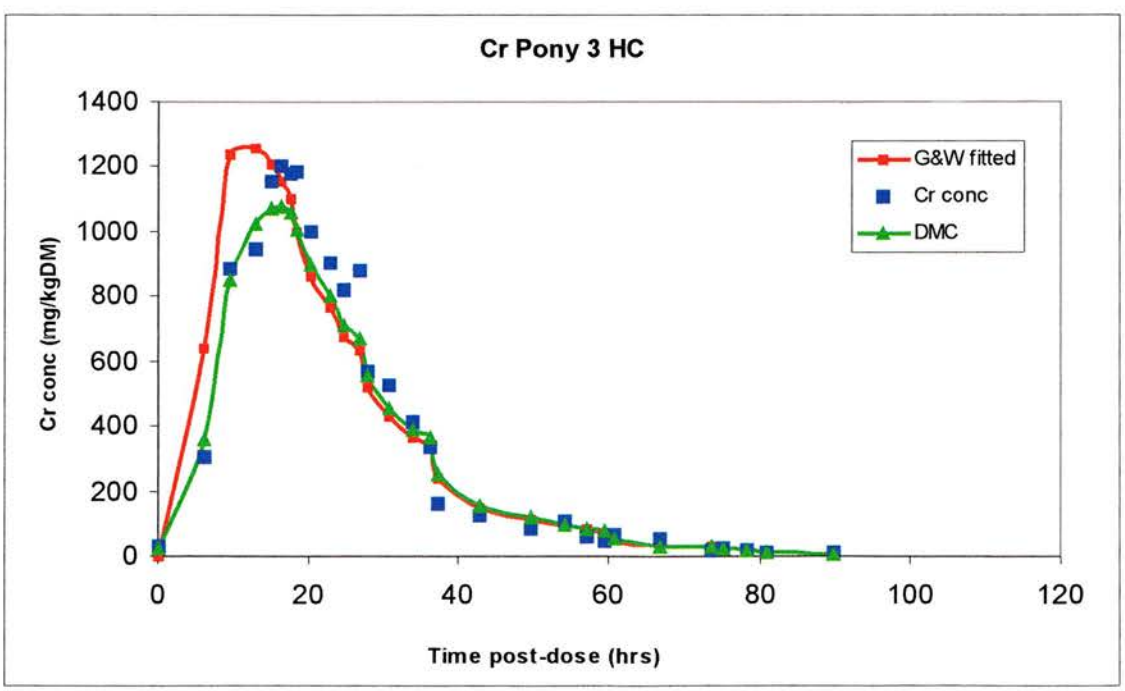
The fibre was then filtered through a nylon stocking and thoroughly washed with tap water. The fibre was then suspended in tap water and 0.5 times the weight of the fibre in ascorbic acid was added, ie. 44g. This was mixed and allowed to stand at room temperature for 1 hour. The fibre was then washed through a nylon stocking using tap water until all the green soluble matter has been washed off. The sample was then dried at 65°C and stored in plastic bags until administered to the ponies.

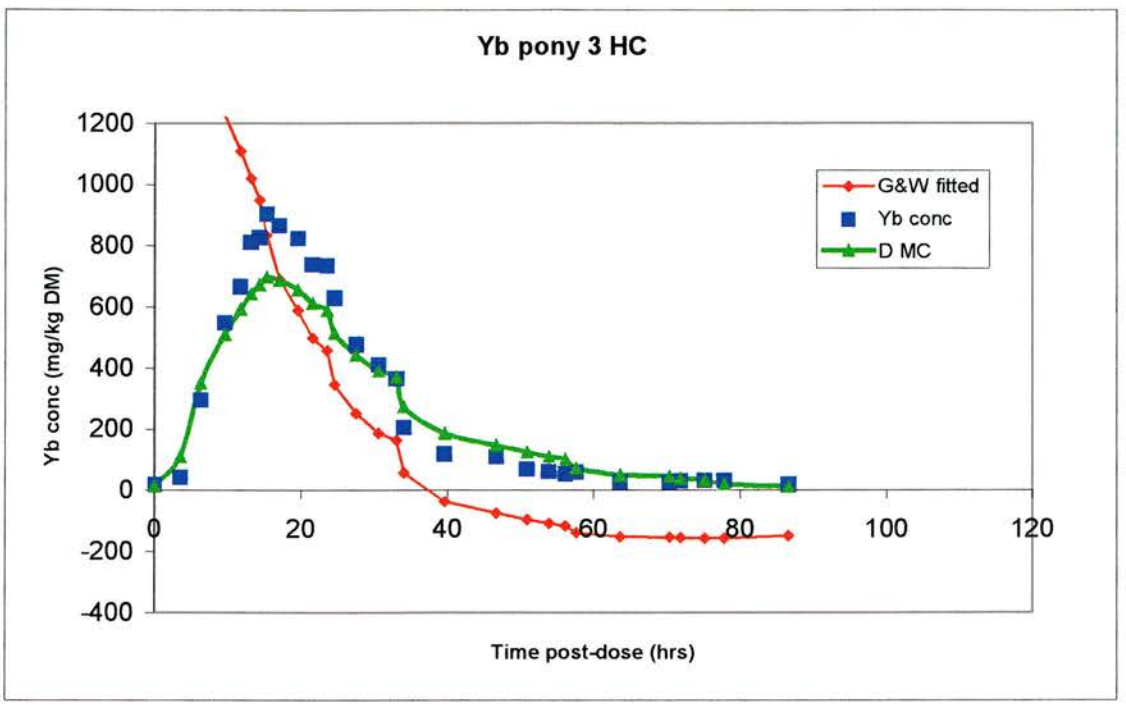
APPENDIX 6. Experiment 3.3.

Faecal excretion curves for chromium and ytterbium marked food for individual ponies.

Key :

Yb ytterbium marked food
Cr chromium marked food
GW Grovum and Williams (1973) two-compartment time-independent model
DMC Dhanoa *et al.* (1985) multi-compartmental time-independent model
G1 gamma 1 time-independent model of Pond *et al.*(1988).
G2 gamma 2 time-dependent model of Pond *et al.*(1988).
G3 gamma 3 time-dependent model of Pond *et al.*(1988).
G4 gamma 4 time-dependent model of Pond *et al.*(1988).
HC hay cubes food
OH:NO a 67:33 mix of oat hulls:naked oats food
SB:HC a 50:50 mix of sugar beet : hay cubes food

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Time post-dose (hrs)
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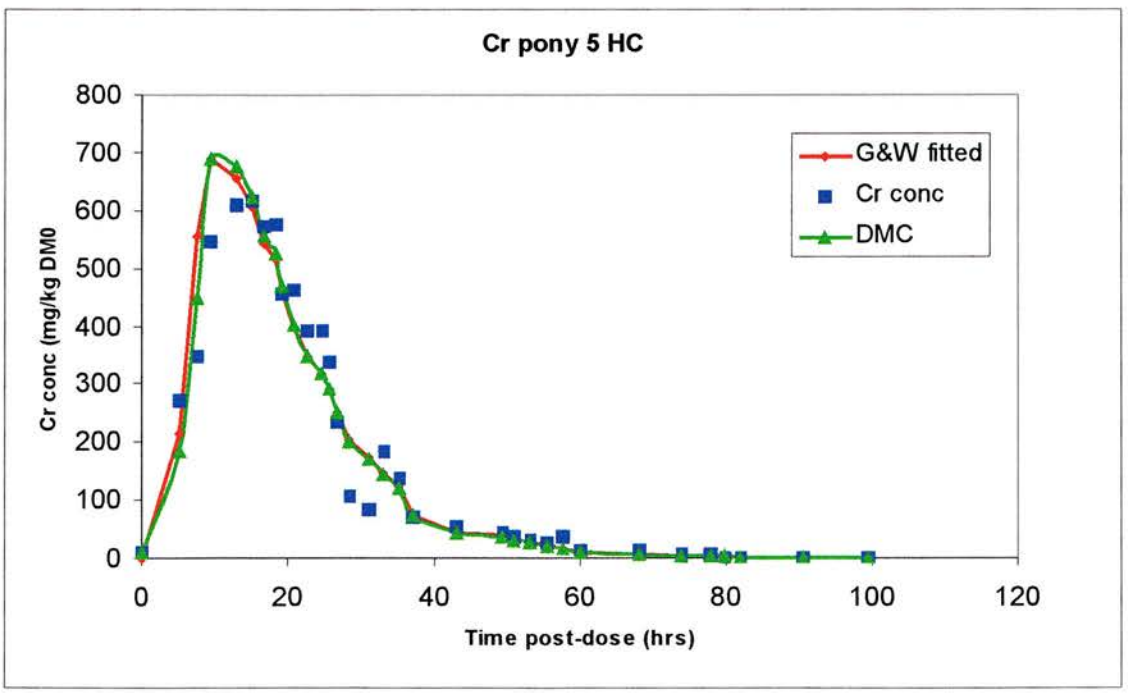
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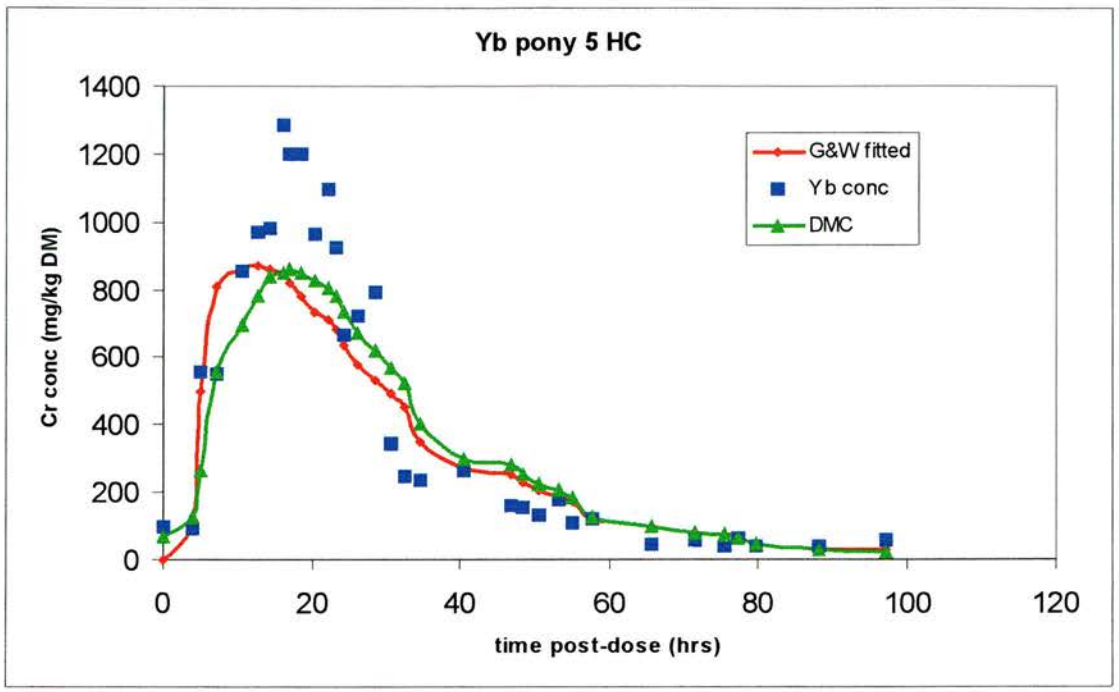
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time post-dose (hrs)
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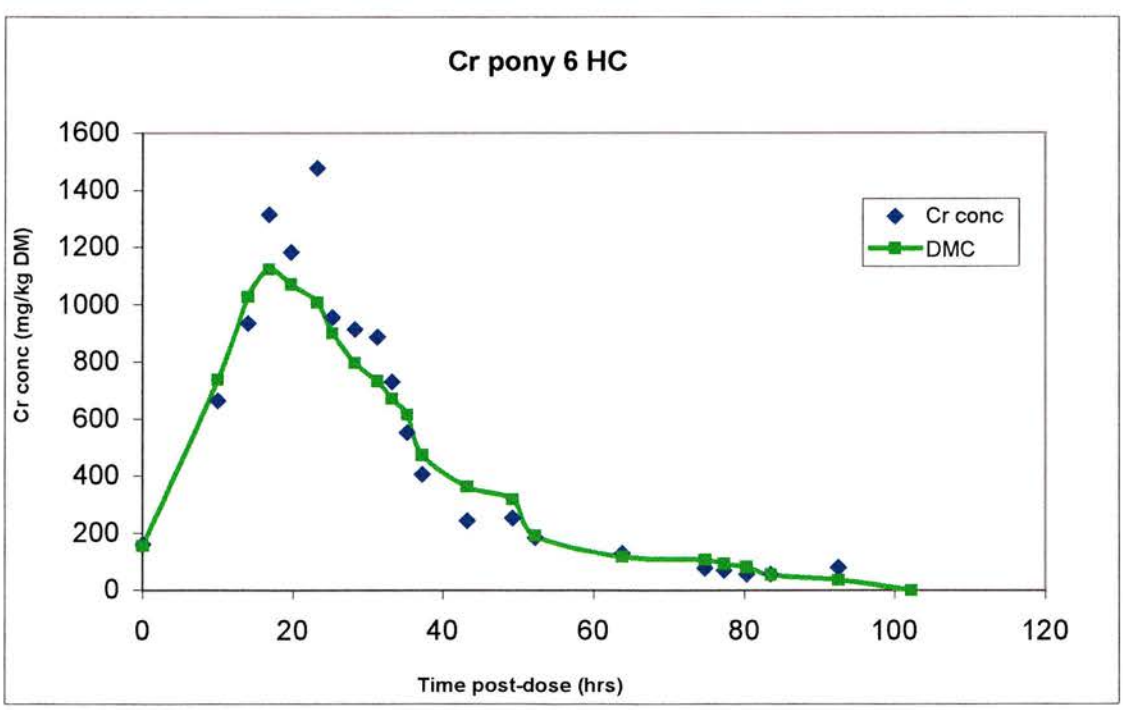
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Cr conc (mg/kg DM)
Time post-dose (hrs)
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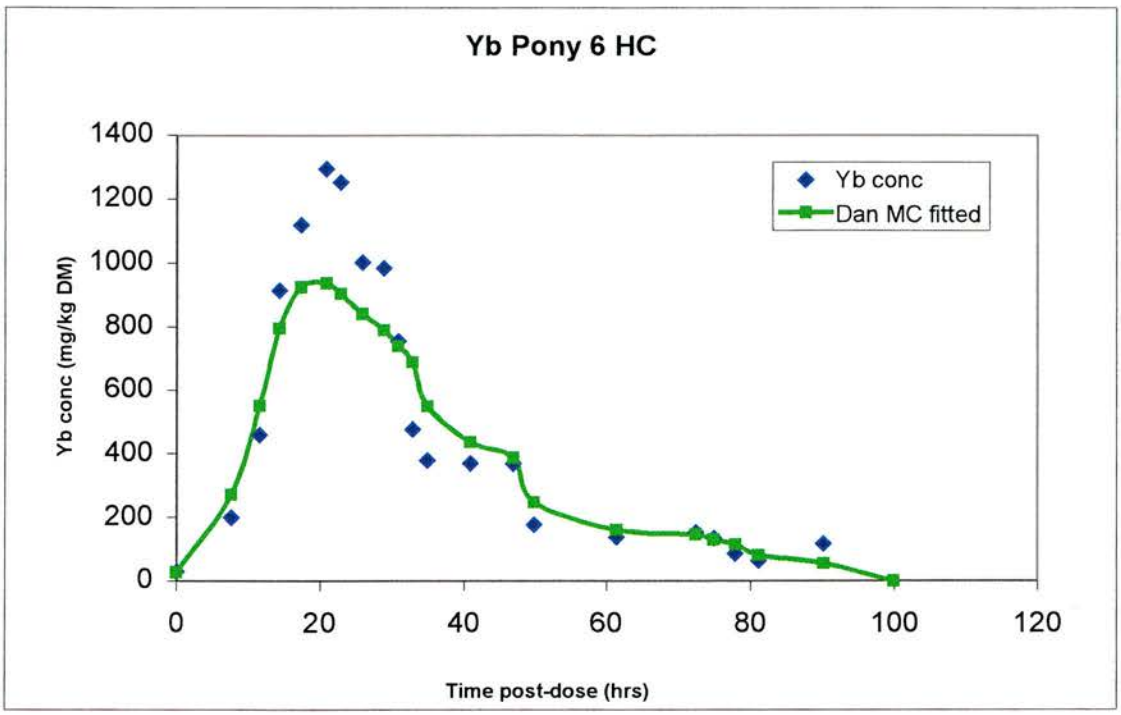
Yb Pony 6 HC
Yb conc
Dan MC fitted
Yb conc (mg/kg DM)
Time post-dose (hrs)
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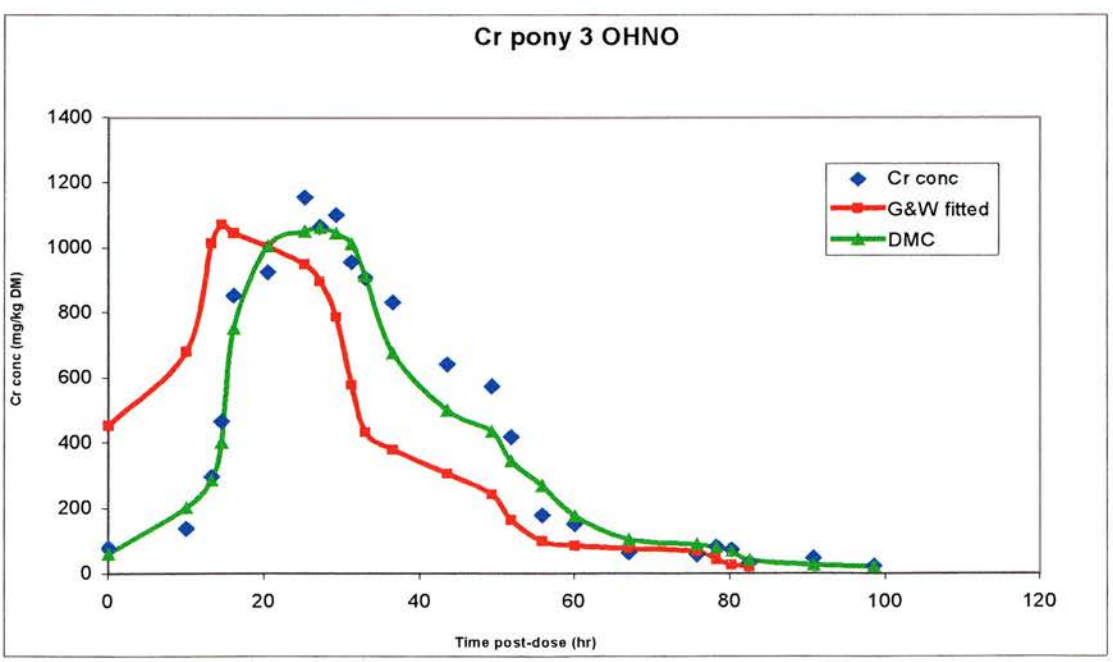
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Cr conc
G&W fitted
DMC
Cr conc (mg/kg DM)
Time post-dose (hr)
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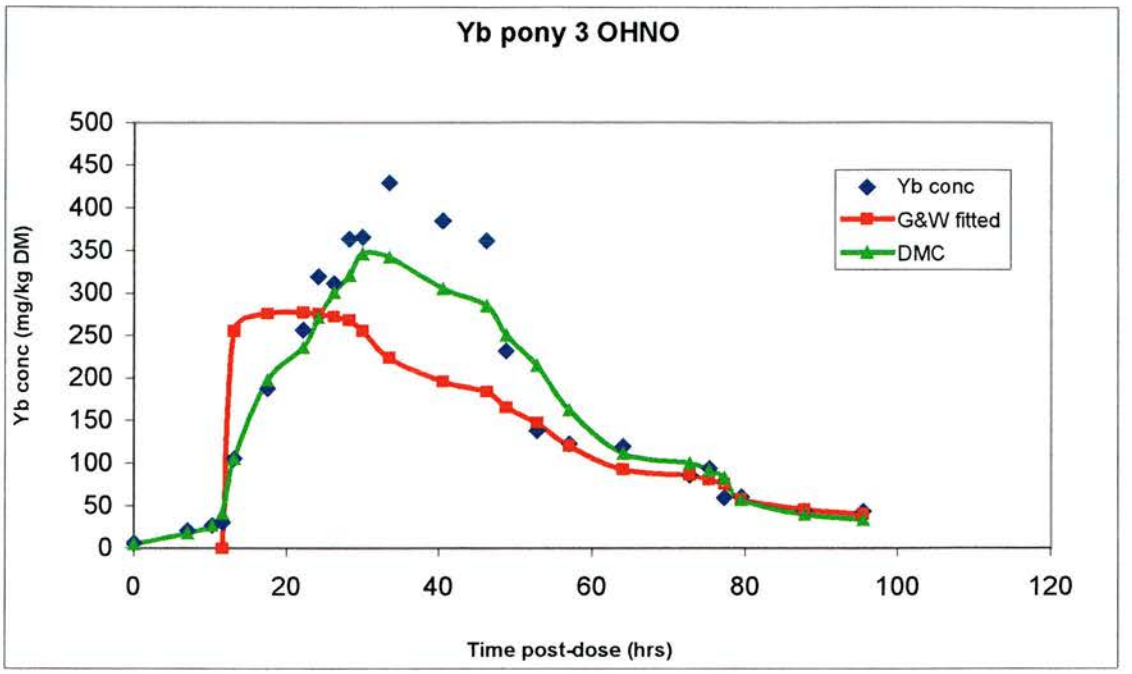
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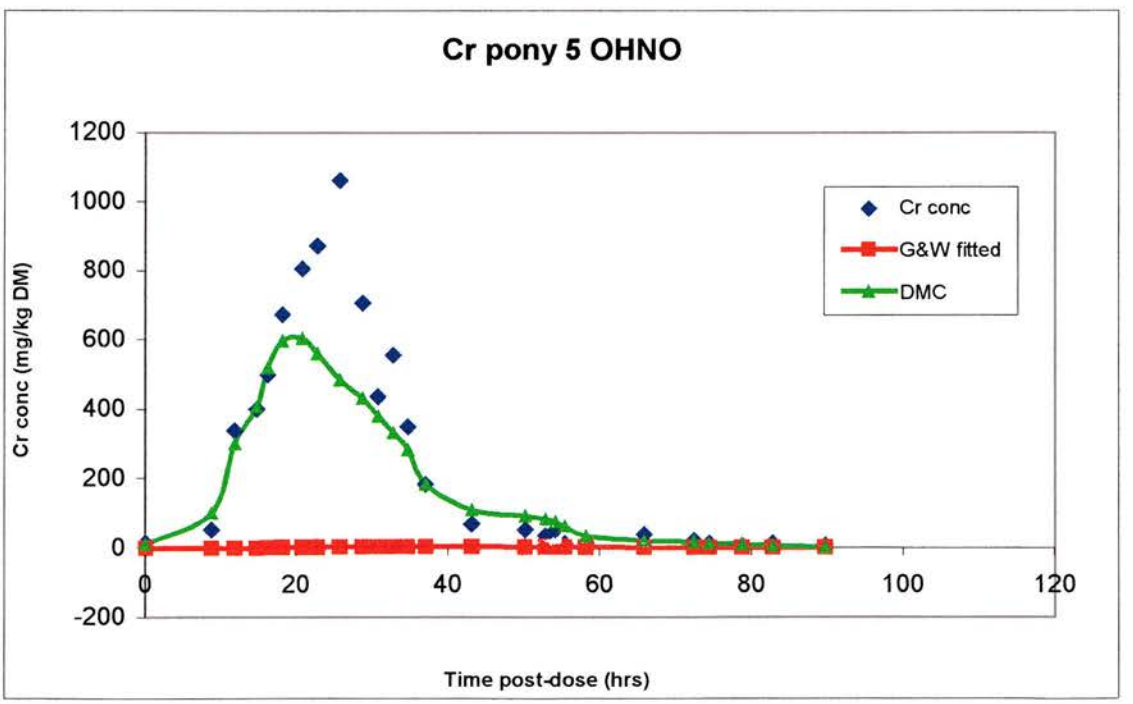
Time post-dose (hrs)
Yb conc
G&W fitted
DMC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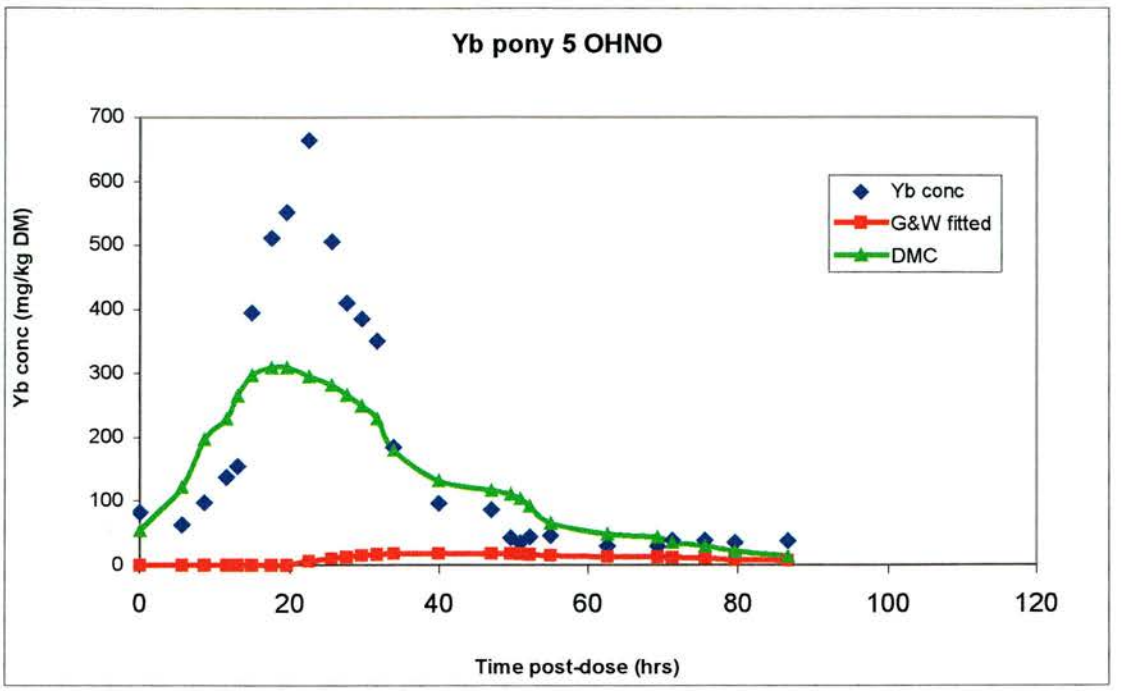
Time post-dose (hrs)
Cr conc
G&W fitted
DMC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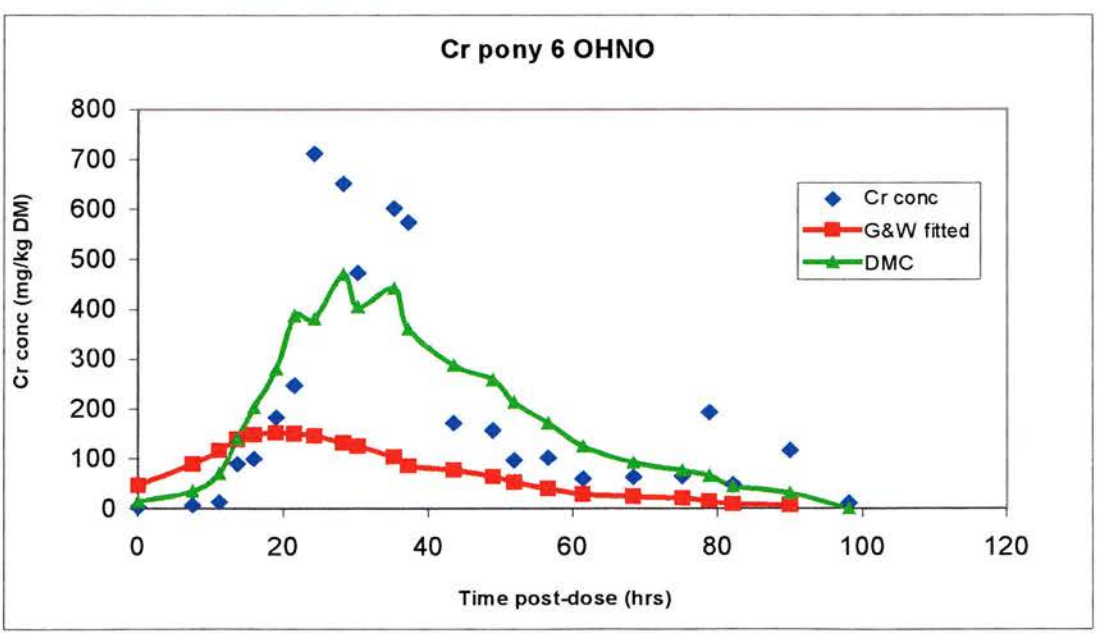
Time post-dose (hrs)
Yb conc
G&W fitted
DMC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Time post-dose (hrs)
Cr conc
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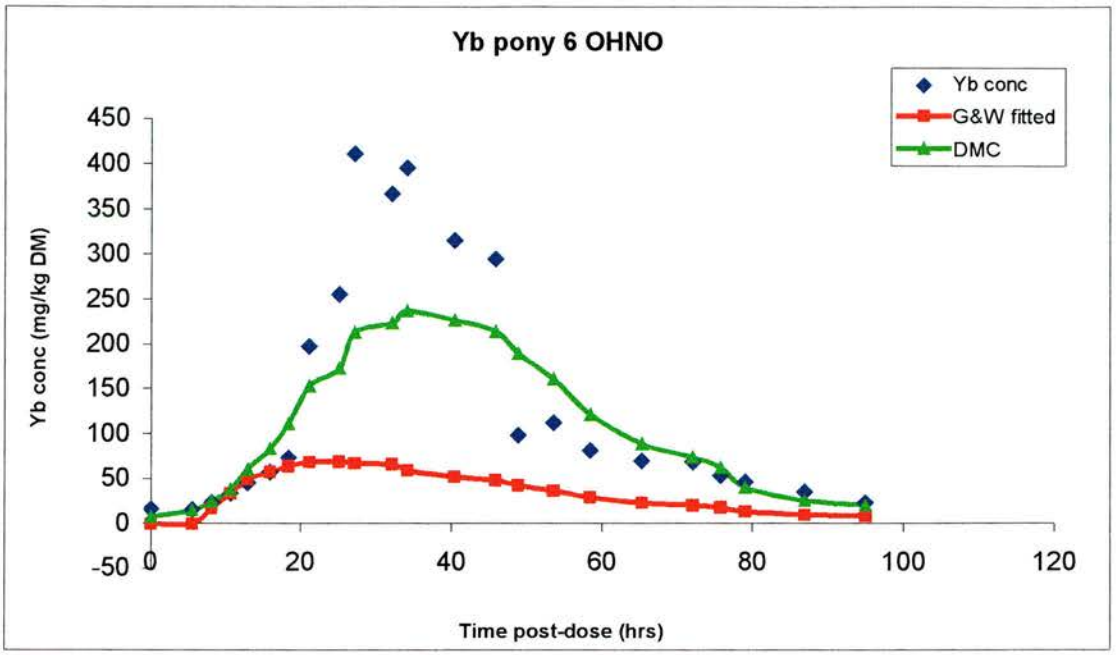
DMC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Yb conc (mg/kg DM)
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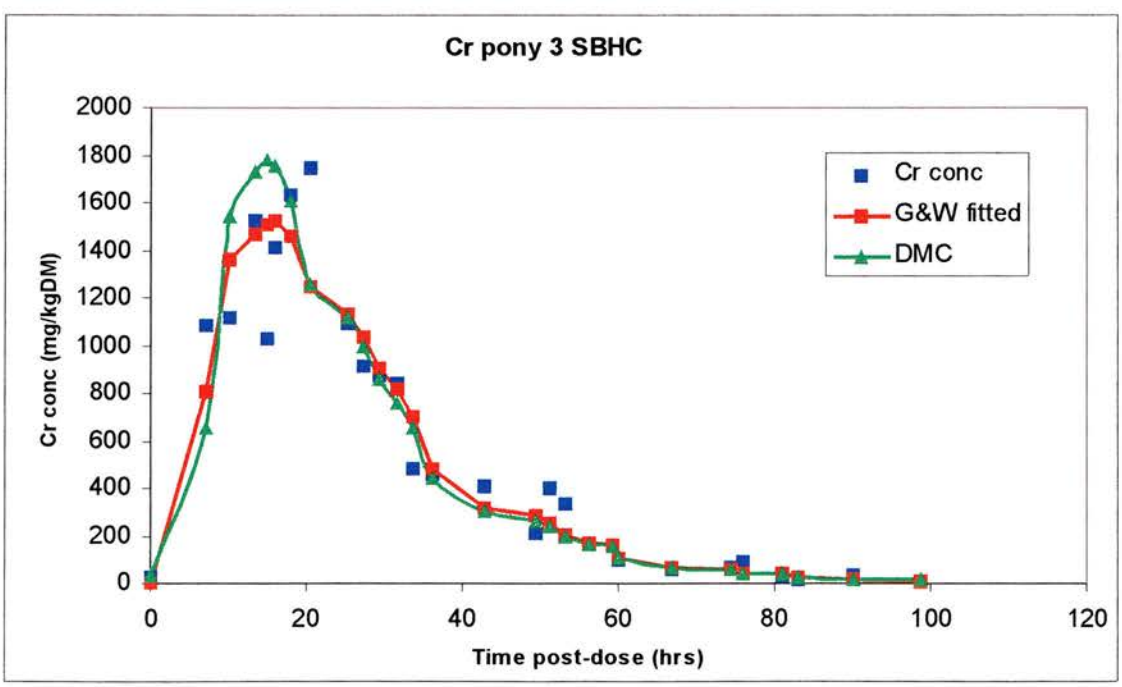
Time post-dose (hrs)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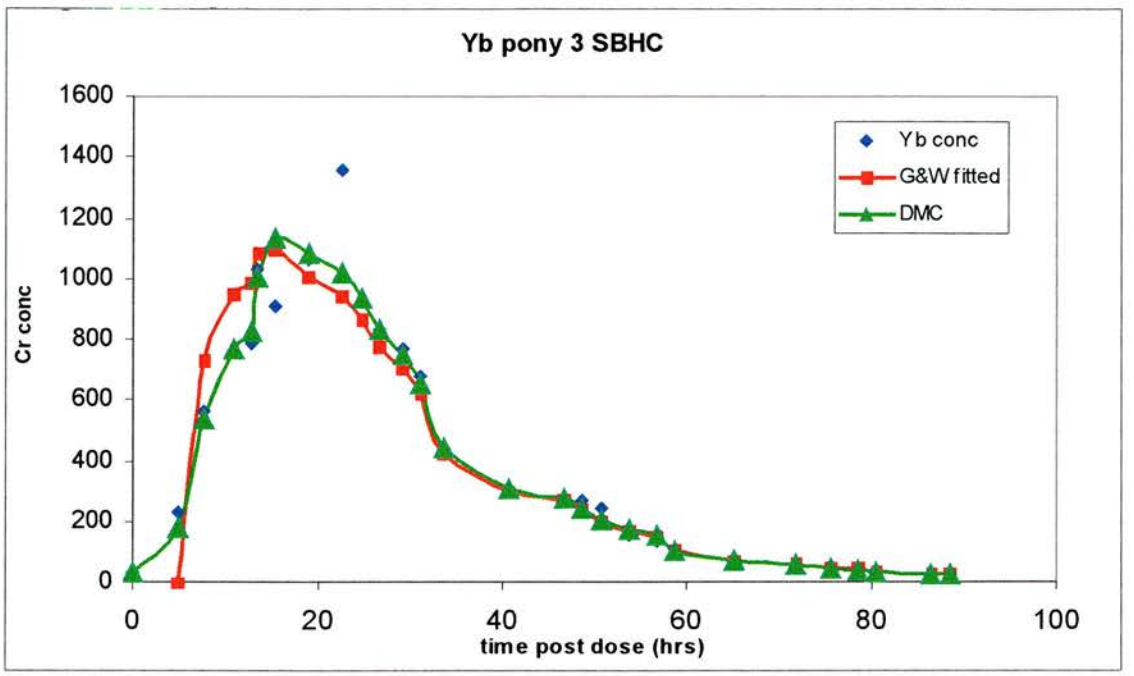
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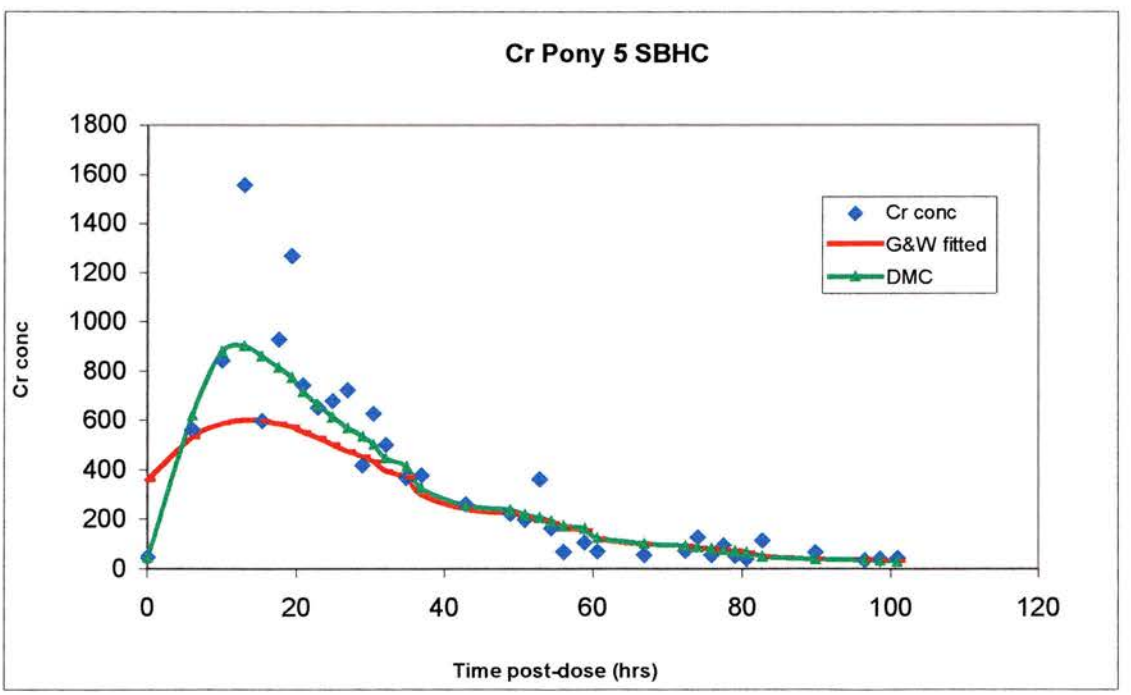
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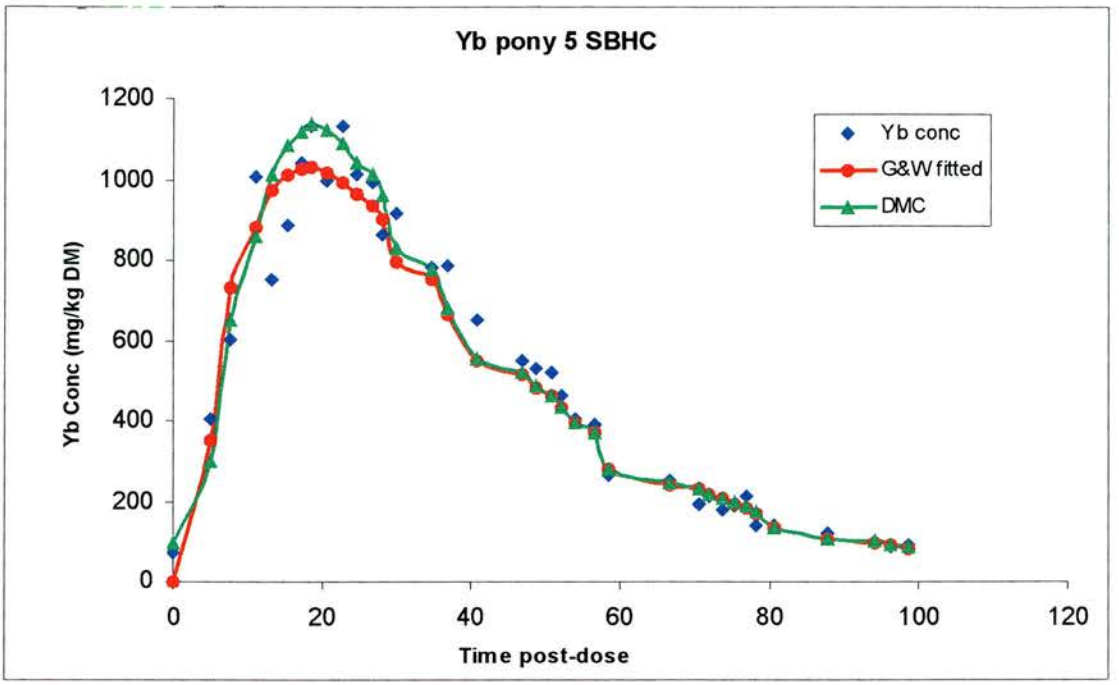
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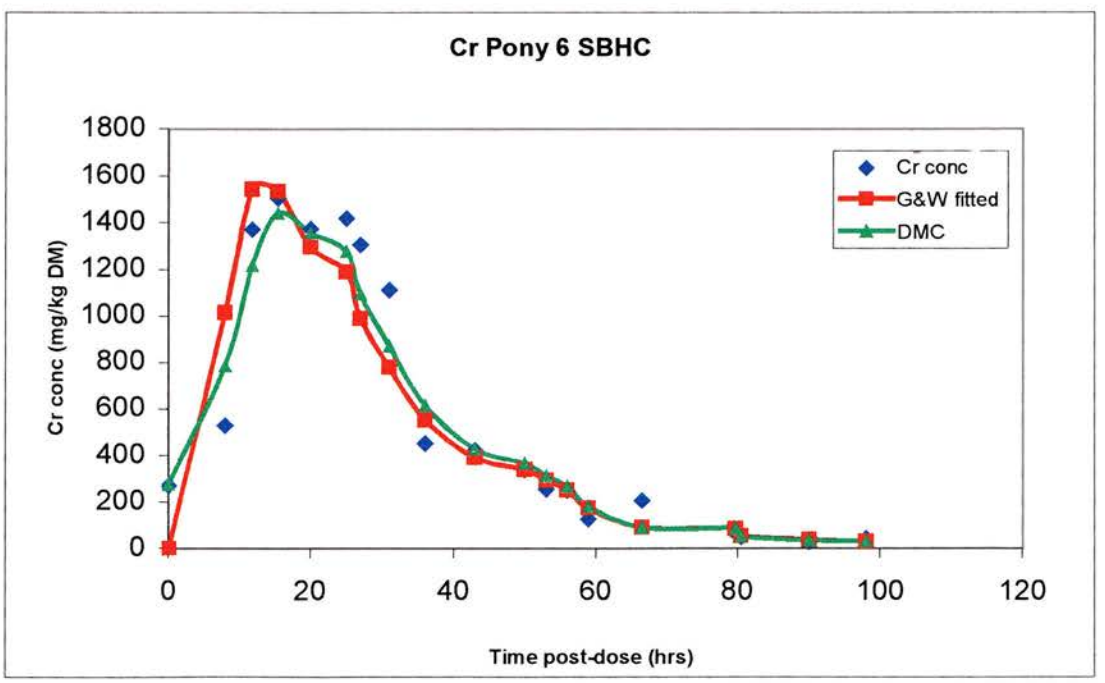
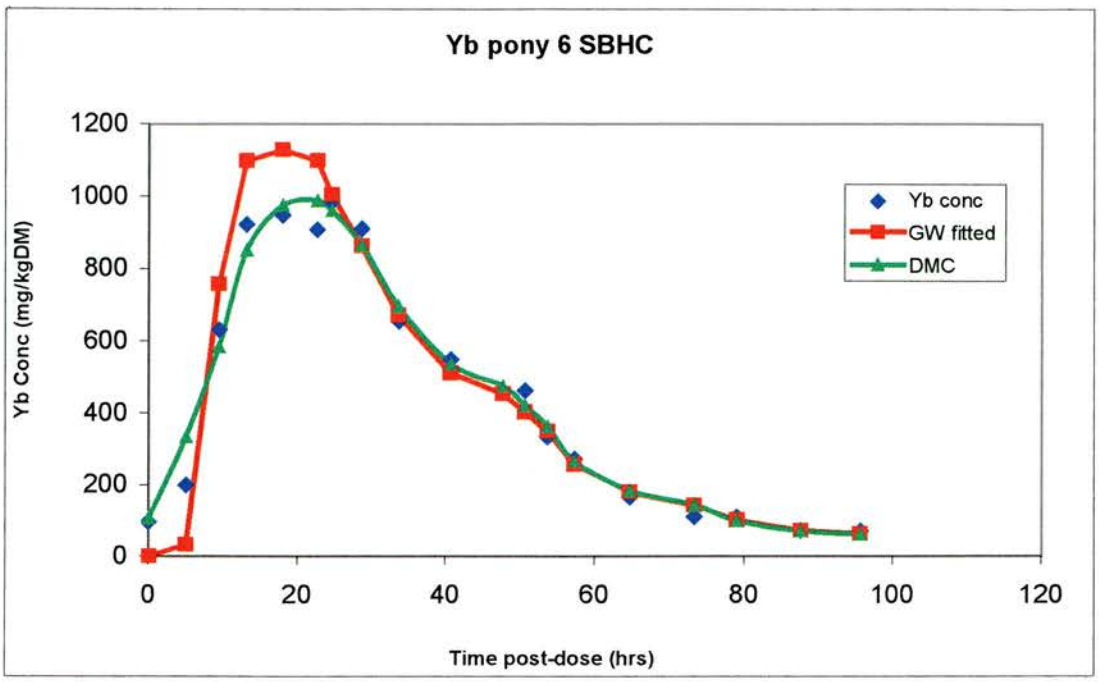
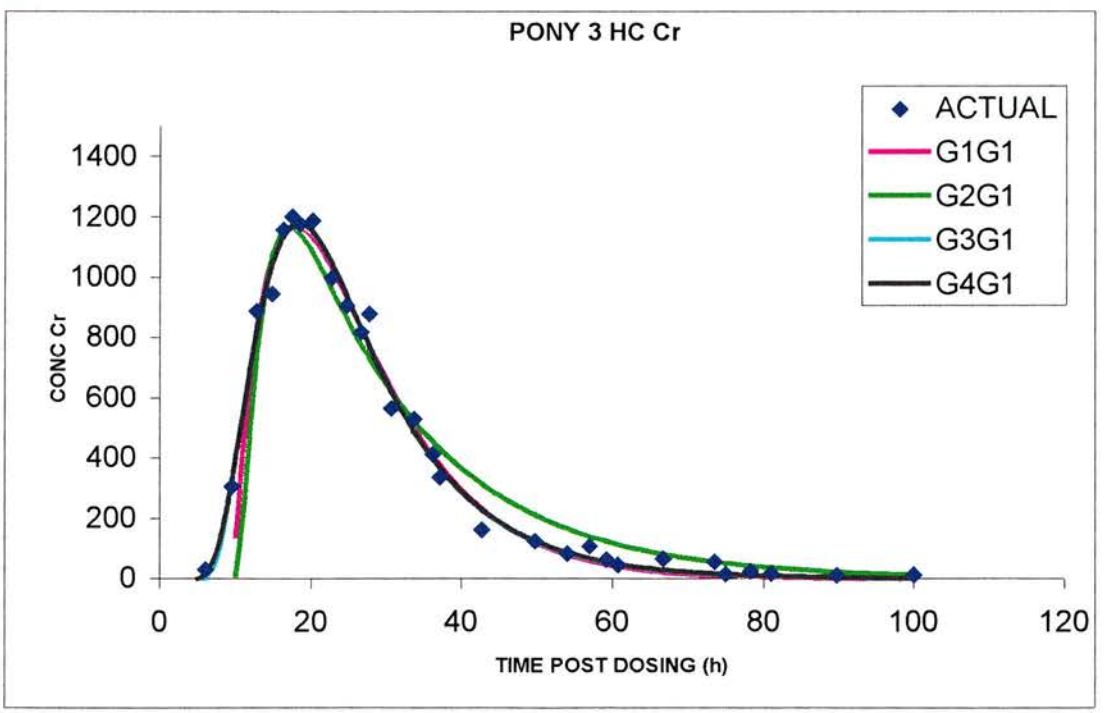
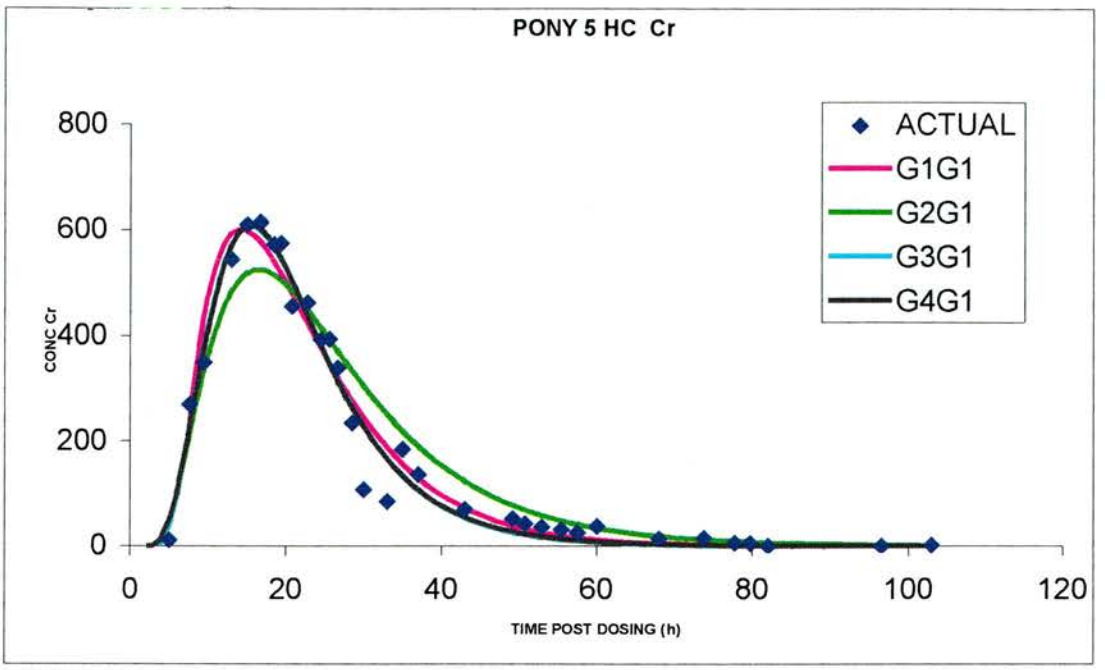
Time post-dose
Yb conc
G&W fitted
DMC


Cr Pony 6 SBHC
Cr conc (mg/kg DM)
1800
1600
1400
1200
1000
800
600
400
200
0
0
20
40
60
80
100
120
Time post-dose (hrs)
Cr conc
G&W fitted
DMC


Yb pony 6 SBHC
Yb Conc (mg/kgDM)
1200
1000
800
600
400
200
0
0
20
40
60
80
100
120
Time post-dose (hrs)
Yb conc
GW fitted
DMC


PONY 3 HC Cr
CONC Cr
1400
1200
1000
800
600
400
200
0
0
20
40
60
80
100
120
TIME POST DOSING (h)
ACTUAL
G1G1
G2G1
G3G1
G4G1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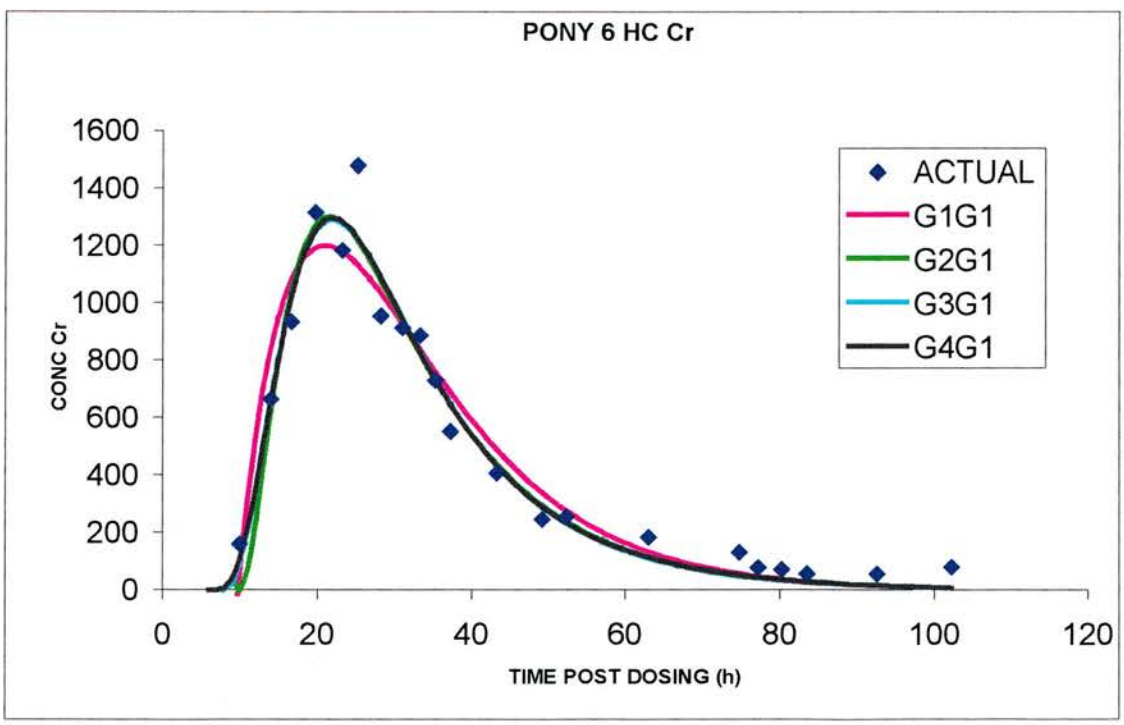
800
600
400
200
0
CONC Cr
0
20
40
60
80
100
120
TIME POST DOSING (h)
ACTUAL
G1G1
G2G1
G3G1
G4G1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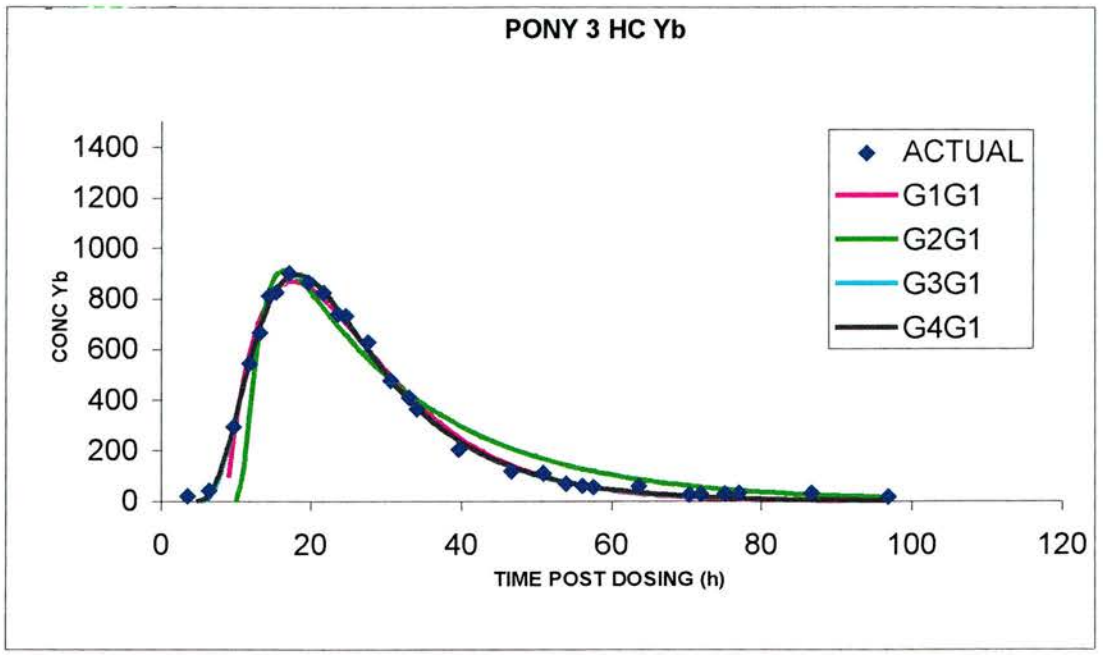
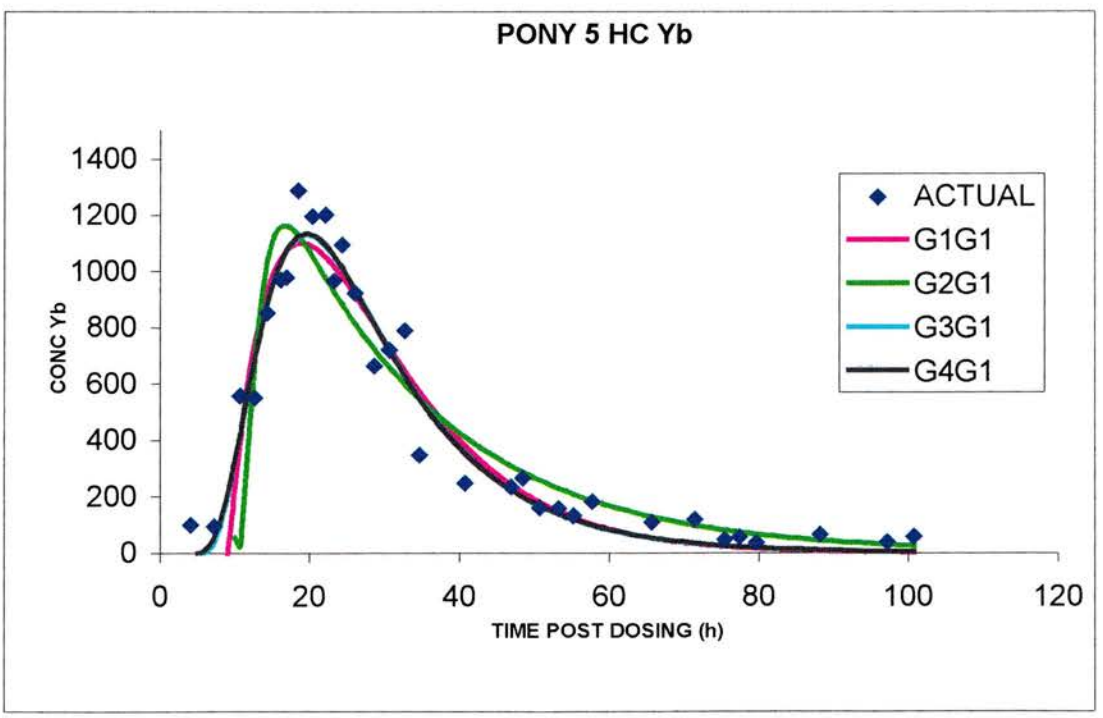
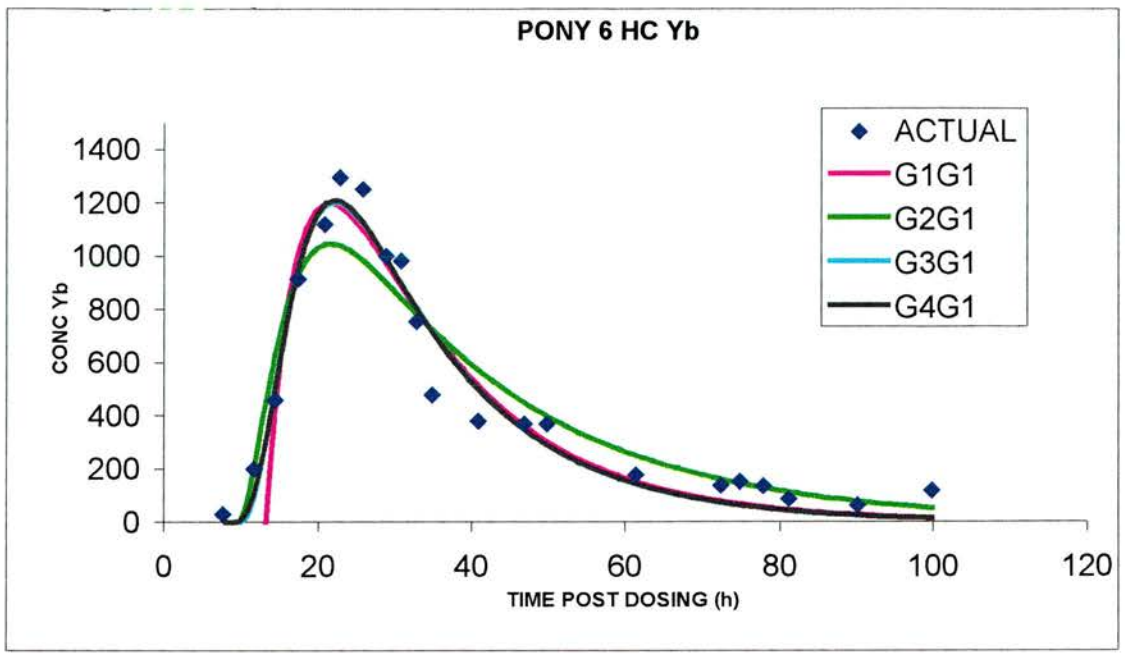
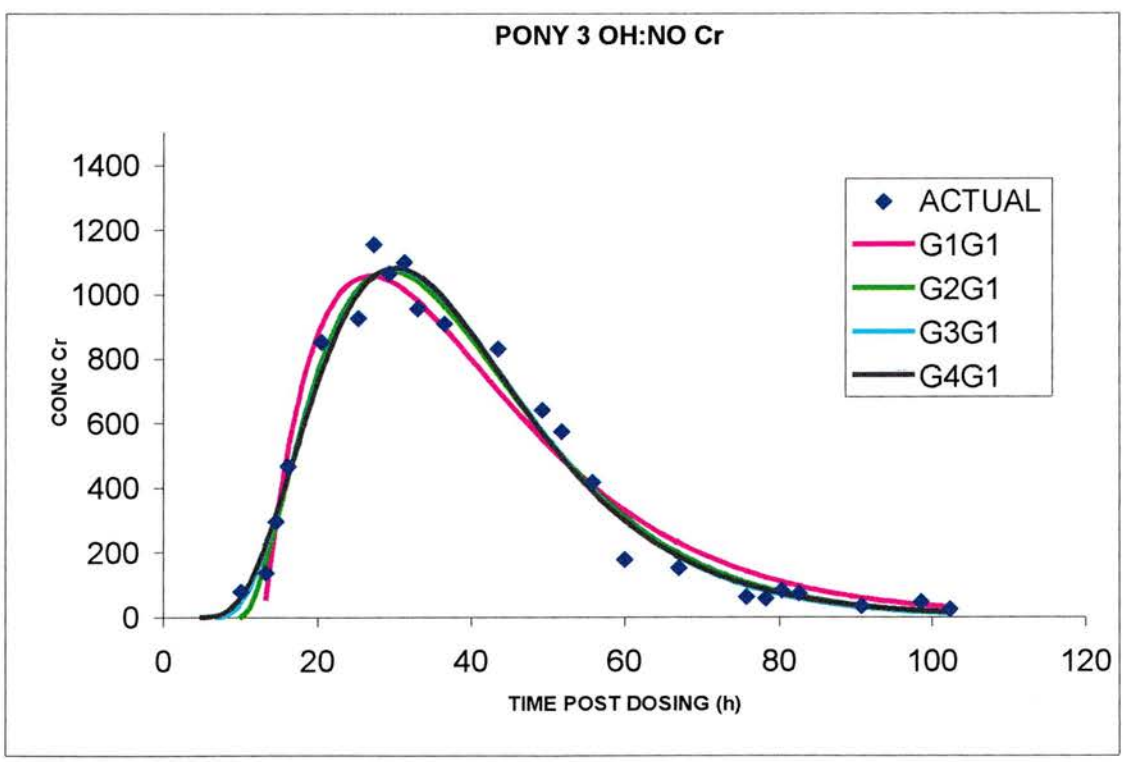
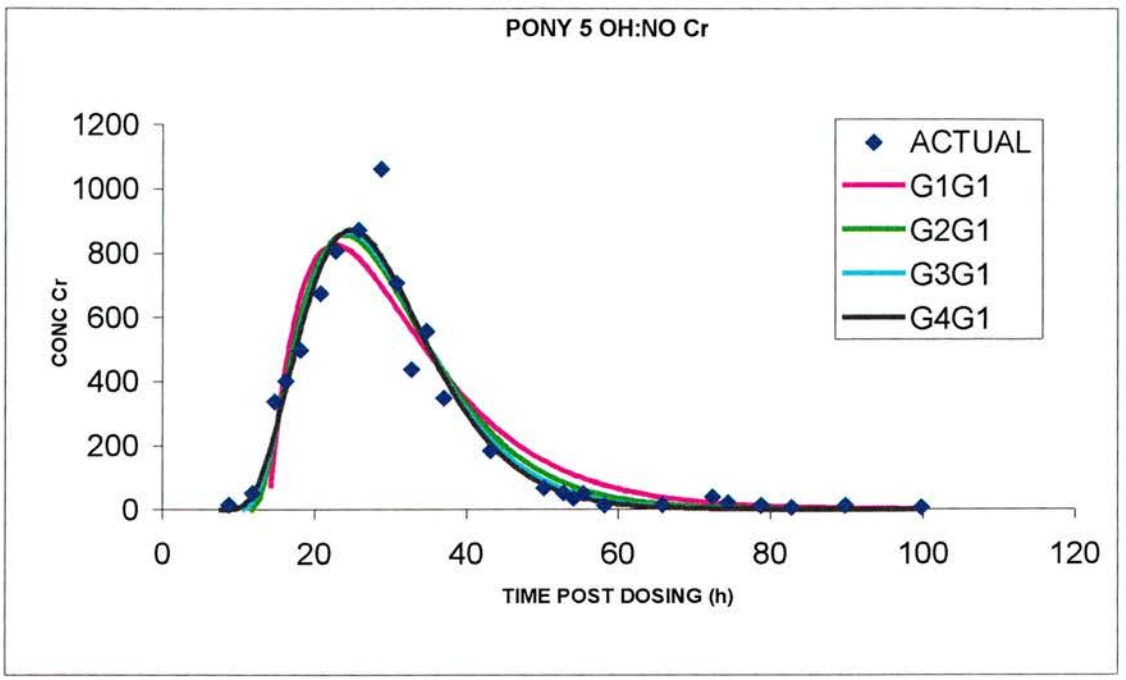
1600
1400
1200
1000
800
600
400
200
0
CONC Cr
0
20
40
60
80
100
120
TIME POST DOSING (h)
ACTUAL
G1G1
G2G1
G3G1
G4G1

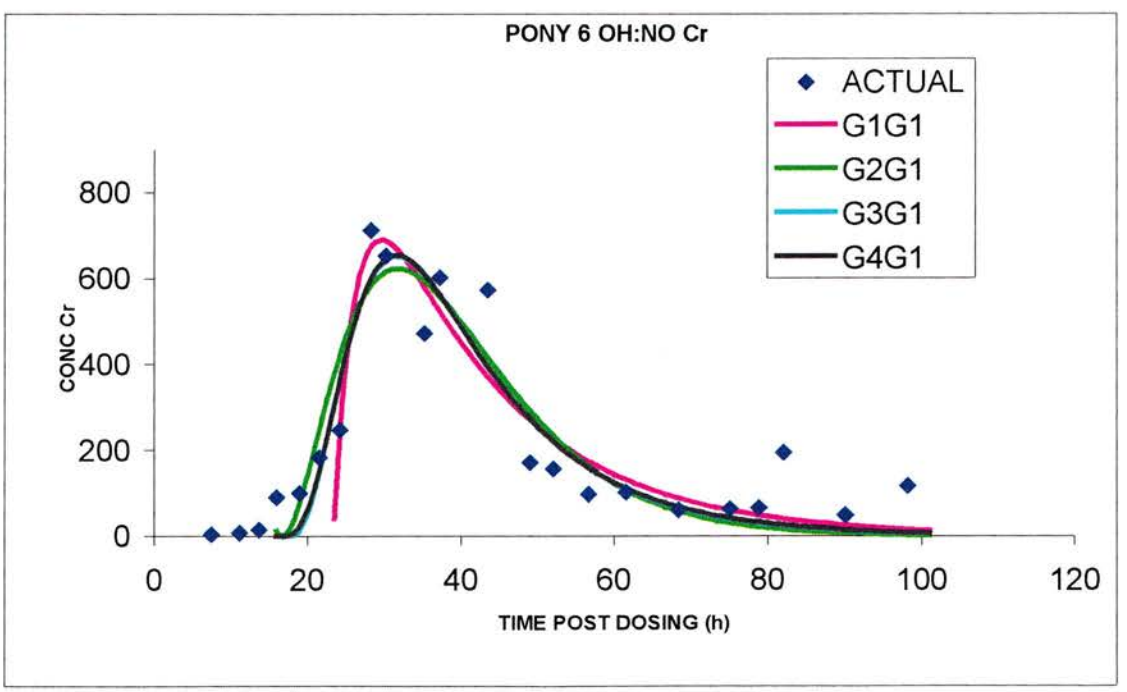

PONY 3 HC Yb
CONC Yb
1400
1200
1000
800
600
400
200
0
0
20
40
60
80
100
120
TIME POST DOSING (h)
ACTUAL
G1G1
G2G1
G3G1
G4G1

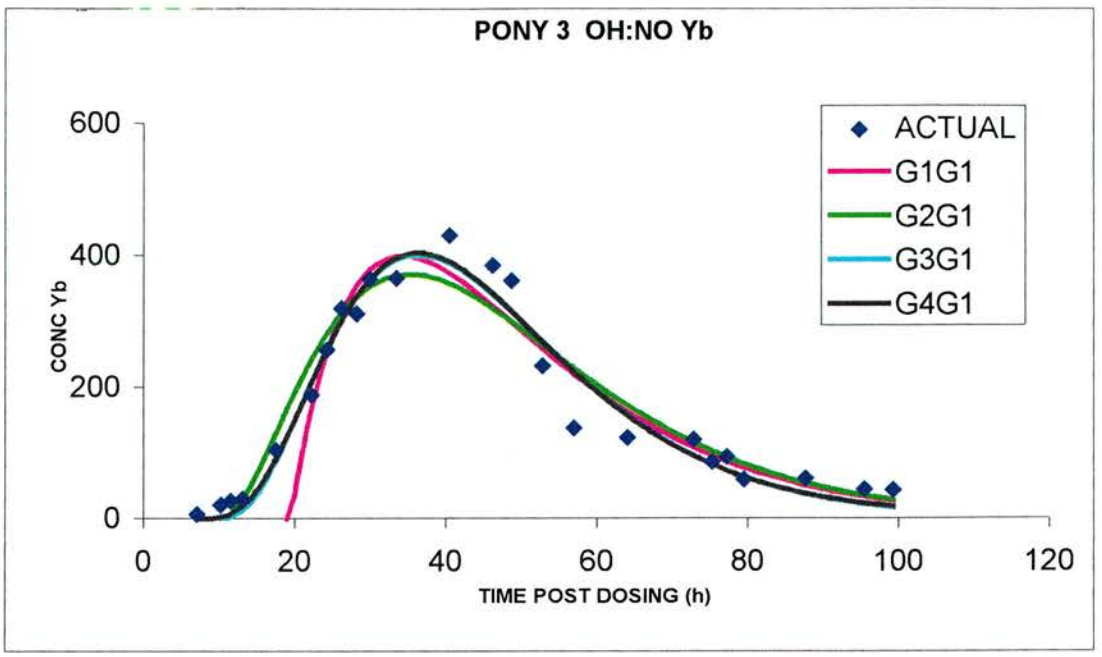

PONY 5 HC Yb
CONC Yb
1400
1200
1000
800
600
400
200
0
0
20
40
60
80
100
120
TIME POST DOSING (h)
ACTUAL
G1G1
G2G1
G3G1
G4G1

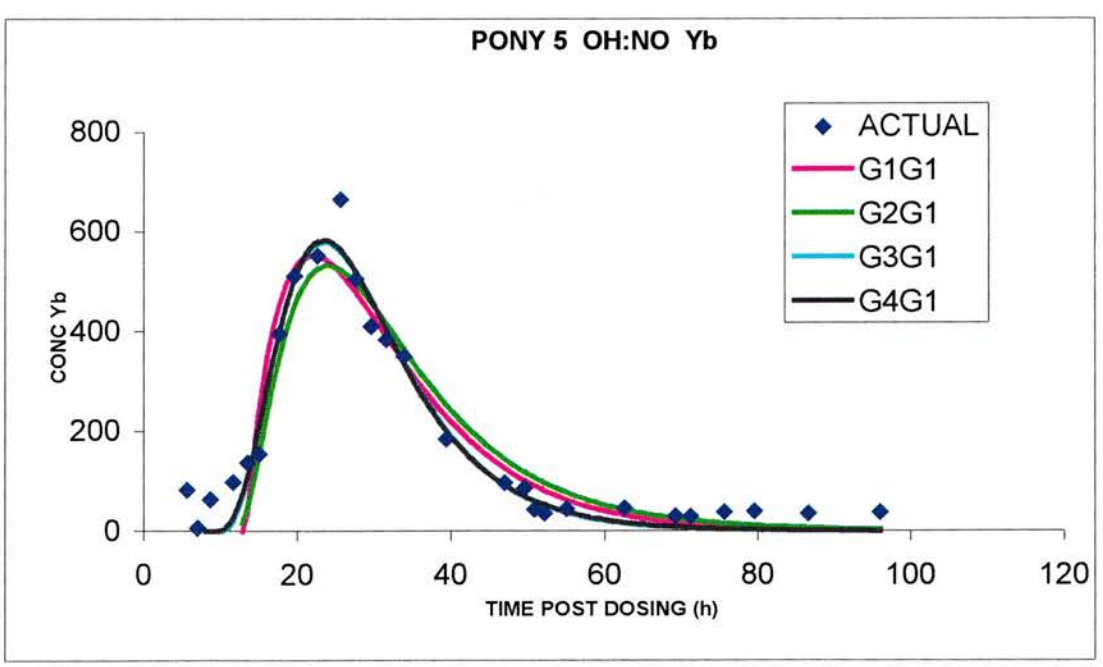

PONY 6 HC Yb
CONC Yb
1400
1200
1000
800
600
400
200
0
0
20
40
60
80
100
120
TIME POST DOSING (h)
ACTUAL
G1G1
G2G1
G3G1
G4G1

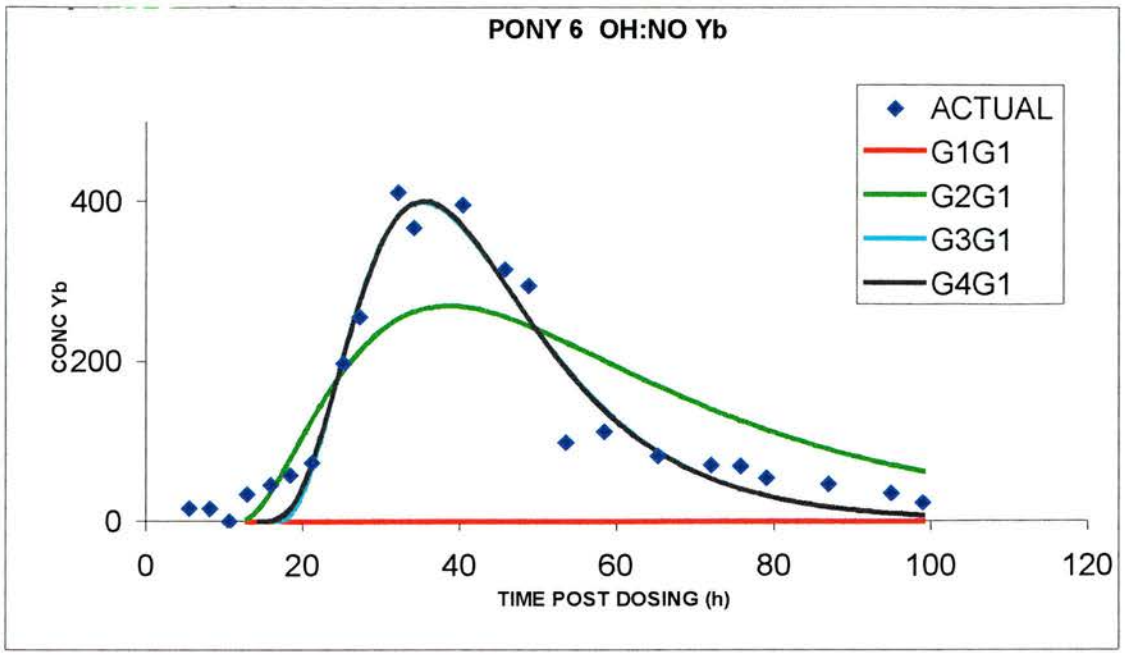

PONY 3 OH:NO Cr
CONC Cr
1400
1200
1000
800
600
400
200
0
0
20
40
60
80
100
120
TIME POST DOSING (h)
ACTUAL
G1G1
G2G1
G3G1
G4G1

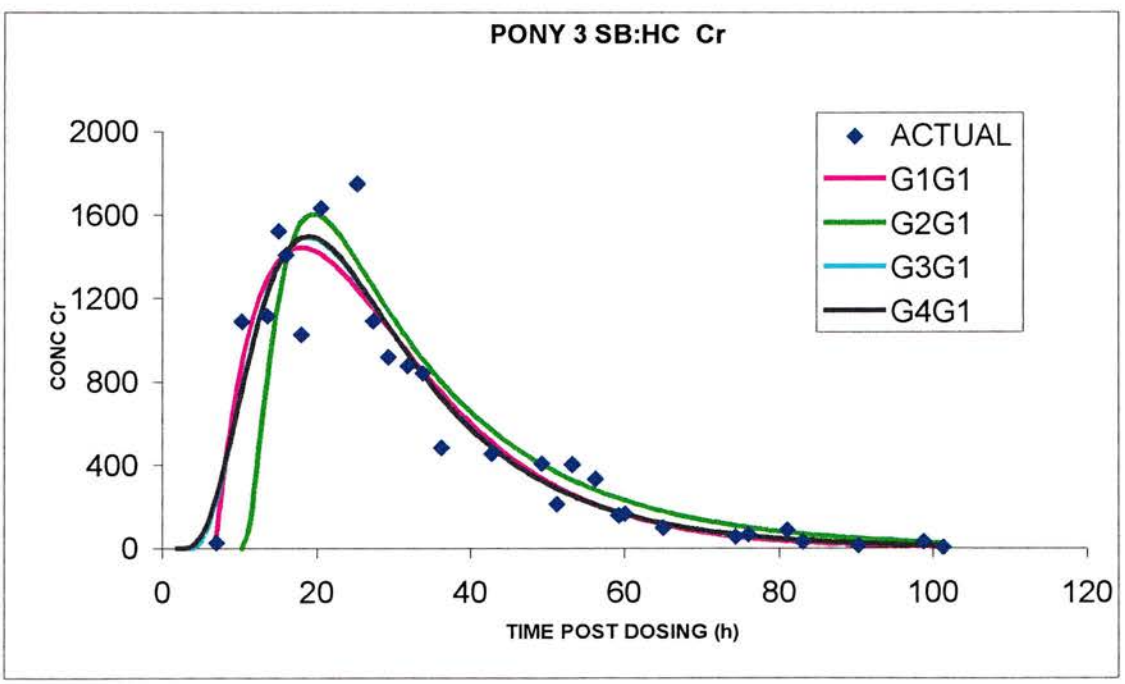

PONY 5 OH:NO Cr
1200
1000
800
600
400
200
0
CONC Cr
0
20
40
60
80
100
120
TIME POST DOSING (h)
ACTUAL
G1G1
G2G1
G3G1
G4G1

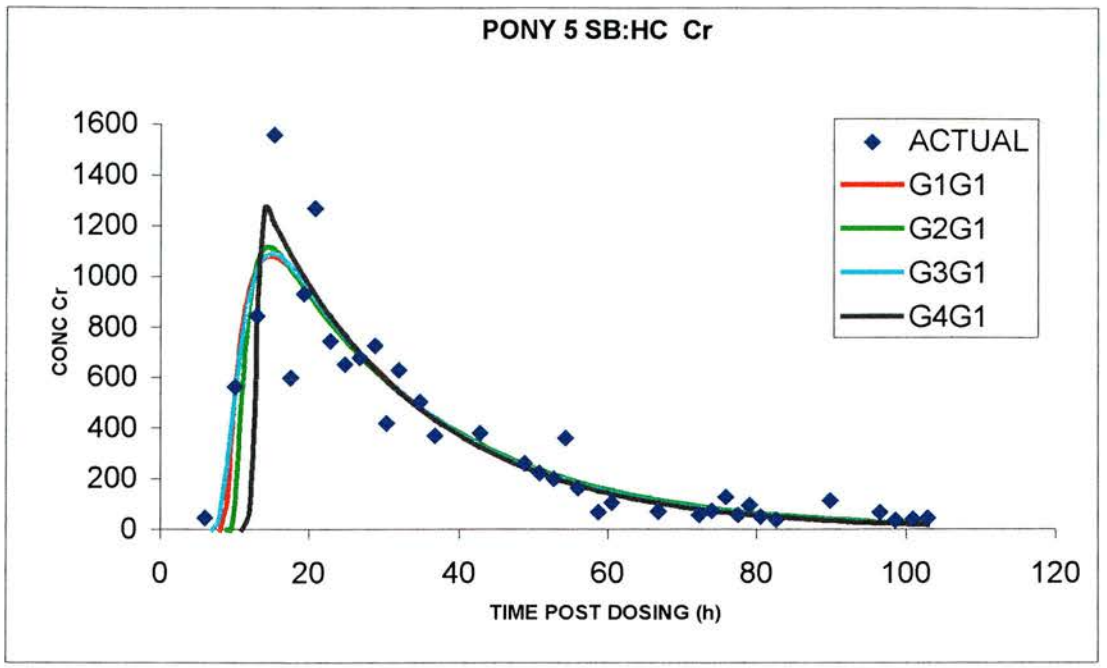

PONY 6 OH:NO Cr
800
600
400
200
0
CONC Cr
0
20
40
60
80
100
120
TIME POST DOSING (h)
ACTUAL
G1G1
G2G1
G3G1
G4G1

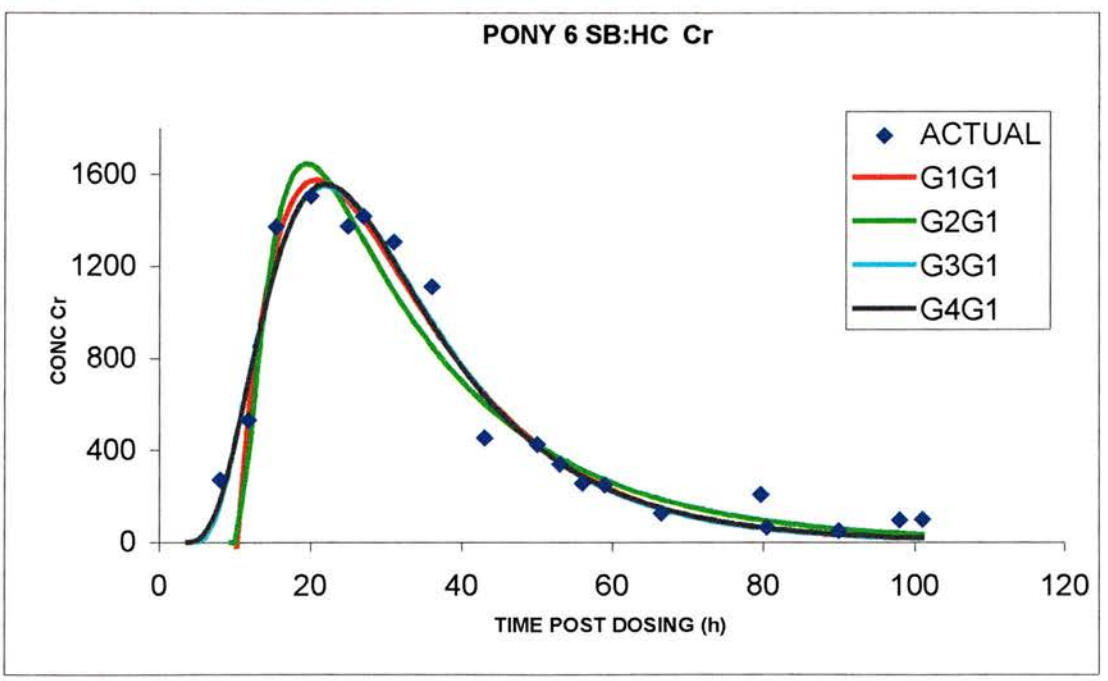

PONY 3 OH:NO Yb
600
400
200
0
CONC Yb
0
20
40
60
80
100
120
TIME POST DOSING (h)
ACTUAL
G1G1
G2G1
G3G1
G4G1

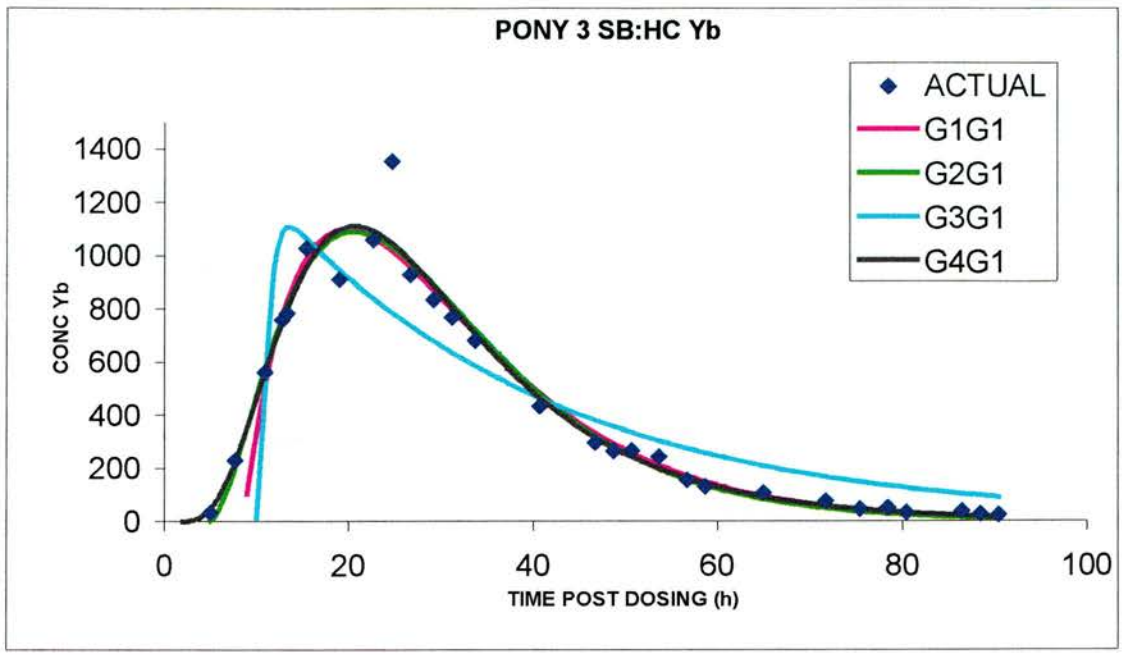

PONY 5 OH:NO Yb
800
600
400
200
0
CONC Yb
0
20
40
60
80
100
120
TIME POST DOSING (h)
ACTUAL
G1G1
G2G1
G3G1
G4G1

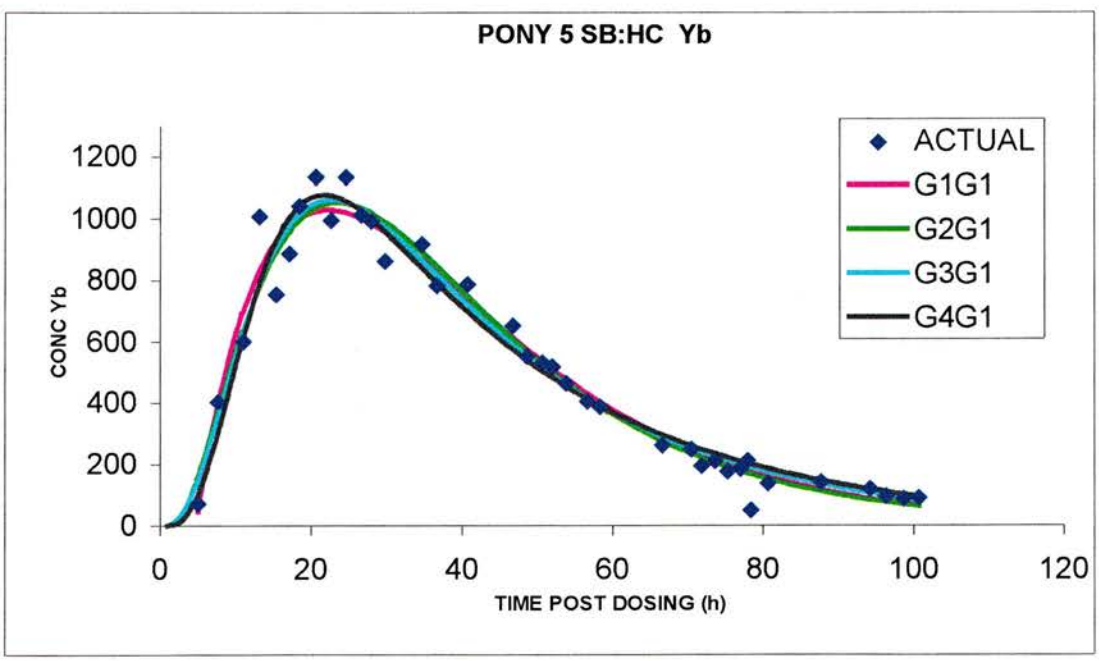

PONY 6 OH:NO Yb
ACTUAL
G1G1
G2G1
G3G1
G4G1
CONC Yb
400
200
0
0
20
40
60
80
100
120
TIME POST DOSING (h)

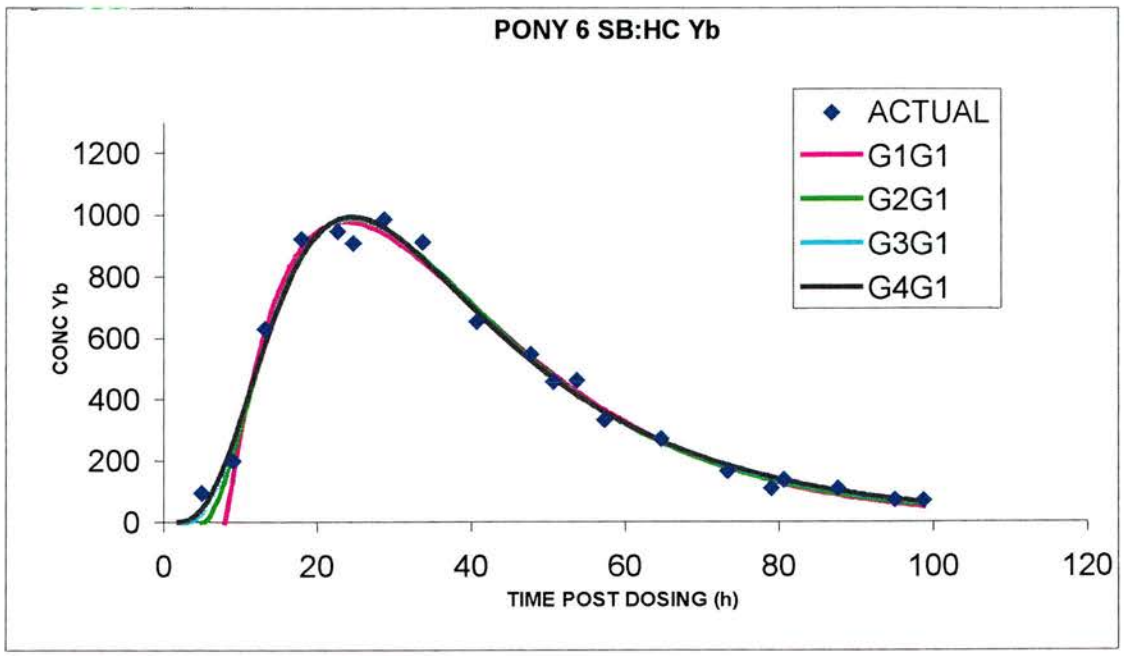

PONY 3 SB:HC Cr
ACTUAL
G1G1
G2G1
G3G1
G4G1
CONC Cr
2000
1600
1200
800
400
0
0
20
40
60
80
100
120
TIME POST DOSING (h)


PONY 5 SB:HC Cr
CONC Cr
1600
1400
1200
1000
800
600
400
200
0
0
20
40
60
80
100
120
TIME POST DOSING (h)
ACTUAL
G1G1
G2G1
G3G1
G4G1


PONY 6 SB:HC Cr
CONC Cr
1600
1200
800
400
0
0
20
40
60
80
100
120
TIME POST DOSING (h)
ACTUAL
G1G1
G2G1
G3G1
G4G1


PONY 3 SB:HC Yb
ACTUAL
G1G1
G2G1
G3G1
G4G1
CONC Yb
1400
1200
1000
800
600
400
200
0
0
20
40
60
80
100
TIME POST DOSING (h)


PONY 5 SB:HC Yb
ACTUAL
G1G1
G2G1
G3G1
G4G1
CONC Yb
1200
1000
800
600
400
200
0
0
20
40
60
80
100
120
TIME POST DOSING (h)


PONY 6 SB:HC Yb
ACTUAL
G1G1
G2G1
G3G1
G4G1
CONC Yb
1200
1000
800
600
400
200
0
0
20
40
60
80
100
120
TIME POST DOSING (h)

APPENDIX 7. Experiment 3.1.

Appendix 7.1 *In vivo* apparent digestibility of rhamnose by four ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Period stratum	3	794397.	264799.	0.87	
Period. *Units* stratum					
Food	3	193560.	64520.	0.21	0.885
Pony:	3	142845	47615.	0.16	0.922
Residual	6	1830496	305083.		
Total:	15	2961298			

Appendix 7.2 *In vivo* apparent digestibility of arabinose by four ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Period stratum	3	23462.	7821.	0.89	
Period. *Units* stratum					
Food	3	211036.	70345.	8.04	0.016
Pony	3	21533.	7178.	0.82	0.528
Residual	6	52469.	8745.		
Total:	15	308500.			

Appendix 7.3 *In vivo* apparent digestibility of xylose by four ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Period stratum	3	49486.	16495.	1.66	
Period. *Units* stratum					
Food	3	665746.	221915	22.29	0.001
Pony	3	513153.	171051.	17.18	0.002
Residual	6	59747.	9958.		
Total:	15	1288131.			

Appendix 7.4 *In vivo* apparent digestibility of mannose by four ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Period stratum	3	49975.	16658.	1.12	
Period. *Units* stratum					
Food	3	159283.	53094.	3.58	0.086
Pony	3	38221.	12740.	0.86	0.511
Residual	6	88960.	14827.		
Total:	1	336437.			

Appendix 7.5 *In vivo* apparent digestibility of galactose by four ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Period stratum	3	46541.	15514.	3.21	
Period. *Units* stratum					
Food	3	75056.	25019.	5.17	0.042
Pony	3	33476.	11159.	2.31	0.176
Residual	6	29021.	4837.		
Total:	15	184095.			

Appendix 7.6 *In vivo* apparent digestibility of glucose by ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage **(CS).**

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Period stratum	3	40675.	13558.	1.57	
Period. *Units * stratum					
Food	3	357145.	119048.	13.80	0.004
Pony	3	11297.	3766.	0.44	0.735
Residual	6	51758.	8626.		
Total:	15	460876.			

Appendix 7.7 *In vivo* apparent digestibility of uronic acids by ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Period stratum	3	8218.	2739.	0.97	
Period. *Units* stratum					
Food	3	165320.	55107.	19.47	0.002
Pony	3	30383.	10128.	3.58	0.086
Residual	6	16985.	2831.		
Totalo:	15	220905.			

Appendix 7.8 *In vivo* apparent digestibility of total non-starch polysaccharides by ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Period stratum	3	43962.	14654.	1.74	
Period *Units* stratum					
Food	3	337519.	112506.	13.39	0.005
Pony	3	42896.	14299.	1.70	0.265
Residual	6	50416.	8403.		
Total:	15	474793.			

Appendix 7.9 Mean variation in live weight (LW) across the four ponies when consuming four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Period stratum	3	288.7	96.2	0.59	
Period *Units* stratum					
Food	3	1505.2	501.7	3.10	0.111
Pony	3	10330.2	3443.4	21.28	0.001
Residual	6	970.9	161.8		
Total:	15	13094.9			

Appendix 7.10. Mean variation in fresh weight intake across the four ponies when consuming four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Period stratum	3	.8.093	2.698	0.90	
Period *Units* stratum					
Food	3	83.629	27.876	9.27	0.011
Pony	3	42.836	14.279	4.75	0.050
Residual	6	18.048	3.008		
Total:	15	152.605			

Appendix 7.11 Mean variation in dry matter intake (g/kg LW $^{0.75}$) across the four ponies when consuming four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Period stratum	3	352.73	117.58	1.60	
Period *Units* stratum					
Food	3	3915.76	1305.25	17.76	0.002
Pony	3	1594.95	531.65	7.23	0.020
Residual	6	441.07	73.51		
Total:	15	6304.51			

Appendix 7.12. *In vivo* apparent digestibility of dry matter (DM) by four ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Period stratum	3	18106.	6035.	1.42	
Period *Units* stratum					
Food	3	172558.	57519.	13.58	0.004
Pony	3	6395.	2132.		0.694
Residual	6	25422.	4237.		
Total:	15	222480.			

Appendix 7.13 *In vivo* apparent digestibility of organic matter by ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Period stratum	3	18025.	6008.	1.33	
Period *Units* stratum					
Food	3	166515.	55505.	12.31	0.005
Pony	3	7872.	2624.	0.58	0.265
Residual	6	27049.	4508.		
Total:	15	219462.			

Appendix 1.14 *In vivo* apparent digestibility of crude protein by ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Period stratum	3	44886.	14962.	4.38	
Period *Units* stratum					
Food	3	598625.	199542.	58.40	0.001
Pony	3	20268.	6756.	1.98	0.236
Residual	5(1)	17085.	3417.		
Total:	14(1)	589081.			

Appendix 7.15 *In vivo* apparent digestibility of acid detergent fibre by ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Period stratum	3	43377.	14459.	1.89	
Period *Units* stratum					
Food	3	288514.	96171.	12.60	0.005
Pony	3	3962.	1321.	0.17	0.911
Residual	6	45793	7632.		
Total:	15	381644.			

Appendix 7.16 *In vivo* apparent digestibility of neutral detergent fibre by ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Period stratum	3	32789.	10930.	1.61	
Period *Units* stratum					
Food	3	242940.	80980.	11.89	0.006
Pony	3	3149.	1050.	0.15	0.923
0.923					
Residual	6	40848.	6808.		
Total:	15	319726.			

Appendix 7.17 *In vivo* apparent digestibility of gross energy by ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Period stratum	3	15883.	5294.	1.13	
Period *Units* stratum					
Food	3	209761.	69920.	14.86	0.003
Pony	3	10187.	3396.	0.72	0.575
Residual	6	28230.	4705.		
Total:	15	264060.			

Appendix 7.18 *In vivo* apparent digestibility of starch by ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Period stratum	3	4417.	14.72	0.18	
Period *Units* stratum					
Food	3	19159.	6385.	0.77	0.549
Pony	3	27691.	9230.	1.12	0.413
Residual	6	49472	8245.		
Total:	15	100737.			

Appendix 7.19 *In vivo* apparent digestibility of calcium by ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Period stratum	3	13828.	4609.	0.16	
Period *Units* stratum					
Food	3	372786.	124262.	4.21	0.078
Pony	3	35762.	11921.	0.40	0.757
Residual	5(1)	147563	29513		
Total:	14(1)	528286			

Appendix 7.20 *In vivo* apparent digestibility of phosphorus by ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Period stratum	3	457619.	152540.		
Period *Units* stratum					
Food	3	1149598.	383199.	23.75	0.002
Pony	3	283926.	94642.	5.87	0.043
Residual	5(1)	80681	16136		
Total:	14(1)	1540510			

Appendix 7.21 *In vivo* apparent digestibility of magnesium by ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Period stratum	3	132391.	44130.	0.92	
Period *Units* stratum					
Food	3	875015.	291672.	6.10	0.030
Pony	3	55955.	18652	0.39	0.765
Residual	6	286829.	47805.		
Total:	15	1350190.			

Appendix 7.22 *In vivo* digestibility of energy intake (MJ/kg DM) by ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Period stratum	3	4.750	1.583	0.93	
Period *Units* stratum					
Food	3	80.072	26.691	15.54	0.003
Pony	3	2.194	0.731	0.43	0.742
Residual	6	10.305	1.718		
Total:	15	97.321			

Appendix 7.23 Mean digestible energy intake (MJ/kg LW) by ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Period stratum	3	0.0036737	0.0012246	1.68	
Period *Units* stratum					
Food	3	0.0197892	0.0065964	9.03	0.012
Pony	3	0.0057507	0.0019169	2.63	0.145
Residual	6	0.0043814	0.007302		
Total:	15	0.0335949			

Appendix 7.24 Mean digestible energy intake (MJ/kg LW $^{0.75}$) by ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Period stratum	3	0.06802	0.002267	1.83	
Period *Units* stratum					
Food	3	0.37257	0.12419	10.03	0.009
0.009					
Pony	3	0.12482	0.04161	3.36	0.096
0.096					
Residual	6	0.07432	0.01239		
Total:	15	0.63972			

Appendix 7.25 Mean dry matter intake (kg/day) by ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Period stratum	3	2.3496	0.7832	1.99	
Period *Units* stratum					
Food	3	27.2107	9.0702	23.03	0.001
Pon	3	17.3535	5.7845	14.69	0.004
Residual	6	2.3629	0.3938		
Total:	15	49.2768			

Appendix 7.26 Mean digestible energy intake (MJ/day) by ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Period stratum	3	429.80	143.27	2.29	
Period *Units* stratum					
Food	3	2508.44	836.15	13.39	0.005
Pony	3	1309.59	436.53	6.99	0.022
0.022					
Residual	6	374.72	62.45		
Total:	15	4622.55			

Appendix 7.27 Mean digestible energy requirement (MJ/day) of ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Period stratum	3	2.200	0.733	0.58	
Period *Units* stratum					
Food	3	11.656	3.885	3.09	0.111
Pony	3	80.048	26.683	21.24	0.001
Residual	6	7.538	1.256		
Total:	15	101.442			

Appendix 7.28 Mean digestible crude protein intake (g/kg DM) by ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Period stratum	3	626.69	208.90	4.15	
Period *Units* stratum					
Food	3	22182.65	7394.22	46.85	0.001
Pony	3	41.37	13.79	0.27	0.842
Residual	5(1)	251.77	50.35		
Total:	14(1)	19822.68			

Appendix 7.29 Mean digestible crude protein intake (g/day) by ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Period stratum	3	37993.	12664	2.39	
Period *Units* stratum					
Food	3	360122.	120041	22.62	0.002
Pony	3	68759	22920.	4.32	0.075
Residual	5(1)	26540.	5308.		
Total:	14(1)	414006			

Appendix 7.30 Mean digestible energy intake as a proportion of digestible energy requirement by ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Period stratum	3	0.36662	0.12221	1.73	
Period *Units* stratum					
Food	3	1.97537	0.65846	9.30	0.011
Pony	3	0.62007	0.20669	2.92	0.122
Residual	6	0.42474	0.7079		
Total:	15	3.38679			

Appendix 7.31 Mean digestible crude protein requirement (g/day) by ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Period stratum	3	100.69	33.56	0.57	
Period *Units* stratum					
Food	3	551.69	183.90	3.15	0.108
Pony	3	3728.69	1242.90	21.28	0.001
Residual	6	350.37	58.40		
Total:	15	4731.44			

Appendix 7.32 Mean digestible crude protein intake as a proportion of digestible crude protein requirement of ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Period stratum	3	0.9633	0.3211	3.00	
Period *Units* stratum					
Food	3	8.3175	2.7735	25.87	0.002
Pony	3	0.8007	0.2669	2.49	0.175
Residual	5(1)	0.5358	0.1072		
Total:	14(1)	9.1494			

Appendix 7.33 Mean dry matter intake (g/kg LW) by ponies offered four types of conserved forage, hay (H), haylage (HY), big-bale silage (BB) and clamp silage (CS).

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Period stratum	3	19.365	6.455	1.49	
Period *Units* stratum	3	205.735	68.578	15.86	
Food	3	71.390	23.797	5.50	0.003
Pony	6	25.947	4.324		0.037
Residual	6	25.947			
Total:	15	322.437			

APPENDIX 8. Experiment 3.2.

Appendix 8.1 Dry matter intake of the three ponies offered three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Food	2	9.56238	4.78119	49.16	0.005
Pony	2	0.46199	0.23099	2.38	0.241
Residual	3(1)	0.29171	0.09724		
Total:	7(1)	8.25600			

Appendix 8.2 *In vivo* apparent digestibility of dry matter by ponies offered offered three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Food	2	117817.	58908.	16.97	0.023
Pony	2	8451.	4226.	1.22	0.410
Residual	3(1)	10414.	3471.		
Total:	7(1)	121109.			

Appendix 8.3 Live weight (kg) of the three ponies offered three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Food	2	5.56	2.78	0.14	0.871
Pony	2	3538.89	1769.44	91.00	0.001
Residual	4	77.78	19.44		
Total:	8	3622.22			

Appendix 8.4 *In vivo* apparent digestibility of organic matter by ponies offered three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Food	2	167396.	83698.	22.36	0.016
Pony	2	7814.	3907.	1.04	0.453
Residual	3(1)	11229.	3743.		
Total:	7(1)	160906.			

Appendix 8.5 *In vivo* apparent digestibility of crude protein by ponies offered three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Food	2	323112.	161556.	31.68	0.010
Pony	2	55875.	27937.	5.48	0.100
Residual	3(1)	15299.	5100.		
Total:	7(1)	393012.			

Appendix 8.6 *In vivo* apparent digestibility of acid detergent fibre by ponies offered three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Food	2	89606.	44803.	2.09	0.270
Pony	2	51308.	25654.	1.20	0.414
Residual	3(1)	64176.	21392.		
Total:	7(1)	203304.			

Appendix 8.7 *In vivo* apparent digestibility of neutral detergent fibre by ponies offered three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	**d.f (m.v)**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Food	2	220186.	110093.	11.11	0.041
Pony	2	25750.	12875.	1.30	0.392
Residual	3(1)	29735.	9912.		
Total:	7(1)	251390.			

Appendix 8.8 *In vivo* apparent digestibility of gross energy by ponies offered three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	**d.f m.v)**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Food	2	132021.	66010.	27.33	0.012
Pony	2	9583.	4792.	1.98	0.283
Residual	3(1)	7246.	2415.		
Total:	7(1)	132200.			

Appendix 8.9 Digestible energy intake (g/kg DM) by ponies offered three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	**d.f (m.v)**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Food	2	19.2682	9.6341	14.63	0.028
Pony	2	3.4326	1.7163	2.61	0.221
Residual	3(1)	1.9761	0.6587		
Total:	7(1)	23.5382			

Appendix 8.10 Crude protein intake (g/kg DM) by ponies offered three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	**d.f (m.v)**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Food	2	2246.52	1123.26	33.57	0.009
Pony	2	369.28	184.64	5.52	0.099
Residual	3(1)	100.38	33.46		
Total:	7(1)	2700.49			

Appendix 8.11 *In vivo* apparent digestibility of rhamnose by ponies offered three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Food	2	1950496.19	975248.09	1.42E+04	.001
Pony	2	103.34	51.67	0.75	0.544
Residual	3(1)	206.67	68.89		
Total:	7(1)	1463192.51			

Appendix 8.12 *In vivo* apparent digestibility of arabinose by ponies offered three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Food	2	309931.	154966.	15.65	0.026
Pony	2	4356.	2178.	0.22	0.814
Residual	3(1)	29699.	9900.		
Total:	7(1)	285274.			

Appendix 8.13 *In vivo* apparent digestibility of xylose by ponies offered three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Food	2	207526.	103763.	3.99	0.143
Pony	2	78754.	39377.	1.52	0.351
Residual	3(1)	77934.	25978		
Total:	7(1)	312625.			

Appendix 8.14 *In vivo* apparent digestibility of mannose by ponies offered three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Food	2	382806.	191403.	1.19	0.456
Pony	2	249322.	124661.	0.78	0.563
Residual	2(2)	320943.	160471.		
Total:	6(2)	949065.			

Appendix 8.15 *In vivo* apparent digestibility of galactose by ponies offered three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Food	2	445478.	222739.	12.40	0.035
Pony	2	9052.	4526.	0.25	0.792
Residual	3(1)	53895.	17965.		
Total:	7(1)	428899.			

Appendix 8.16 *In vivo* apparent digestibility of glucose by ponies offered three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Food	2	282247.	141123.	6.07	0.088
Pony	2	6085.	3042.	0.13	0.882
Residual	3(1)	69728.	23243.		
Total:	7(1)	285659.			

Appendix 8.17 *In vivo* apparent digestibility of uronic acids by ponies offered three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Food	2	3704097.	1852049.	29.35	0.011
Pony	2	1710095.	855047.	13.55	0.031
Residual	3(1)	189323.	63108.		
Total:	7(1)	5534867.			

Appendix 8.18 *In vivo* apparent digestibility of total non-starch polysaccharides by ponies offered three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Food	2	314611.	157306.	16.90	0.023
Pony	2	10541.	5270.	0.57	0.619
Residual	3(1)	27924.	9308.		
Total:	7(1)	283766.			

Appendix 8.19 pH of caecal fluid measured hourly, from ponies offered three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	**d.f (m.v)**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Pony stratum	2	0.08076	0.04038	2.45	
Period stratum	2	0.00345	0.00172	0.10	
Pony.period.time stratum					
Time	1	0.17307	0.17307	10.52	0.018
Food	2	0.76880	0.38440	23.36	0.001
Time. Food	2	0.27236	0.13618	8.28	0.019
Residual	6(2)	0.09873	0.01645		
Total:	15(2)	1.38920			

Appendix 8.20 Total volatile fatty acid content of caecal fluid measured hourly, from ponies offered three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	**d.f (m.v)**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Pony stratum	2	196.48	98.24	1.33	
Period stratum	2	290.41	145.20	1.97	
Pony.period.time stratum					
Time	1	60.62	60.62	0.82	0.400
Food	2	2191.83	1095.91	14.86	0.005
Time. Food	2	497.10	248.55	3.37	0.104
Residual	6(2)	442.39	73.73		
Total:	15(2)	3522.15			

Appendix 8.21 Proportion of acetate (mmol/mol) in caecal fluid measured hourly from ponies offered three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	1651.3	825.7	0.97	
Period stratum	2	4074.3	2037.2	2.40	
Pony.period.time stratum					
Time	1	503.3	503.3	0.59	0.471
Food	2	15512.6	7756.3	9.14	0.015
Time. Food	2	591.0	295.5	0.35	0.719
Residual	6(2)	5093.4	848.9		
Total:	15(2)	21027.2			

Appendix 8.22 Proportion of propionate (mmol/mol) in caecal fluid measured hourly from ponies offered three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	475.5	237.8	0.36	
Period stratum	2	3280.4	1640.2	2.45	
Pony.period.time stratum					
Time	1	813.2	813.2	1.22	0.312
Food	2	17710.9	8855.4	13.24	0.006
Time. Food	2	52.4	26.2	0.04	0.962
Residual	6(2)	4013.9	669.0		
Total:	15(2)	22357.5			

Appendix 8.23 Proportion of butyrate (mmol/mol) in caecal fluid measured hourly from ponies offered three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	357.1	178.5	1.58	
Period stratum	2	171.7	85.9	0.76	
Pony.period.time stratum					
Time	1	37.0	37.0	0.33	0.588
Food	2	1728.2	864.1	7.64	0.022
Time. Food	2	295.3	147.6	1.30	0.338
Residual	6(2)	678.8	113.1		
Total:	15(2)	2982.5			

Appendix 8.24 pH content of caecal fluid measured 5 hours post-fooding in ponies fed three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	0.10839	0.05420	0.98	
Period stratum	2	0.00008	0.00004	0.00	
Pony.period.time stratum					
Food	2	0.14524	0.07262	1.32	0.289
Day	3	0.17816	0.05939	1.08	0.380
Residual	21(5)	1.5596	0.05505		
Total:	30(5)	1.55231			

Appendix 8.25 Total volatile fatty acid content of caecal fluid measured 5 hours post-fooding in ponies fed three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	40.0	20.0	0.06	
Period stratum	2	162.0	81.3	0.26	
Pony.period.time stratum					
Food	2	2531.0	1265.5	3.97	0.034
Day	2	1739.3	579.8	1.82	0.173
Residual	22(4)	7004.2	318.4		
Total:	31(4)	10752.0			

Appendix 8.26 Proportion of acetate (mmol/mol) in caecal fluid measured 5 hours post-fooding in ponies fed three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	6217.	3108.	1.30	
Period stratum	2	27070.	13535.	5.65	
Pony.period.time stratum					
Food	2	12478.	6239.	2.60	0.097
Day	3	7227.	2409.	1.01	0.409
Residual	22(4)	52715.	2396.		
Total:	31(4)	102181.			

Appendix 8.27 Proportion of propionate (mmol/mol) in caecal fluid measured 5 hours post-fooding in ponies fed three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	1864.	932.	0.42	
Period stratum	2.	17004.	8502.	3.84	
Pony.period.time stratum					
Food	2	28672.	14336.	6.47	0.006
Day	3	9056.	3019.	1.36	.280
Residual	22(4)	48733.	2215.		
Total:	31(4)	98895.			

Appendix 8.28 Proportion of butyrate (mmol/mol) in caecal fluid measured 5 hours post-fooding in ponies fed three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	1661.5	830.8	3.13	
Period stratum	2	2941.2	1470.6	5.55	
Pony.period.time stratum					
Food	2	5772.4	2886.2	10.89	0.001
Day	3	1761.9	587.3	2.22	0.115
Residual	22(4)	5832.0	265.1		
Total:	31(4)	16432.4			

Appendix 8.29 Lactate content (mmol/l) in caecal fluid measured 5 hours post-fooding in ponies fed three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	29.424	14.712		
Period stratum	2	10.801	5.400	0.76	
Pony.period.time stratum					
Food	2	80.661	40.331	5.66	0.010
Day	3	15.809	5.270	0.74	0.540
Residual	22(4)	156.789	7.127		
Total:	31(4)	289.144			

Appendix 8.30 **L**actate levels (mmol/l) in caecal fluid measured hourly in ponies fed three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	**d.f (m.v)**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Pony stratum	2	0.6685	0.3343	1.41	
Period stratum	2	1.2189	0.6095	2.57	
Pony.period.time stratum					
Time	1	8.0093	8.0093	33.74	0.001
Food	2	26.7778	13.3889	56.39	0.001
Time.Food	2	16.2767	8.1384	34.28	0.001
Residual	6(2)	1.4245	0.2374		
Total:	15(2)	51.7003			

Appendix 8.31 *In vivo* apparent digestibility of starch by ponies offered three botanically diverse fibrous diets, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	**d.f (m.v)**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Food	2	39324.	19662.	8.91	0.55
Pony	2	2195.	1097.	0.50	0.651
Residual	3(1)	6616.	2205.		
Total:	7(1)	46624.			

APPENDIX 9. Experiment 3.3.

Appendix 9.1 Dry matter volume (kg) in the caecum of ponies offered three botanically diverse fibrous foods, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Food	2	0.08780	0.04390	2.22	0.256
Pony	2	0.13606	0.06803	3.44	0.167
Residual	3(1)	0.05933	0.01978		
Total	7(1)	0.28309			

Appendix 9.2 Digesta passage rate per hour through the caecae of ponies offered three botanically diverse fibrous foods, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Food	2	293.1	146.6	1.45	0.362
Pony	2	9.8	4.9	0.05	0.953
Residual	3(1)	303.1	101.0		
Total	7(1)	551.6			

Appendix 9.3 Mean retention of digesta in the caecae of ponies offered three botanically diverse fibrous foods, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Food	2	6.971	3.485	1.46	0.362
Pony	2	0.948	0.474	0.20	0.830
Residual	3(1)	7.184	2.395		
Total	7(1)	13.777			

Appendix 9.4 R^2 values for actual vs fitted marker concentrations in the caecae of ponies offered three botanically diverse fibrous foods, hay cubes (HC), sugar beet food (SBF) and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	d.f (m.v)	s.s.	m.s.	v.r.	F pr.
Food	2	0.063230	0.031615	8.99	0.054
Pony	2	0.014765	0.007382	2.10	0.269
Residual	3(1)	0.010549	0.003516		
Total	7(1)	0.078474			

Appendix 9.5 Total tract mean retention time measured using ytterbium marked food when ponies were offered two botanically diverse fibrous foods, hay cubes (HC), and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Pony stratum	2	900.74	450.37	15.77	
Pony.*Units* stratum					
Food	1	1189.66	1189.66	41.65	<.001
Model	6	334.80	55.80	1.95	0.110
Food.model	6	8.96	1.49	0.05	0.999
Residual	26	742.61	28.56		
Total	41	3176.77			

Appendix 9.6 Large intestine mean retention time measured using chromium marked food when ponies were offered two botanically diverse fibrous foods, hay cubes (HC), and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Pony stratum	2	1025.284	512.642	87.41	
Pony *Units* stratum					
Food	1	1097.424	1097.424	187.13	<.001
Model	6	68.949	11.492	1.96	0.109
Food model	6	13.237	2.206	0.38	0.887
Residual	26	152.480	5.865		
Total	41	2357.374			

Appendix 9.7 Goodness of fit (R^2) values for 5 mathematical models fitted to faecal excretion data obtained from ponies given a caecal pulse dose of chromium marked food and fed two botanically diverse fibrous foods, hay cubes (HC), and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Pony stratum	2	0.047706	0.0233853	13.94	
Pony *Units* stratum					
Model	4	0.006774	0.001694	0.99	0.438
Food	1	0.029453	0.029453	17.21	<.001
Model food	4	0.003426	0.000856	0.50	0.736
Residual	18	0.030803	0.001711		
Total	29	0.118163			

Appendix 9.8 Goodness of fit (R^2) values for 5 mathematical models fitted to faecal excretion data obtained from ponies given an oral pulse dose of ytterbium marked food and fed two botanically diverse fibrous foods, hay cubes (HC), and a 67:33 mix of oat hulls:naked oats (OH:NO).

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Pony stratum	2	0.025464	0.012732	4.63	
Pony Units stratum					
Model	4	0.041419	0.010355	3.77	0.021
Food	1	0.018650	0.018650	6.79	0.018
Model food	4	0.005181	0.001295	0.47	0.756
Residual	18	0.049476	0.002749		
Total	29	0.140191			

Appendix 9.9 Goodness of fit (R^2) values for 6 mathematical models fitted to faecal excretion data obtained from ponies given an oral pulse dose of ytterbium marked food and fed a 50:50 mix of sugar beet:hay cubes.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Pony stratum	2	0.011383	0.005692	3.29	
Pony *Units* stratum					
Model	5	0.008568	0.001714	0.99	0.470
Residual	10	0.017303	0.001730		
Total	17	0.037254			

Appendix 9.10 Total tract mean retention time measured using ytterbium marked food when ponies were a 50:50 mix of sugar beet: hay cubes.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Pony stratum	2	398.041	199.021	38.35	
Pony Units stratum					
Model	7	81.021	11.574	2.23	0.095
Residual	14	72.660	5.190		
Total	23	551.722			

Appendix 9.11 Goodness of fit (R^2) values for 6 mathematical models fitted to faecal excretion data obtained from ponies given a caecal pulse dose of chromium marked food and fed a 50:50 mix of sugar beet:hay cubes.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Pony stratum	2	0.046627	0.023313	14.22	
Pony *Units* stratum					
Model	5	0.012898	0.002580	1.57	0.253
Residual	10	0.016400	0.001640		
Total	17	0.075924			

Appendix 9.12 Large intestine mean retention time measured using chromium marked food when ponies were offered a 50:50 mix of sugar beet:hay cubes.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Pony stratum	2	67.252	33.626	24.12	
Pony *Units* stratum					
Model	7	25.742	3.677	2.64	0.058
Residual	14	19.517	1.394		
Total	23	112.511			

APPENDIX 10. Experiment 3.4.

Appendix 10.1 Losses of OM (g/kg) from hay cubes,, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags given a cold-water rinse in an automatic washing machine.

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	298.1	149.0	0.28	
Rep *Units* stratum					
Food	3	73712.4	24570.8	46.22	<.001
Residual	6	3189.5	531.6		
Total:	11	77200.0			

Appendix 10.2 Losses of CP (g/kg) from hay cubes,, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags given a cold-water rinse in an automatic washing machine.

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1750.	875.	0.42	
Rep *Units* stratum					
Food	3	735019.	245006.	117.22	<.001
Residual	6	12541.	2090.		
Total:	11	749310.			

Appendix 10.3 Losses of ADF (g/kg) from hay cubes,, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags given a cold-water rinse in an automatic washing machine.

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	2623.	1312.	0.26	
Rep *Units* stratum					
Food	3	35119.	11706.	2.28	0.180
Residual	6	30844.	5141.		
Total:	11	68587.			

Appendix 10.4 Losses of NDF (g/kg) from hay cubes,, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags given a cold-water rinse in an automatic washing machine.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Rep stratum	2	3001.	1501.	1.39	
Rep *Units* stratum					
Food	3	52065.	17355.	16.03	0.003
Residual	6	6497.	1083.		
Total:	11	61563.			

Appendix 10.5 Losses of rhamnose (g/kg) from hay cubes,, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags given a cold- water rinse in an automatic washing machine.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Rep stratum	2	124071.	62035.	0.89	
Rep *Units* stratum					
Food	3	1160522.	386841.	5.57	0.036
Residual	6	416954.	69492.		
Total:	11	1701547.			

Appendix 10.6 Losses of arabinose (g/kg) from hay cubes,, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags given a cold- water rinse in an automatic washing machine.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Rep stratum	2	3355.4	1677.7	6.09	
Rep *Units* stratum					
Food	3	19344.0	6448.0	23.42	0.001
Residual	6	1651.7	275.3		
Total:	11	24351.1			

Appendix 10.7 Losses of xylose (g/kg) from hay cubes,, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags given a cold- water rinse in an automatic washing machine.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Rep stratum	2	2453.	1227.	0.27	
Rep *Units* stratum					
Food	6	42875.	14292.	3.14	0.109
Residual	6	27348.	4558.		
Total:	11	72676.			

Appendix 10.8 Losses of mannose (g/kg) from hay cubes,, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags given a cold- water rinse in an automatic washing machine.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Rep stratum	2	31951.	15976.	0.77	
Rep *Units* stratum					
Food	3	1221655.	407218.	19.51	0.002
Residual	6	125225.	20871.		
Total:	11	1378831.			

Appendix 10.9 Losses of galactose (g/kg) from hay cubes,, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags given a cold- water rinse in an automatic washing machine.

ource of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Rep stratum	2	1743.8	871.9	1.23	
Rep *Units* stratum					
Food	3	193001.4	64333.8	91.01	<.001
Residual	6	4241.5	706.9		
Total:	11	198986.7			

Appendix 10.10 Losses of glucose (g/kg) from hay cubes,, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags given a cold- water rinse in an automatic washing machine.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Rep stratum	2	1386.6	693.3	1.00	
Rep *Units* stratum					
Food	3	37664.3	12554.8	18.13	0.002
Residual	6	4154.1	692.4		
Total:	11	43205.1			

Appendix 10.11 Losses of uronic acids (g/kg) from hay cubes,, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags given a cold- water rinse in an automatic washing machine.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Rep stratum	2	7871.	3935.	0.64	
Rep *Units* stratum					
Food	3	278693.	92898.	15.13	0.003
Residual	6	36838.	6140.		
Total:	11	323402.			

Appendix 10.12 Losses of TNSP (g/kg) from hay cubes,, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags given a cold- water rinse in an automatic washing machine.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Rep stratum	2	2586.8	1293.4	1.46	
Rep *Units* stratum					
Food	3	33819.5	11273.2	12.71	0.005
Residual	6	5322.0	887.0		
Total:	11	41728.4			

Appendix 10.13 Losses of DM (g/kg) from hay cubes,, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags given a cold-water rinse in an automatic washing machine.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Pony stratum	2	444.1	222.1	0.51	
Pony *Units* stratum					
Food	3	78792.6	26264.2	60.25	<.001
Residual	6	2615.5	435.9		
Total:	11	81852.2			

Appendix 10.14 Losses of DM (g/kg) from hay cubes,, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine of ponies

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Pony stratum	2	1249.3	624.6	1.52	
Pony *Units* stratum					
Food	3	44557.8	14852.6	36.25	<.001
Residual	6	2458.7	409.8		
Total:	11	48265.7			

Appendix 10.15 Transit time in hours of mobile bags bags containing hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, after passing through the small intestine of ponies.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Pony stratum	2	2.9054	1.4527	1.60	
Pony *Units* stratum					
Food	3	3.2578	1.0859	1.20	0.388
Residual	6	5.4412	0.9069		
Total:	11	11.6044			

Appendix 10.16 Losses of DM (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the total tract of ponies.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Pony stratum	2	4002.7	2001.3	3.46	
Pony *Units* stratum					
Food	3	246266.8	82088.9	142.04	<.001
Residual	6	3467.5	577.9		
Total:	11	253737.0			

Appendix 10.17 Transit time in hours of mobile bags containing hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, after passing through the total tract of ponies.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Pony stratum	2	504.08	252.04	11.38	
Pony *Units* stratum					
Food	3	236.92	78.97	3.57	0.087
Residual	6	132.87	22.14		
Total:	11	873.86			

Appendix 10.18 Losses of DM (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine and total tract of ponies.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Pony stratum	2	532,0	266.0	0.35	
Pony *Units* stratum					
Food	3	41508.6	13836.2	18.20	<.001
Site	1	778608.3	778608.3	1023.90	<.001
Food Site	3	249316.0	83105.3	109.29	<.001
Residual	14	10646.1	760.4		
Total:	23	1080611.0			

Appendix 10.19 Losses of OM (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine and total tract of ponies.

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	918.0	459.0	0.51	
Pony *Units* stratum					
Food	3	56459.4	18819.8	20.77	<.001
Site	1	829754.0	829754.0	915.92	<.001
Food site	3	271717.0	90572.3	99.98	<.001
Residual	13(1)	11777.0	905.9		
Total:	22(1)	1091099.1			

Appendix 10.20 Losses of CP (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine and total tract of ponies.

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	8568.	4284.	0.69	
Pony *Units* stratum					
Food	3	289645.	96548.	15.54	<.001
Site	1	231536.	231536.	37.28	<.001
Food Site	3	145127.	48376.	7.79	0.003
Residual	14	86959.	6211.		
Total:	23	761835.			

Appendix 10.21 Losses of ADF (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine and total tract of ponies.

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	25588.	12794.	3.32	
Pony *Units* stratum					
Food	3	234446.	78149.	20.29	<.001
Site	1	769360.	769360.	199.76	<.001
Food site	3	156039.	52013.	13.51	<.001
Residual	13(1)	50067.	3851.		
Total:	22(1)	1153430.			

Appendix 10.22 Losses of NDF (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine and total tract of ponies.

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	4899.	2449.	1.32	
Pony *Units* stratum					
Food	3	384212.	128071.	68.91	<.001
Site	1	1019041.	1019041.	548.27	<.001
Food Site	3	164589.	54863.	29.52	<.001
Residual	14	26021.	1859.		
Total:	23	1598762.			

Appendix 10.23 Losses of rhamnose (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine and total tract of ponies.

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	131376.	65688.	1.07	
Pony *Units* stratum					
Food	3	1836233.	612078.	9.96	<.001
Site	1	2483267.	2483267.	40.42	<.001
Food Site	3	1779598.	593199.	9.65	<.001
Residual	14	860186.	61442.		
Total:	23	7090660.			

Appendix 10.24 Losses of arabinsoe (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine and total tract of ponies.

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	60426.	30213.	4.45	
Pony *Units* stratum					
Food	3	1 45731.	48577.	7.15	0.004
Site	1	2047271.	2047271.	301.28	<.001
Food Site	3	256357.	85452.	12.58	<.001
Residual	14	95132.	6795.		
Total:	23	2604916.			

Appendix 10.25 Losses of xylose (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine and total tract of ponies.

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	61362.	30681.	1.99	
Pony *Units* stratum					
Food	3	161362.	53787.	3.49	0.044
Site	1	1801440.	1801440.	116.97	<.001
Food Site	3	233914.	77971.	5.06	0.014
Residual	14	215604.	15400.		
Total:	23	2473682.			

Appendix 10.26 Losses of mannose (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine and total tract of ponies.

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	30704.	15352.	2.50	
Pony *Units* stratum					
Food	3	833621.	277874.	45.23	<.001
Site	1	1480712.	1480712.	241.02	<.001
Food Site	3	564517.	188172.	30.63	<.001
Residual	14	86009.	6143.		
Total:	23	2995564.			

Appendix 10.27 Losses of galactose (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine and total tract of ponies.

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	37682.	18841.	2.78	
Pony *Units* stratum					
Food	3	35660.	11887.	1.76	0.202
Site	1	1965908.	1965908.	290.49	<.001
Food Site	3	395433.	131811.	19.48	<.001
Residual	14	94747.	6768.		
Total:	23	2529429.			

Appendix 10.28 Losses of glucose (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine and total tract of ponies.

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	71311.	35656.	3.10	
Pony *Units* stratum					
Food	3	75564.	25188.	2.19	0.135
Site	1	2177495.	2177495.	189.18	<.001
Food Site	3	303420.	101140.	8.79	0.002
Residual	14	161140.	11510.		
Total:	23	2788931.			

Appendix 10.29 Losses of uronic acids (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine and total tract of ponies.

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	34361.	17810.	0.35	
Pony *Units* stratum					
Food	3	2206382.	735461.	15.05	<.001
Site	1	1746793.	1746793.	35.75	<.001
Food Site	3	249726.	83242.	1.70	0.212
Residual	14	684119.	48866.		
Total:	23	4921381.			

Appendix 10.30 Losses of TNSP (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine and total tract of ponies.

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	54986.	27493.	4.21	
Pony *Units* stratum					
Food	3	292435.	97478.	14.93	<.001
Site	1	1760633.	1760633.	269.59	<.001
Food Site	3	158468.	52823.	8.09	0.002
Residual	14	91433.	6531.		
Total:	23	2357955.			

Appendix 10.31 Variation in parameter a obtained from the Ørskov and McDonald (1979) degradation equation, for hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the total tract of ponies.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Food	3	2444.5	814.8	0.83	0.523
Pony	2	173.5	86.7	0.09	0.916
Residual	6	5868.6	978.1		
Total:	11	8486.5			

Appendix 10.32 Variation in parameter b obtained from the Ørskov and McDonald (1979) degradation equation, for hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the total tract of ponies.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Food	3	1716.7	572.2	1.18	0.393
Pony	2	233.9	117.0	0.24	0.793
Residual	6	2910.5	485.1		
Total	11	4861.2			

Appendix 10.33 Variation in parameter c obtained from the Ørskov and McDonald (1979) degradation equation, for hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the total tract of ponies.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Food	3	0.0013582	0.0004527	0.80	0.535
Pony	2	0.0003485	0.0001743	0.31	0.745
Residual	6	0.0033753	0.0005625		
Total:	11	0.0050820			

Appendix 10.34 Variation in parameters a+b obtained from the Ørskov and McDonald (1979) degradation equation, for hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the total tract of ponies.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Food	3	715.6	238.5	1.25	0.372
Pony	2	84.0	42.0	0.22	0.809
Residual	6	1146.0	191.0		
Total:	11	1945.7			

Appendix 10.35 Variation effective degradability at a MRT of 10 hours obtained from the Ørskov and McDonald (1979) degradation equation, for hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the total tract of ponies.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Food	3	1531.6	510.5	1.59	0.287
Pony	2	91.7	45.8	0.14	0.870
Residual	6	1923.5	320.6		
Total:	11	3546.7			

Appendix 10.36 Variation effective degradability at a MRT of 20 hours obtained from the Ørskov and McDonald (1979) degradation equation, for hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the total tract of ponies.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Food	3	1322.1	440.7	2.79	0.131
Pony	2	65.2	32.6	0.21	0.819
Residual	6	946.3	157.7		
Total:	11	2333.7			

Appendix 10.37 Variation effective degradability at a MRT of 40 hours obtained from the Ørskov and McDonald (1979) degradation equation, for hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the total tract of ponies.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Food	3	1371.79	452.26	6.60	0.025
Pony	2	33.71	16.86	0.24	0.791
Residual	6	415.67	69.28		
Total:	11	1821.18			

Appendix 10.38 Variation effective degradability at a MRT of 60 hours obtained from the Ørskov and McDonald (1979) degradation equation, for hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the total tract of ponies.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Food	3	1518.71	506.24	10.89	0.008
Pony	2	19.51	9.76	0.21	0.816
Residual	6	278.93	46.49		
Total:	11	1817.15			

Appendix 10.39 Losses of DM (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine of ponies expressed as a proportion of total tract losses.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Pony stratum	2	149.08	75.54	4.57	
Pony *Units* stratum					
Food	3	4726.56	1575.52	96.57	<.001
Residual	6	97.89	16.32		
Total:	11	4973.53			

Appendix 10.40 Losses of OM (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine of ponies expressed as a proportion of total tract losses.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Pony stratum	2	163.81	81.90	3.69	
Pony *Units* stratum					
Food	3	4491.30	1497.10	67.53	<.001
Residual	5(1)	110.85	22.17		
Total:	10(1)	4285.19			

Appendix 10.41 Losses of CP (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine of ponies expressed as a proportion of total tract losses.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Pony stratum	2	1023.2	511.6	4.39	
Rep *Units* stratum					
Food	3	5023.2	1674.4	14.38	0.004
Residual	6	698.9	116.5		
Total:	11	6745.2			

Appendix 10.42 Losses of ADF (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine of ponies expressed as a proportion of total tract losses.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Pony stratum	2	376,6	188.3	0.84	
Pony *Units* stratum					
Food	3	980.4	326.8	1.46	0.331
Residual	5(1)	1119.6	223.9		
Total:	10(1)	2430.4			

Appendix 10.43 Losses of NDF (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine of ponies expressed as a proportion of total tract losses.

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	755.1	377.5	1.29	
Pony *Units* stratum					
Food	3	617.8	205.9	0.70	0.585
Residual	6	1760.6	293.4		
Total:	11	3133.5			

Appendix 10.44 Losses of rhamnose (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine of ponies expressed as a proportion of total tract losses.

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	3973.	1987.	1.10	
Pony *Units* stratum					
Food	3	24752.	8251.	4.55	0.055
Residual	6	10884.	1814.		
Total:	11	39609.			

Appendix 10.45 Losses of arabinose (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine of ponies expressed as a proportion of total tract losses.

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	2636.42	1318.21	13.84	
Pony *Units* stratum					
Food	3	1161.69	387.23	4.06	0.068
Residual	6	571.63	95.27		
Total:	11	4369.74			

Appendix 10.46 Losses of xylose (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine of ponies expressed as a proportion of total tract losses.

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	9003.	4501.	2.08	
Pony *Units* stratum					
Food	3	5937.	1979.	0.91	0.489
Residual	6	13005.	2167.		
Total:	11	27945.			

Appendix 10.47 Losses of mannose (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine of ponies expressed as a proportion of total tract losses.

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	505.1	252.5	1.95	
Pony *Units* stratum					
Food	3	14081.8	4693.9	36.19	<.001
Residual	6	778.1	129.7		
Total:	11	15365.0			

Appendix 10.48 Losses of galactose (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine of ponies expressed as a proportion of total tract losses.

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	1407.1	703.6	5.64	
Pony *Units* stratum					
Food	3	4510.3	1503.4	12.05	0.006
Residual	6	748.7	124.8		
Total:	11	6666.1			

Appendix 10.49 Losses of glucose (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine of ponies expressed as a proportion of total tract losses.

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	5003.0	2501.5	7.76	
Pony *Units* stratum					
Food	3	3547.7	1182.6	3.67	0.082
Residual	6	1935.3	322.6		
Total:	11	10486.0			

Appendix 10.50 Losses of uronic acids (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine of ponies expressed as a proportion of total tract losses.

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	1741.	870.		
Pony *Units* stratum					
Food	3	40139.	13380.	6.63	0.025
Residual	6	12100.	2017.		
Total:	11	53979.			

Appendix 10.51 Losses of TNSP (g/kg) from hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the small intestine of ponies expressed as a proportion of total tract losses.

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Pony stratum	2	4836.6	2418.3	5.49	
Pony *Units* stratum					
Food	3	68.5	22.8	0.05	0.983
Residual	6	2643.3	440.6		
Total:	11	7548.4			

Appendix 10.52 Variation in particle size between hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the washing machine, small intestine and total tract of ponies.

Source of variation	**d.f**	**s.s**	**m.s**	**v.r**	**F pr**
foods	3	60875	20292	16.11	0.003
Foods	2	977	488	0.39	0.694
Reps	6	7557	1259		
Total	11	69408			

Appendix 10.53 Variation in water holding capacity between hay cubes, plain sugar beet pulp, Soya hulls and a 67:33 mix of oat hulls:naked oats, contained in polyester mesh bags after passing through the washing machine, small intestine and total tract of ponies.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Foods	3	32.67830	10.89277	283.11	<.001
Reps	2	0.07695	0.03847	1.00	0.422
Residual	6	0.023085	0.03848		
Total:	11	32.98610			

APPENDIX 11. Experiment 3.5.

Appendix 11.1 Variation in parameter A derived from the France *et al.* (1993) equation applied to gas production data obtained from incubating hay, a 75:25 mix of hay:sugar beet, a 50:50 mix of hay:sugar beet a 25:75 mix of hay:sugar beet and sugar beet, with pony faecal inoculum for 135 and 49 hours and in the presence or absence of added nitrogen.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Time stratum	1	36116.9	36116.9	154.33	
Time.units.stratum					
Treat	1	538.1	538.1	2.30	0.136
Food	4	138007.4	34501.9	147.43	<0.001
Lin	1	137422.1	137422.1	587.2	<0.001
Quad	1	19.1	19.1	0.08	0.777
Deviations	2	566.3	283.1	1.21	0.307
Treat food	4	1180.7	295.2	1.26	0.298
Lin	1	888.3	888.3	3.80	0.057
Quad	1	19.2	19.2	0.08	0.776
Deviations	2	273.1	136.6	0.58	0.562
Residual	49	11467.5	234.0		
Total	59	187310.6			

Appendix 11.2 Variation in parameter B derived from the France *et al.* (1993) equation applied to gas production data obtained from incubating hay, a 75:25 mix of hay:sugar beet, a 50:50 mix of hay:sugar beet a 25:75 mix of hay:sugar beet and sugar beet, with pony faecal inoculum for 135 and 49 hours and in the presence or absence of added nitrogen.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Time stratum	1	161897.9	547.3	330.98	
Time.units.stratum					
Treat	1	547.3	2785.6	1.12	0.295
Food	4	11142.5	8643.9	5.69	<0.001
Lin	1	8643.9	2312.0	17.67	<0.001
Quad	1	2312.0	93.3	4.73	0.035
1Deviations	2	186.6	367	0.19	0.827
Treat food	4	1469.7	4	0.75	0.562
Lin	1	717.8	717.8	1.47	0.232
Quad	1	1.8	1.8	0.00	0.952
Deviations	2	750.1	375.0	0.77	0.470
Residual	49	23968.5	489.2		
Total	59	199025.8			

Appendix 11.3 Variation in parameter Q derived from the France *et al.* (1993) equation applied to gas production data obtained from incubating hay, a 75:25 mix of hay:sugar beet, a 50:50 mix of hay:sugar beet a 25:75 mix of hay:sugar beet and sugar beet, with pony faecal inoculum for 135 and 49 hours and in the presence or absence of added nitrogen.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Time stratum	1	0.0504304	0.0504304	214.42	
Time.units.stratum					
Treat	1	0.0004251	0.0004251	1.81	0.185
Food	4	0.0376954	0.0094239	40.07	<0.001
Lin	1	0.0272541	0.0272541	115.88	<0.001
Quad	1	0.0094595	0.0094595	40.22	<0.001
1Deviations	2	0.0009819	0.0004909	2.09	0.135
Treat food	4	0.0001141	0.0000285	0.12	0.974
Lin	1	0.0000257	0.0000257	0.11	0.742
Quad	1	0.0000305	0.0000305	0.13	0.721
Deviations	2	0.0000580	0.0000290	0.12	0.884
Residual	49	0.0115247	0.0002352		
Total	59	0.1001898			

Appendix 11.4 Variation in parameter b derived from the France *et al.* (1993) equation applied to gas production data obtained from incubating hay, a 75:25 mix of hay:sugar beet, a 50:50 mix of hay:sugar beet a 25:75 mix of hay:sugar beet and sugar beet, with pony faecal inoculum for 135 and 49 hours and in the presence or absence of added nitrogen.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Time stratum	1	0.0595791	0.0595791	212.76	
Time.units.stratum					
Treat	1	0.0004913	0.0004913	1.75	0.191
Food	4	0.0433137	0.0108284	38.67	<0.001
Lin	1	0.0309155	0.0309155	110.40	<0.001
Quad	1	0.0111150	0.0111150	39.69	<0.001
1Deviations	2	0.0012832	0.0006416	2.29	0.112
Treat food	4	0.0000954	0.0000239	0.09	0.987
Lin	1	0.0000164	0.0000164	0.06	0.810
Quad	1	0.0000332	0.0000332	0.12	0.732
Deviations	2	0.0000459	0.0000229	0.08	0.922
Residual	49	0.0137212	0.0002800		
Total	59	0.1172008			

Appendix 11.5 Variation in parameter c derived from the France *et al.* (1993) equation applied to gas production data obtained from incubating hay, a 75:25 mix of hay:sugar beet, a 50:50 mix of hay:sugar beet a 25:75 mix of hay:sugar beet and sugar beet, with pony faecal inoculum for 135 and 49 hours and in the presence or absence of added nitrogen.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Time stratum	1	1.68555	1.68555	168.33	
Time.units.stratum					
Treat	1	0.00297	0.00297	0.30	0.589
Food	4	1.23579	0.30895	30.85	<0.001
Lin	1	0.91270	0.91270	91.15	<0.001
Quad	1	0.31997	0.31997	31.95	<0.001
1Deviations	2	0.00312	0.00156	0.16	0.856
Treat food	4	0.00195	0.00049	0.05	0.995
Lin	1	0.00000	0.00000	0.00	0.993
Quad	1	0.00001	0.00001	0.00	0.977
Deviations	2	0.00194	0.00097	0.10	0.908
Residual	49	0.49065	0.01001		
Total	59				

Appendix 11.6 Variation in lag time derived from the France *et al.* (1993) equation applied to gas production data obtained from incubating hay, a 75:25 mix of hay:sugar beet, a 50:50 mix of hay:sugar beet a 25:75 mix of hay:sugar beet and sugar beet, with pony faecal inoculum for 135 and 49 hours and in the presence or absence of added nitrogen.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Time stratum	1	53.9412	53.9412	73.98	
Time.units.stratum					
Treat	1	0.1717	0.1717	0.24	0.630
Food	4	49.0777	12.2694	16.83	<0.001
Lin	1	28.3435	28.3435	38.88	<0.001
Quad	1	7.8434	7.8434	10.76	0.002
1Deviations	2	12.8908	6.4454	8.84	<0.001
Treat food	4	1.8289	0.4572	0.63	0.645
Lin	1	0.0418	0.0418	0.06	0.812
Quad	1	0.4630	0.4630	0.64	0.429
Deviations	2	1.3240	0.6620	0.91	0.410
Residual	49	35.7254	0.7291		
Total	59				

Appendix 11.7 Variation in the fractional rate of gas production derived from the France *et al.* (1993) equation applied to gas production data obtained from incubating hay, a 75:25 mix of hay:sugar beet, a 50:50 mix of hay:sugar beet a 25:75 mix of hay:sugar beet and sugar beet, with pony faecal inoculum for 135 and 49 hours and in the presence or absence of added nitrogen.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Time stratum	1	0.00981760	0.00981760	269.66	
Time.units.stratum					
Treat	1	0.00015010	0.00015010	4.12	0.048
Food	4	0.00732605	0.00183151	50.31	<0.001
Lin	1	0.00455840	0.00455840	125.21	<0.001
Quad	1	0.0025720	0.00225720	62.00	<0.001
1Deviations	2	0.0051045	0.00025522	7.01	0.002
Treat food	4	0.00007385	0.00001846	0.51	0.731
Lin	1	0.00002784	0.00002784	0.76	0.386
Quad	1	0.00003548	0.00003548	0.97	0.328
Deviations	2	0.00001053	0.00000527	0.14	0.866
Residual	49	0.00178394	0.00003641		
Total	59	0.01915154			

Appendix 11.8 Variation in the time to reach 50% of total gas production derived from the France *et al.* (1993) equation applied to gas production data obtained from incubating hay, a 75:25 mix of hay:sugar beet, a 50:50 mix of hay:sugar beet a 25:75 mix of hay:sugar beet and sugar beet, with pony faecal inoculum for 135 and 49 hours and in the presence or absence of added nitrogen.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Time stratum	1	850.51	850.51	69.27	
Time.units.stratum					
Treat	1	56.53	56.53	4.60	0.037
Food	4	926.57	231.64	18.87	<0.001
Lin	1	399.02	399.02	32.50	<0.001
Quad	1	373.87	373.87	30.45	<0.001
1Deviations	2	153.68	76.84	6.26	0.004
Treat food	4	102.90	25.73	2.10	0.096
Lin	1	51.59	51.59	4.20	0.046
Quad	1	48.90	48.90	3.98	0.052
Deviations	2	2.41	1.21	0.10	0.907
Residual	49	601.62	12.28		
Total	59	2538.14			

Appendix 11.9 Variation in the time to reach 95% of total gas production, derived from the France *et al.* (1993) equation applied to gas production data obtained from incubating hay, a 75:25 mix of hay:sugar beet, a 50:50 mix of hay:sugar beet a 25:75 mix of hay:sugar beet and sugar beet, with pony faecal inoculum for 135 and 49 hours and in the presence or absence of added nitrogen.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Time stratum	1	25983.8	25983.8	109.22	
Time.units.stratum					
Treat	1	691.6	691.6	2.91	0.095
Food	4	44638.0	11159.5	46.91	<0.001
Lin	1	28486.0	28486.0	119.74	<0.001
Quad	1	13439.5	13439.5	56.49	<0.001
1Deviations	2	2712.5	1356.2	5.70	0.006
Treat food	4	1832.8	458.2	1.93	0.121
Lin	1	1009.7	1009.7	4.24	0.045
Quad	1	805.5	805.5	3.39	0.072
Deviations	2	17.5	8.8	0.04	0.964
Residual	49	11657.0	237.9		
Total	59	84803.2			

Appendix 11.10 Variation in DM loss derived from the France *et al.* (1993) equation applied to gas production data obtained from incubating hay, a 75:25 mix of hay:sugar beet, a 50:50 mix of hay:sugar beet a 25:75 mix of hay:sugar beet and sugar beet, with pony faecal inoculum for 135 and 49 hours and in the presence or absence of added nitrogen.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Time stratum	1	1948.60	1948.60	101.97	
Time.units.stratum					
Treat	1	15.15	15.15	0.79	0.378
Food	4	17165.48	4291.37	224.57	<0.001
Lin	1	17115.27	17115.27	895.63	<0.001
Quad	1	0.65	0.65	0.03	0.855
1Deviations	2	49.56	24.78	1.30	0.283
Treat food	4	9.83	2.46	0.13	0.971
Lin	1	4.01	4.01	0.21	0.649
Quad	1	3.19	3.19	0.17	0.685
Deviations	2	2.63	1.31	0.07	0.934
Residual	49	936.38	19.11		
Total	59	20075.44			

Appendix 11.11 Variation in the extent of degradation derived from the France *et al.* (1993) equation applied to gas production data obtained from incubating hay, a 75:25 mix of hay:sugar beet, a 50:50 mix of hay:sugar beet a 25:75 mix of hay:sugar beet and sugar beet, with pony faecal inoculum for 135 and 49 hours and in the presence or absence of added nitrogen.

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Time stratum	1	6.756	6.756	1.20	
Time.units.stratum					
Treat	1	3.743	3.743	0.67	0.418
Food	4	4575.692	1143.923	203.78	<0.001
Lin	1	4490.296	4490.296	799.92	<0.001
Quad	1	56.551	56.551	10.07	0.003
1Deviations	2	28.845	14.422	2.57	0.087
Treat food	4	12.256	3.064	0.55	0.703
Lin	1	0.102	0.102	0.02	0.893
Quad	1	8.018	8.018	1.43	0.238
Deviations	2	4.136	2.068	0.37	0.694
Residual	49	275.057	5.613		
Total	59	4873.505			

Appendix 11.12 Variation in the fractional rate of gas production from hay (calculated by difference) when incubated in a 75:25 mix of hay:sugar beet, a 50:50 mix of hay:sugar beet a 25:75 mix of hay:sugar beet with pony faecal inoculum for 135 and 49 hours and in the presence or absence of added nitrogen [values derived from the France *et al.* (1993) model]

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Time stratum	1	0.0381377	0.0381377	83.20	
Time.units.stratum					
Treat	1	0.0000001	0.0000001	0.00	0.991
Food	3	0.0065624	0.0021875	4.77	0.006
Lin	1	0.0025873	0.0025873	5.64	0.023
Quad	1	0.0033735	0.0033735	7.36	0.010
1Deviations	1	0.0006017	0.0006017	1.31	0.259
Treat food	3	0.0004664	0.0001555	0.34	0.797
Lin	1	0.0000022	0.0000022	0.00	0.946
Quad	1	0.0003674	0.0003674	0.80	0.376
Deviations	1	0.0000968	0.0000968	0.21	0.648
Residual	39	0.0178772	0.0004584		
Total	47	0.0178772			

Appendix 11.13. Variation in the DM loss from hay (calculated by difference) when incubated in a 75:25 mix of hay:sugar beet, a 50:50 mix of hay:sugar beet a 25:75 mix of hay:sugar beet with pony faecal inoculum for 135 and 49 hours and in the presence or absence of added nitrogen [values derived from the France *et al.* (1993) model]

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Time stratum	1	7121.65	7121.65	143.61	
Time.units.stratum					
Treat	1	0.02	0.02	0.00	0.985
Food	3	544.95	181.65	3.66	0.020
Lin	1	16.89	16.89	0.34	0.563
Quad	1	50.04	50.04	1.01	0.321
1Deviations	1	478.02	478.02	9.64	0.004
Treat food	3	78.67	26.22	0.53	0.665
Lin	1	0.30	0.30	0.01	0.938
Quad	1	31.77	31.77	0.64	0.428
Deviations	1	46.60	46.60	0.94	0.338
Residual	39	1934.01	49.59		
Total	47	9679.30			

Appendix 11.14 Variation in the extent of DM loss from hay (calculated by difference) when incubated in a 75:25 mix of hay:sugar beet, a 50:50 mix of hay:sugar beet a 25:75 mix of hay:sugar beet with pony faecal inoculum for 135 and 49 hours and in the presence or absence of added nitrogen [values derived from the France *et al.* (1993) model]

Source of variation	**d.f**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Time stratum	1	23.35	23.35	0.72	
Time.units.stratum					
Treat	1	51.83	51.83	1.60	0.213
Food	3	266.57	88.86	2.75	0.056
Lin	1	56.14	56.14	1.74	0.195
Quad	1	89.22	89.22	2.76	0.105
1Deviations	1	121.21	121.21	3.75	0.060
Treat food	3	145.03	48.34	1.50	0.231
Lin	1	2.17	2.17	0.07	0.797
Quad	1	105.73	105.73	3.27	0.078
Deviations	1	37.13	37.13	1.15	0.290
Residual	39	1260.54	32.32		
Total	47	1747.32			

APPENDIX 12. Publications to date from the work in this thesis.

Moore-Colyer, M.J.S.; Longland,A.C.; Hyslop,J. and Cuddeford, D.(2000) Intra-caecal fermentation parameters in ponies fed botanically diverse fibre-based diets. *Animal Food Science and Technology,* **84:** 183-197.

Moore-Colyer, M.J.S. and Longland, A.C.L. *In vivo* apparent digestibility of four types of conserved forage by ponies. *Animal Science*, **71:** 527-535

Moore-Colyer, M.J.S. Degradation of four dietary fibre sources by ponies as measured by the mobile bag technique. *Proceedings of the Equine Nutrition and Physiology Society*, Fort Worth Texas. May 1997

Moore-Colyer, M.J.S. Longland,A.C.; Hyslop,J. and Cuddeford, D. The degradation of organic matter and crude protein of four botanically diverse foodstuffs in the fore gut of ponies as measured by the mobile bag technique. *Proceedings of the British Society of Animal Science,* Scarborough. March 1997.

Moore-Colyer, M.J.S.; Longland,A.C.; Hyslop,J. and Cuddeford, D. Degradation of protein and NSP from four botanically diverse fibres by ponies as measured by the mobile bag technique. *Proceedings of the British Society of Animal Science,* Reading July 1997.

Moore-Colyer, M.J.S. Dietary fibre in performance horse diets. *Horse race Betting and Levy Board Workshop in Equine Nutrition,* London. September 1998.

Moore-Colyer, M.J.S.; Longland,A.C.; Hyslop,J. and Cuddeford, D *In vivo* apparent digestibility of proximate constituents and non-starch polysaccharides in ponies offered botanically diverse fibre-based diets. *Proceedings of the British Society of Animal Science* March 1999.

Hyslop,J., McLean, B.M.L., **Moore-Colyer, M.J.S.**; Longland,A.C.;. and Cuddeford, D and Hollands, T. Measurement of caecal outflow rate in ponies using Chromium mordanted foods. *Proceedings of the British Society of Animal Science* March 1999.