METHODOLOGIES TO DETERMINE DIGESTION OF STARCH IN PONIES

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Declaration

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

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Abstract

Cereal and legume starch frequently constitute the majority of the concentrate portion of diets for equines yet large intakes of starch can be associated with changes to the hindgut environment. Cereal and legume grains are often subjected to some form of physical processing with a view to increasing their digestibility and this has been the subject of a number of studies. However, there are few reports of the effects of such processing on either the hindgut environment or the site of starch degradation in the equine. Therefore, the objectives of this thesis were twofold, firstly to investigate the effects of physical processing of grains on intra-caecal fermentation parameters and secondly to develop *in sacco* methodologies to enable degradation parameters of processed cereals to be determined in different segments of the equine digestive tract. In the first set of experiments three caecally fistulated ponies were offered 4 kg DM per day of either 100% hay cubes (HC) or one of three diets consisting of 50:50 barley:HC mix. The barley in the mixed diets was either rolled (RB), micronised (MB) or extruded (EB). Inclusion of RB in the diet significantly ($P<0.05$) reduced intra-caecal pH and acetate molar proportions whilst lactate concentration and propionate molar proportions were increased ($P<0.05$) compared with the HC diet measured 5 hours post feeding. Physical processing of grains did not alter their total tract *in vivo* apparent digestibility nor their digestible energy (average 14.8 MJ/kg DM) or digestible crude protein (average 86 g/kg DM) contents. Mean retention times (MRT) of digesta were determined for the four diets and were similar at 46.1, 42.3, 46.9 and 43.0 h for HC, RB, MB and EB respectively. In the second set of experiments micronised or extruded barley, maize and peas were incubated *in situ* in the caecum of ponies to determine their degradation parameters. Compared to unprocessed feeds, micronisation significantly increased ($P<0.05$) the effective degradability (ED) of starch (STC) but reduced the ED of crude protein (CP) in barley and maize. Likewise extrusion of maize increased and decreases the ED of maize starch and CP respectively. However, extrusion of barley had no effect on the ED of

the STC fraction but decreased that of the CP. Neither extrusion or micronisation had an effect on the ED of starch or protein in peas. It was noted that incubation sequence had no significant effect on any of the measured degradation parameters of these starch-based feeds. In a third set of experiments a mobile bag technique was developed to determine the degradation parameters of the above feedstuffs both pre-caecally and over the whole digestive tract of ponies. Bags were administered directly into the stomach of two caecally fistulated ponies *via* a naso-gastric tube. The bags were then retrieved either at the ileo-caecal junction *via* a magnet placed in the caecal fistula or in the faeces. Site of recovery of bags had a significant effect ($P<0.05$) on DM, STC and CP losses and these effects were particularly marked for maize. Both micronisation and extrusion increased pre-caecal DM and STC losses from barley, maize and peas compared to unprocessed grains. Micronisation and extrusion of maize resulted in increased pre-caecal and decreased total tract losses of CP compared to unprocessed maize. Precaecal degradation parameters for barley were determined demonstrating an increase in the ED of DM for barleys which had been micronised or extruded. The lower ED of unprocessed barley in the pre-caecal segment of the equine digestive tract may account for the differences in intra-caecal fermentation parameters recorded in the first set of experiments. Further development of *in sacco* techniques should allow the digestion of feedstuff in the equine digestive tract to be partitioned enabling the identification of processing methods which minimise alterations in intra-caecal fermentation parameters.

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List of abbreviations

α	alpha
a	rapidly soluble degradation parameter
ADF	acid detergent fibre
ADFIV	apparent acid detergent fibre digestibility *in vivo*
AFRC	Agricultural and Food Research Council
AME	apparent metabolisable energy
ANOVA	analysis of variance
β	beta
b	slowly degradable degradation parameter
BR	batch reactor
°C	degrees Celsius
c	degradation rate constant
CFSTR	continuous flow stirred tank reactor
cm	centimetre(s)
Co-EDTA	cobalt - ethylenediaminetetra - acetic acid
CP	crude protein
CPD	crude protein disappearance
CPIV	apparent crude protein digestibility *in vivo*
Cr	chromium
Cr_2O_3	chromic oxide
Cr-EDTA	chromium - ethylenediaminetetra - acetic acid
DCP	digestible crude protein
DE	digestible energy
DHG	dehydrated grass
DM	dry matter
DMD	dry matter disappearance
DMI	dry matter intake

DMIV	apparent dry matter digestibility *in vivo*
DSI	daily starch intake
EB	extruded barley
ED	effective degradability
EM	extrude maize
EMS	error mean square
EP	extruded peas
FFAP-CB	free fatty acid phase – chemically bonded
FMRT	mean retention time in the fast compartment
g	gramme(s)
GE	gross energy
GEIV	apparent gross energy digestibility *in vivo*
GI	gastrointestinal tract
GN	grass nuts
h	hour(s)
H_2O	water
H_2SO_4	sulphuric acid
HC	hay cubes
HCl	hydrochloric acid
HNO_3	nitric acid
i.d	internal diameter
k	time independent passage rate constant
kg	kilogramme(s)
λ	time dependent passage rate constant (lambda)
l	litre(s)
L	lag time
LED	light emitting diode
LW	live weight
MB	micronised barley

MBT	mobile bag technique
MFB	micronised flaked barley
min	minute(s)
MJ	mega joule(s)
ml	millilitre(s)
MLP	Maximum likelihood programme
mg	milligramme(s)
MM	micronised maize
mm	millimetre(s)
mmol	millimole(s)
mol	mole(s)
MP	micronised peas
MRT	mean retention time
MTT	mean transit time
NIR	near infrared spectroscopy
NDF	neutral detergent fibre
NDFIV	apparent neutral detergent fibre digestibility *in vivo*
NRC	National Research Council
OM	organic matter
OMIV	apparent organic matter digestibility *in vivo*
P	potential degradability
PC	pre-caecal
PEG	polyethylene glycol
PFR	plug flow reactor
psi	pounds per square inch
R^2	correlation coefficient
RB	rolled barley
rpm	revolutions per minute
sed	standard error of difference

sig	significance level
SMRT	mean retention in the slow compartment
STC	starch
STCD	starch disappearance
STCIV	apparent starch digestibility *in vivo*
t	incubation time
TD	time delay
TI	time independent
TMRT	total mean retention time
TT	transit time
TT	total tract
TVFA	total volatile fatty acids
µl	microlitre
µm	micrometre(s)
UB	unprocessed barley
UK	United Kingdom
UM	unprocessed maize
UP	unprocessed peas
USA	United States of America
VFA	volatile fatty acids
WCOT	wall coated open tubular
WM	washing machine
Yb	ytterbium

Chapter 1. General Introduction

1.1 Introduction

Increased mechanisation of agriculture during the 20th century resulted in a change in the role of the horse in the United Kingdom from a working, agricultural animal to that of a sport or leisure horse. As a result, the motivation for government – funded research into the nutrition of equids was less than other classes of farm livestock, most of equine nutrition research in the United Kingdom being funded sporadically by charitable bodies or a limited pool of feed companies. As a consequence, equine nutrition has lagged behind that of farm ruminants, pigs and poultry. However, results and methodologies that have emerged from research on farm animals provide a useful platform from which to conduct research on equids.

In contrast to the decline in working horse numbers since the end of the second world war, the numbers of horses kept for leisure purposes has increased. It is estimated that there are 1 million horses and ponies and 2.4 million horse riders in Britain (National Equestrian Survey, 1999). Of these 1 million horses, 90% are owned by the private sector. Annual expenditure by both the professional and private sector is estimated to be £1.94 million with £1.2 million accounting for direct costs such as feed, feed supplements, medication and farriers.

Table 1.1 Approximate number of horses registered with the main competitive segments of the horse industry in Britain

Discipline		Approximate numbers
Horse trials	Registered	7,000
	Advanced	700
Dressage	Registered	5,500
	Advanced	150
Show Jumping		15,500
Endurance		2,500
Horse Driving Trials		800
Polo	Estimated	7,500
	High Goal	1,500
Race Training		17,000

(After Harris, 1997)

The horse has evolved and adapted to a grazing environment where its natural diet contains large amounts of water, soluble proteins, lipids, sugars and structural carbohydrates, but little starch. By contrast, the modern domesticated horse consumes a variety of feeds which typically include fresh or conserved forage and varying amounts of cereal grains which contain between 470 and 730g starch/kg DM. It is known that feeding too much starch can cause hindgut dysfunction resulting in a number of disorders such as acidosis, gastric ulceration, colic and laminitis (Garner *et al.*, 1977; Carroll *et al.*, 1987; Clarke *et al.*, 1990; Rowe *et al.*, 1994). Indeed it has been recommended that to avoid such problems equines should receive no more than 2.0g starch/kg bodyweight per meal (Meyer *et al.* 1995).

However, starch is included in equine diets to meet the additional energy requirements of hard working horses. In a recent review of feeding practices in Great Britain, typical diets being fed to race and event horses were detailed (Harris, 1997).

For the race horse, forage formed approximately 48% of the diet with concentrates (either oats, compound mixes or both) accounting for the remaining 52%. In one racing yard, horses received between 6 - 9 kg of oats per day. If it is assumed that oats contain approximately 55% starch then these horses would be receiving 3.3 - 4.9 kg of starch per day. With the cereal portion of the diet being given in three meals per day and taking an average horse weight of 500 kg, then starch intakes of these horses would have been between 2.2 - 3.3 g/kg LW per meal. This is higher than the upper maximum limit of 2.0 g starch /kg LW per meal recommended by Meyer *et al.* (1995) to prevent hindgut dysfunction.

It is therefore important that diets for equines a) meet the animal's energy requirements and b) maintain gut health. Thus, it is important to understand the manner in which feeds are degraded and absorbed in the equine digestive tract in order that diets for equines may be formulated appropriately.

1.2 The digestive tract of the horse

The horse is generally described as being a non-ruminant herbivore and as such has a relatively simple monogastric digestive tract with an enlarged hindgut which contains a microbial population (bacteria and protozoa) for fibre digestion. In the horse, enzymatic digestion of feed precedes fermentation of the undigested feed residue by the gut micro-flora. The small intestine is the primary site of digestion for starch, soluble carbohydrates, protein, fats and vitamins whereas the primary site of fibre digestion is the hindgut. Estimates of the sites of digestion and absorption of various nutrients are given in Table 1.2.1.

Table 1.2.1. Estimates of percentage of nutrients digested and absorbed within the equine digestive tract.

Dietary Fraction	Small intestine (percent)	Caecum and Colon (percent)
Starch[a]	42 - 96	4 - 58
Soluble carbohydrates	65 - 75	25 - 35
Dietary fibre	15 - 25	78 - 85
Protein	60 - 70	30 - 40
Fats	Up to 100	
Calcium	95 - 99	1 - 5
Magnesium	90 - 95	5 - 10
Phosphorus	20 - 50	50 -80
Vitamins	Up to 100	

(after Hintz, 1975; [a]Data from Potter *et al.*, 1992)

The horse has a relatively small stomach for its size, accounting for approximately 8% of the gastrointestinal tract's capacity. It has been suggested that some fermentation of ingested feedstuffs by gastric bacteria does take place in the stomach and that the end product of this fermentation is lactic acid (Alexander, 1963). In the grazing horse most digesta is held in the stomach for a relatively short time but it is rarely empty and some digesta may remain for 2 - 6 h (Frape, 1998). When fresh ingesta enters the stomach, digesta within the stomach moves into the small intestine.

The small intestine accounts for approximately 30% of the equine digestive tract in terms of volume and accounts for 75% of the total length. Movement of digesta through the small intestine is rapid with some digesta reaching the caecum within 45 min (Frape, 1998). Enzymatic digestion of starch, soluble carbohydrates, protein and fat takes place within the small intestine. Two intestinal amylases are involved in starch digestion in the horse, adsorbed pancreatic α- amylase and glucoamylase (Roberts, 1974). Amylase activity in the horse has always been quoted as "being low

compared to the pig". Roberts (1974) compared amylase activity at six different sites in the small intestine of the horse, rabbit, dog and pig. On average, the amylase activity in equines was approximately 18% of that measured in pigs. Kienzle *et al.* (1994) also measured amylase activity in equine pancreatic tissue and equine jejunal chyme and reported activity ranging from 85 - 909 U/g wet tissue and 1 - 70 U/g wet tissue for pancreatic tissue and jejunal chyme respectively. They found that variations in amylase activity between animals was large. The same workers reported an increase in amylase activity 4 - 5 h post feeding. They also reported higher amylase activities in horses fed a diet containing cereals compared to those receiving a "hay – only" diet but found no significant differences in amylase activities in diets containing different cereals. The activity of disaccharidases in the equine small intestine has been investigated by Roberts and co-workers (Roberts *et al.*, 1974; Roberts, 1975) and Kienzle and Radicke (1993). These studies concluded that disaccharidase activity was comparable to that found in other species such as human, pig and dog (Roberts *et al.*, 1974) and that small intestine capacity for the digestion of sugars was relatively high (Kienzle and Radicke, 1993). It has therefore been suggested that amylase activity is the limiting factor of starch digestion in the horse (Kienzle and Radicke, 1993). However, for equids in the wild this does not pose a particular problem as starch forms a very small part of their diet.

It has been suggested that the capacity for starch digestion in the small intestine of the equine can be exceeded, and any undigested starch will pass into the large intestine (Potter *et al.*, 1992). It has been demonstrated that the digestibility of starch over the whole digestive tract is high in equids and similar for varying starches of different botanical origin (Arnold *et al.*, 1981), indicating that starch degradation also occurs in the hindgut. If the digestion of starch in the small intestine is exceeded then undigested starch will enter the hindgut.

The hindgut of the horse includes the caecum, the dorsal and ventral colons, the small colon and the rectum. The caecum, large colon and small colon represent approximately 16%, 40% and 6% (respectively) of the volume of the whole gastrointestinal tract (Shi and Noblet, 1994). Microbial fermentation of digesta takes place in the caecum and colon. The end products of fermentation of fibre in the hindgut are volatile fatty acids (VFA), lactate, methane and carbon dioxide. The VFA ratios are normally in the range 70:20:10 - 75:15:10 for aceteate:propionate:butyrate but these ratios can be altered by diet manipulation (Bergman, 1990). In the wild equid, where fibre forms the bulk of the diet, the large intestine is the major site of digestion and absorption. However, with high starch low forage diets fed to hard working horses, the end products of hindgut fermentation are an increase in lactate concentrations and a resulting decrease in intra-caecal pH (Radicke *et al.*, 1991).

Despite the widely held view that excessive starch fermentation in the hindgut of equines may lead to acidosis, colic and laminitis (Garner *et al.*, 1977; Clarke *et al.*, 1990; Potter *et al.*, 1992) there are few systematic studies investigating the effect of starch fermentation in the equine large intestine (Radicke *et al.*, 1991). Furthermore, it has also been suggested that processing of cereals may lead to an increase of starch digestion in the equine small intestine (Householder *et al.*, 1977; Kienzle *et al.*, 1992; Meyer *et al.*, 1993; Meyer *et al.*, 1995) however, to date, only one study has examined the effect of processing on the fermentation of cereals by the hindgut microflora (Radicke *et al.*, 1991).

1.3 Techniques for estimating starch digestibility in the equine small intestine

Starch digestibility in the equine small intestine has been estimated by the use of markers (Nyberg, 1993; Kienzle, 1994; Nyberg *et al.*, 1995; de Almeida *et al.*, 1998). When markers are used to estimate digestibility the assumption is made that the marker moves through the digestive tract in the same way as feed particles. However, this may not always be the case (Owens and Hanson, 1992). Recent work in

Germany has shown that factors such as time of sampling from the small intestine may result in an over- or underestimation of starch digestibility (Kienzle, 1994). The problems of using fistulae situated in the small intestine have also been highlighted by the same workers. Such problems include incomplete collection of digesta at the fistula and either inadequate frequency or duration of collections. A further problem associated with the fistulation of the small intestine is the subsequent immobility of the intestine due to its anchoring to the abdominal wall, which may result in a gut torsion and subsequent death of the animal (D. Cottrell, pers. comm.). Recently however, the mobile bag technique has been used to measure the disappearance of feedstuffs from the pre-caecal segment of the equine digestive tract (Macheboeuf *et al.*, 1995; Moore-Colyer *et al.*, 1997a). In the mobile bag technique, small amounts of feeds are sealed into porous polyester bags, which are then administered directly into the stomach of equines *via* a naso-gastric tube. These bags move through the stomach and small intestine and are recovered either from the caecum *via* a fistula or in the faeces. This technique has the advantage that nutrient disappearances from the bags are measured directly rather than being related to marker passage. However, a drawback to this technique is the variability in feedstuff disappearance from each bag and the time at which each bag is recovered from the collection site. To date, most researchers have averaged feedstuff disappearances across bags and have ignored the time factor (Macheboeuf *et al.*, 1995; Araujo *et al.*, 1996a; Araujo *et al.*, 1996b; Longland *et al.*, 1997; Moore-Colyer *et al.*, 1997a; Moore-Colyer *et al.*, 1997b). However, to be able to compare feeds directly, disappearances must be on a common time basis. This could be achieved by adapting degradation models developed for *in situ* methods in ruminants to mobile bag data from equines.

1.4 Scope of the thesis

In order to address the questions surrounding the relative degradability of cereal grains before and after processing, the aims of this thesis were to investigate the effects of processing of grains on hindgut fermentation in ponies and to develop

appropriate methodologies to measure starch digestibility in different segments of the equine digestive tract.

Therefore, this thesis is composed of a literature review and four experimental chapters which describe experiments which;

- Establish *in vivo* apparent digestibility values of a starch-based feedstuff that had undergone various form of physical processing
- Compare intra-caecal fermentation parameters in ponies receiving either an all forage diet or a diet containing physically processed cereals
- Determine rates of digesta passage through the equine digestive tract
- Further develop methodologies for *in sacco* (both *in situ* and mobile bag) techniques to measure feedstuff degradation in different segments of the equine digestive tract
- Develop models to estimate effective degradabilities of feedstuffs in different segments of the equine digestive tract.

The review of the literature is in two sections. The first section describes the effects of physical processing on starch based grains and the digestibility of starch based feedstuffs in the equine digestive tract. The second section reviews methodologies to measure degradation of feedstuffs in different segments of the digestive tract. The results of each experiment are discussed in detail at the end of each experimental chapter and finally the major findings are discussed together within the wider context of the general discussion to be found at the end of the text of this thesis.

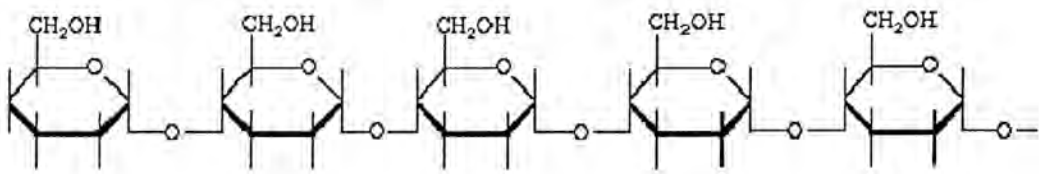
Chapter 2. Literature Review

2.1 Physical processing of starch based feedstuffs

2.1.1 Starch structure

Starch is the major storage polysaccharide of many cereal grains and legume seeds. The physical and chemical structure of starch has been investigated for many years and is the subject of many books and reviews. (see Banks and Greenwood, 1967; French, 1973; Whistler *et al.*, 1984; Zobel, 1988). The chemical structure of starch and the concept of a linear fraction (amylose) and a branched fraction (amylopectin) as distinct molecular types were established by 1940. Amylose consists of α1-4 linked D-glucose monomers forming a linear chain. The α1-4 linkage rotates each glucose slightly resulting in a helical formation of the chain.

Figure 2.1.1: Structure of amylose

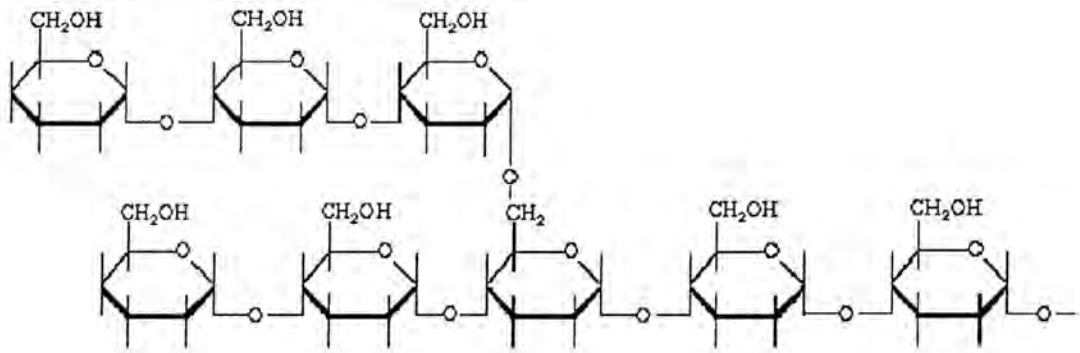


Banks & Greenwood (1967) suggested that many possible conformations of the starch chain exist due to the rotation of the glucose units about the C-O linkage in the glycosidic bond. A particular conformation may be stabilised by intra- or inter-molecular forces or through reactions with various complexing agents such as iodine or butanol (French, 1973). In his review of starch structure, Zobel (1988) suggested that amylose has a double helical structure with six glucose monomers per turn. Zobel (1988) went on to suggest that there was no definite axis of rotation for the

helix, and that it could be left- or right-handed, termed A- or B-type polymorphs, respectively. This is in agreement with the earlier work of Rundle (cited by Banks and Muir, 1980) who demonstrated that amylose had a helical structure and was composed of a double helix with hydrogen bonds forming across the double helix. The double helix structure of amylose suggested by both Rundle (1980) and Zobel (Zobel, 1988) is similar to that of DNA but predated the discovery of this structure by almost a decade (Banks and Muir, 1980).

Amylopectin also consists of glucose residues but it is linked by both α1-4 bonds and α1-6 bonds. Amylopectin has an α1-4 linked structure with α1-6 linked branches *ca.* every 30 monomers.

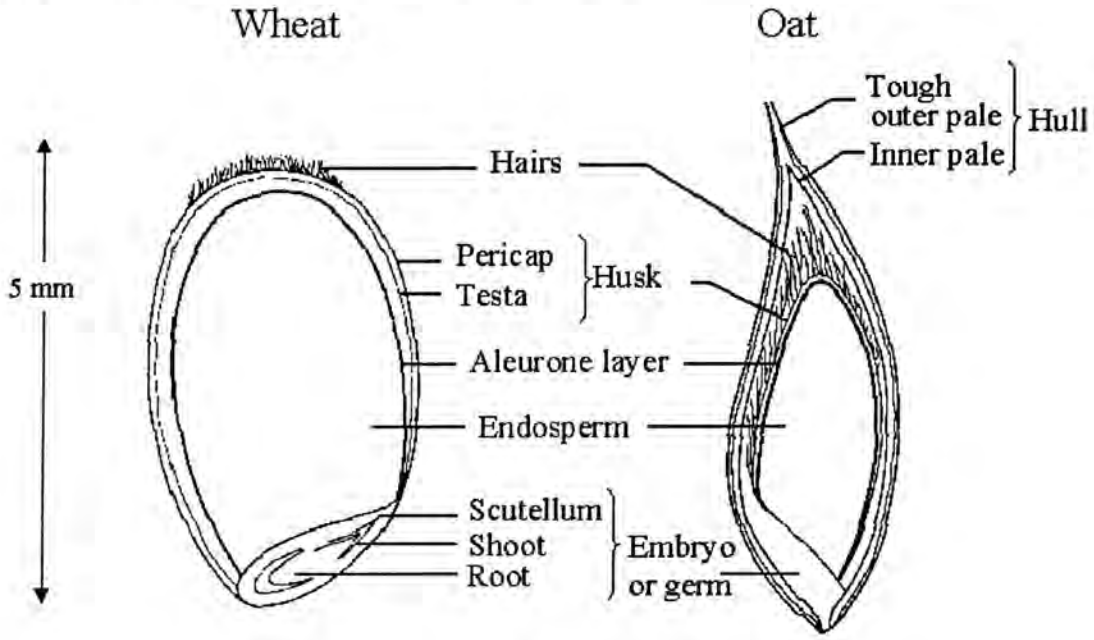
Figure 2.1.2: Structure of amylopectin



2.1.1.1 Starch Granule

Amylose and amylopectin are held together by hydrogen bonds forming highly organised granules which are found within the endosperm of cereal grains (see Figure 2.1.3).

Figure 2.1.3: Macro-structure of a cereal grain



The endosperm accounts for the largest proportion of the cereal grain and can be sub-divided into four layers, the aleurone layer, the peripheral endosperm (or the subaleurone layer), the underlying corneous endosperm and the innermost floury endosperm.

The aleurone layer does not contain starch granules but instead contains autolytic enzymes, amylases and protease inhibitors, water-soluble vitamins, minerals and spherical bodies that contain both protein and lipid. The starch granules in the peripheral and corneous endosperm are surrounded by protein storage bodies and are embedded in a dense matrix of dried endosperm cells. The protein matrix is composed of roughly spherical protein bodies and appears to develop in the aleurone layer and migrate through the endosperm. The protein matrix also includes non-starch polysaccharides and is relatively impervious to water and hydrolytic enzymes. The floury endosperm has little cellular structure and has the highest density of starch

granules where they are most accessible to enzymatic attack. The relative proportions of the peripheral, corneous and floury endosperm layers vary among cereal species. This difference in endosperm composition may well account for the differences in the susceptibility of cereal grains to enzymatic hydrolysis.

Starch granules have both crystalline and amorphous regions, which are dependent on the relative proportions of amylose and amylopectin. Most cereal starches contain approximately 25% amylose and 75% amylopectin. Waxy maize starch contains no amylose yet it exhibits the same crystalline pattern as ordinary maize. Amylomaize, on the other hand contains large quantities of amylose and has a less ordered structure. It has therefore been suggested that amylopectin is responsible for the crystalline structure of starch granules (Banks and Muir, 1980). Intact starch granules have one of three crystalline structures A, B, or C, the occurrence of which depends on the botanical source of the starch. The C-type is an intermediate of A and B type and is present in some tubers and nuts as well as in smooth pea and various bean starches.

Table 2.1.1: Crystallinity of granular starches showing A, B, or C type structures

Starch	Crystallinity (%)	Amylose (%)
A-type		
Oat	33	23
Rye	34	26
Wheat	36	23
Waxy Maize	37	0
Sorghum	37	25
Rice	38	17
Maize	40	27
Waxy Maize	40	0
B-type		
Amylomaize	15-22	55-75
Potato	28	22
C-type		
Sweet potato	38	20
Tapioca	37	25
Horse chestnut	38	18

(Reproduced from Zobel, 1988)

It is apparent form Table 2.1.1 that the A-type occurs most commonly in cereal starches, and the B-type in potato and amylomaize starches. The crystalline structure of starch is important for its gelatinisation.

2.1.1.2 Starch gelatinisation

Starch gelatinisation occurs when a solution of starch is gradually heated resulting in the disruption of the granular structure. Water enters the amorphous regions of the granule causing it to swell. As only small amounts of water are involved initially, the

process is reversible. Once a critical temperature has been exceeded, the process is no longer reversible and the hydrogen bonds within the molecules are ruptured causing more water to enter the molecule resulting in irreversible swelling and eventual bursting of the granule. Other phenomena associated with gelatinisation are pasting and retrogradation. All three phenomena have been used by starch scientists to define individual starches and to measure changes in chemically modified starches. Until recently however, there has been no consensus amongst starch scientists as to the definitions of all three phenomena. In order to redress this inconsistency Atwell *et al.* (1988) conducted a survey and developed the following definitions for gelatinisation, pasting and retrogradation.

- Starch gelatinisation is the collapse (disruption) of molecular orders within the starch granule and is manifested by irreversible changes in properties such as granular swelling, native crystallite melting, loss of birefringence and starch solubilisation. The point of initial gelatinisation and the range over which it occurs is governed by starch concentration, method of observation, granule type, and heterogenities within the granule population under observation.

- Pasting is the phenomenon following gelatinisation in the dissolution of starch. It involves granular swelling, exudation of molecular components from the granule and eventually total disruption of the granules.

- Starch retrogradation is a process which occurs when the molecules comprising gelatinised starch begin to re-associate in an ordered structure. In its initial phase, two or more starch chains may form a simple juncture point which then may develop into more extensively ordered regions. Ultimately, under favourable conditions, a crystalline order appears.

The disruption of the crystalline structure of the starch granule allows the ingress of enzymes capable of hydrolysing the component molecules of starch.

2.1.1.3 Starch hydrolysis

Both amylose and amylopectin are hydrolysed by amylases. Mammals produce α-amylase which hydrolyses the internal α1-4 linkages of starch to yield maltose, maltotriose and α-dextrins which, are in turn, hydrolysed to glucose by maltase and α-dextrinase. Leach & Schoch (1961) observed two types of enzymatic hydrolysis of starch, A and B. In type A, extensive erosion and fragmentation of the granule occurred (observed in sorghum and maize), whereas in type B a selective, granule by granule process was observed in potatoes, wheat, rice , sago and arrowroot. Fuwa *et al.* (1978) described starch hydrolysis as either an endocorrosion process where by amylase can only gain access to the starch granule through small pinholes on the granule surface or by an exocorrosion process where the whole granule is hydrolysed.

Gallant *et al.* (1973) investigated the effect of piglet pancreatic juice *in vitro* on starch granules from different botanical sources. Their results showed that the extent of damage to starch granules was different for each botanical starch source and that the damage was either by exocorrosion alone or a combination of exocorrosion and endocorrosion. In a study carried out by Kienzle *et al.* (1998) where the enzymatic hydrolysis of starch in the small intestine of the horse was examined, it was found that different botanical sources were hydrolysed in different ways. In maize and barley the starch granules were digested by endocorrosion whereas oat starch underwent extensive exocorrosion. Kienzle *et al.* (1998) also reported starch digestibility coefficients of oats, maize and barley as being 0.88 -0.85, 0.47 and 0.22 respectively when measured in the small intestine of horses. The differences in the digestibility of these starch sources may well be due to the differences in the mode of enzymatic hydrolysis.

The disruption of the starch granule structure by heat treatment has also been demonstrated to increase the susceptibility of the granule to hydrolysis (Colonna *et al.*, 1992). The effect of ensiling and expansion of maize was investigated by Kienzle *et al.*(1997; 1998) They found that starch granules from maize that had been ensiled had many more pin holes than those from whole maize. The digestibility coefficient of the same maize silage in the small intestine of the horse was 0.80 (Kienzle *et al.*, 1997). Expansion was found to completely disrupt the starch granule resulting in a digestibility coefficient of 0.90 in the small intestine of the horse (Kienzle *et al.*, 1998).

2.1.2 Physical processing methods of cereal and legume grains

There are at least 18 different methods of processing grain. Hale and Theurer, (1972) listed these methods under the headings of dry or wet processing (see Table 2.1.2.1).

Table 2.1.2.1 Methods of grain processing categorised according to dry or wet process (after Hale and Theurer, 1972)

Dry processing	Wet Processing
Whole grain	Soaking
Grinding	Steam Rolling
Dry rolling or cracking	Steam processing and flaking
Popping/Expanding	Reconstitution
Extruding	Exploding
Micronising	Pressure cooking
Roasting	Early harvesting
Pelleting	Ensiling
Thermalizing	

Although these authors classified extrusion as a dry process, modern extrusion methods would be more correctly classed as a wet process. It is not however the

purpose of this literature review to compare all of these methods but to concentrate on those used most commonly in the production of feeds for equines, in particular micronisation and extrusion.

2.1.2.1 Rolling, grinding, pelleting and expanding

The simplest mechanical processes include rolling and grinding. In both cases the overall cereal structure is disrupted and the starch granules are exposed. These simple processes also remove other physical barriers to exogenous enzymes such as outer fibrous husks.

More sophisticated process generally include a combination of thermal and mechanical processes and include pelleting and expanding. These physical processes generally result in damage to the starch granules and, in some cases, gelatinisation. Pellets generally contain more than one single ingredient, so all of the ingredients are ground and mixed prior to pelleting and the resultant meal is either pelleted (cold pelleting) or is conditioned and then pelleted. Although cold pelleting usually refers to the making of pellets without steam conditioning of the meal, heat is generated due to the frictional resistance of the meal to compression through the die (Wood, 1987). Conditioning as part of the pelleting process can be defined as the process of converting the mixed meal with the use of heat, water, pressure and time to a physical state that facilitates compaction of the feed mash. Conditioning increases production capacity and simultaneously affects the physical, nutritional and hygienic quality of the pelleted feed (Skoch *et al.*, 1981). If the conditioning process also includes some form of residence time within the conditioning chamber then changes in starch and protein properties will normally be enhanced (Thomas *et al.*, 1997). Heffner and Pfost (1973) reported that some starch gelatinisation occurred during steam conditioning of diets for poultry layer but more extreme gelatinisation was seen to occur during pelleting. This was in contrast to the findings of Skoch *et al.* (1981) who

found that no significant starch gelatinisation occurred during steam conditioning, regardless of steam level or pelleting rate. For starch gelatinisation to occur, the minimum moisture content required appears to be between 30 - 50% and the minimum temperature ~ 80 - 90°C (Collison and Chilton, 1974). Under most pelleting conditions it is unlikely that starch gelatinisation occurs to any significant degree even with a long conditioning period (Wood, 1987).

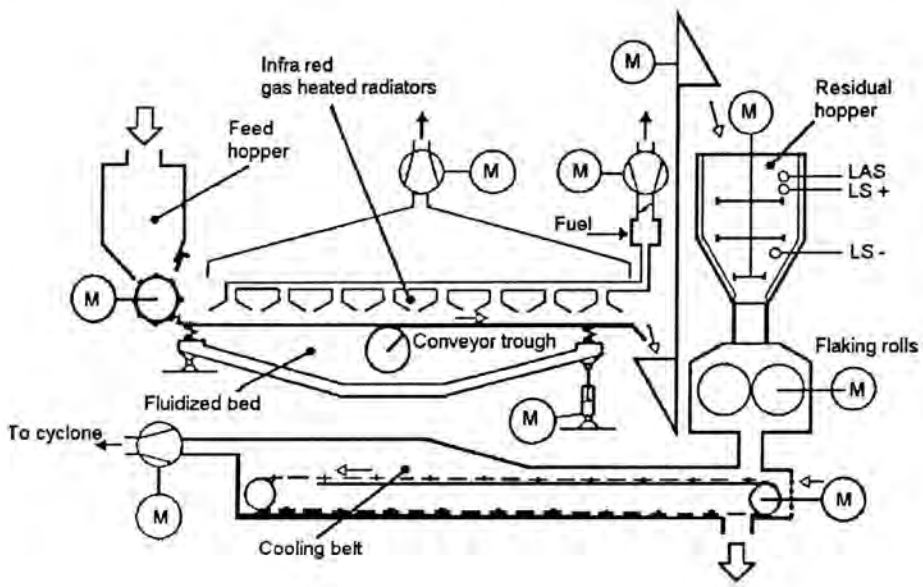
Whilst the overall effect of pelleting on starch gelatinisation is unclear, pelleting has been shown to improve feed conversion ratios in pigs and poultry (Skoch *et al.*, 1983a; Skoch *et al.*, 1983b; Graham *et al.*, 1989; Smits *et al.*, 1994) when compared to feeding the same ingredients in the form of a meal. Other studies have been carried out in ruminants comparing pelleted feeds with the same constituents provided as a "textured" feed which found no differences in DM intake, average daily gain or milk production between the pelleted diet or "textured" diet (Gardner *et al.*, 1997). In comparison to the number of studies in pigs, few studies have been carried out in equines comparing pelleted feeds with "textured" feeds. Wolter *et al.* (1978) used a complete diet, which was offered to ponies in a pelleted, extruded, or textured feed (flaked concentrates and pelleted forage) form. The apparent digestibility of nutrients, glycaemic response and VFA profiles were recorded for each pony. They found no difference between the pelleted feed and the textured feed for any of the dietary constituents measured (DM, OM, CP, cellulose and starch), although differences between the digestibility of the pelleted and the extruded feed were recorded for DM, CP and cellulose: values being lower for the extruded feed. Nor did they find any differences in glycaemic response and caecal VFA profiles. Hintz and Loy (1966) offered a complete pelleted diet and the same ration in a non-pelleted form to horses and recorded no differences in digestibility, with the exception of ether extract. They did, however, note a faster rate of passage when the pelleted ration compared to the non-pelleted ration was fed. Wolter *et al.* (1974) also noted that the

rate of passage in equines was faster when ground or pelleted forages were fed compared to long hay.

2.1.2.2 Micronisation

Unlike pelleting, during micronisation the starch becomes gelatinised by a hydrothermal process. The raw grain is first cleaned to remove any debris and then placed on a moving belt where it is sprayed with water. The moistened grain is then placed in a silo and left until the water is absorbed (normally 24 hours). Depending on the botanical source of the cereal this "dampening" process is repeated until the grain has a moisture content of 18-20%, whereupon the grain is placed on a conveyor belt and is micronised by being passed underneath ceramic tiles which are heated by gas infra-red burners. The burners heat the ceramic tiles to temperatures of 850°C which produce infra-red rays at 1.8-3.4μm which are within the band width normally associated with microwaves, hence the term micronisation. When the infra-red rays penetrate the grain they cause the molecules within the grain to vibrate at a frequency of 80-170 million megacycles per second (The Micronizing Company (UK) Ltd.) causing a rapid rise in water vapour pressure within the grain which ruptures the starch granules resulting in gelatinisation of the starch . By the time the grain has reached the end of the belt it has attained a temperature of 85-90°C, and as it falls off the belt it is loosely rolled causing further disruption of the starch granule. (see Figure 2.1.4)

Figure 2.1.4 Diagrammatic representation of a microniser



Factors affecting the efficiency of the micronising process include the depth of the grain on the belt (bed depth, cm), the speed of the belt (cm/min), and the differential setting of the rollers for flaking. Not all cereals are ideal for micronisation due to their composition e.g. cereals which have a fibrous outer husk, such as oats, tend to burn under the microniser and are rarely used in this process. It appears from the literature that the factors influencing micronisation have not undergone systematic study in the same way as other processes such as pelleting and extrusion. Papasolomontos (1977) compared the digestibility of starch of unprocessed, micronised and micronised and flaked barley and maize using an *in vitro* enzymatic assay. Micronisation alone increased *in vitro* starch digestibility by 58% and 52% for barley and maize respectively. Micronising and flaking resulted in a further increase of 36% and 146% in *in vitro* starch digestibility for barley and maize respectively. Papasolomontos (1977) suggested that flaking the grain while still hot disrupted the whole kernel structure ensuring maximal starch granule hydration and gelatinisation.

This would appear to be in agreement with the studies on steam flaking of cereals. Osman *et al.* (1970) showed that with steam flaking or pressure cooking of barley and sorghum the degree of flaking was the important factor in improving *in vitro* enzymatic starch degradation. These results have been confirmed by other *in vitro* enzymatic studies (Aman and Graham, 1990; Zinn, 1990) where flake density/quality has been investigated. In all cases, the flaked grains investigated had been ground prior to incubation with enzymes suggesting that the response was due to the flaking and not to the increase in surface area of the grains *per se*. *In vivo* studies in finishing steers showed that average daily gains were not different between dry rolled or steam flaked cereals but feed conversion ratios were higher when steam flaked cereals were fed (Osman *et al.*, 1970; Aman and Graham, 1990; Zinn, 1991).

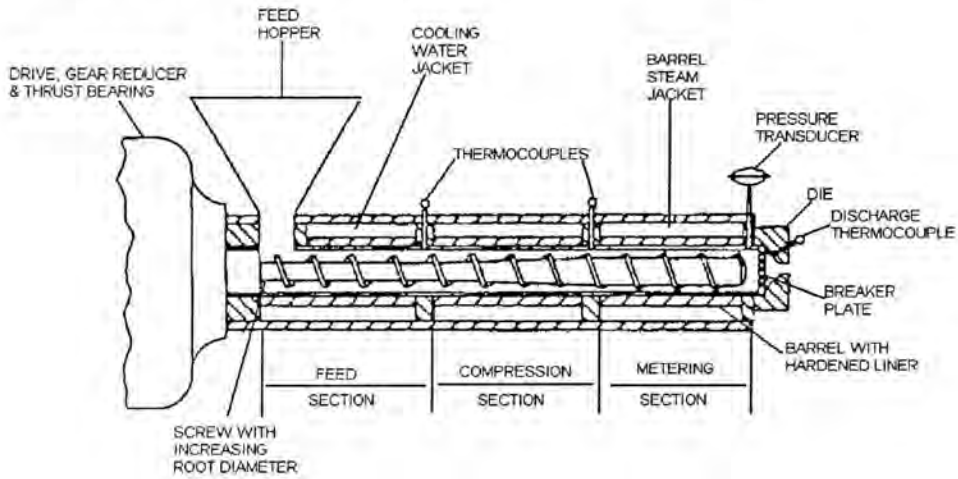
2.1.2.3 Extrusion

During extrusion cooking the grain is cooked under pressure at high temperatures. The grain is first ground into a flour and then steam is added until a dough-like mixture is formed, which is then passed through an extruder barrel. The barrel contains either a single or twin screw and is divided into sections. Each of the sections is individually controlled for temperature and pressure. At the end of the screw the "cooked" dough is forced through a die at extreme pressure (400 - 1000psi), whereupon the extruded material is dried in forced draught ovens. (See Figure 2.1.5)

Extrusion cooking results in gelatinisation and mechanical shearing of the starch granules. Many variables are involved in extrusion cooking, including barrel temperature, pressure, screw differential, screw type and moisture content. An extruder cooker may be considered as a continuous reactor capable of simultaneously transporting, mixing and shearing. This process which uses elevated temperatures and pressures with short residence times and low moisture contents (10 - 20%), disentangles amylose and amylopectin. Moisture content is of greater concern in

single-screw extruders. Particle size may also be critical in single barrel extruders; if the material is very coarse, the starch granules may not become fully gelatinised.

Figure 2.1.5 Diagrammatic representation of an extruder



Although extrusion cooking of cereals is more often associated with the making of pet food than with farm animal or the horse feed, the extrusion cooking of cereals for the latter is now gaining in popularity. Several of the leading equine feed manufacturers now produce at least one extruded product. However, there are few studies which have studied the effects of extrusion on cereal digestibility in horses. Bouwan (1978) conducted a study, using Shetland ponies, which compared the total tract digestibility of unprocessed with that of extruded cereals. Results indicated that extrusion did not improve the digestibility of dry matter, organic matter, crude protein or crude fibre but that the digestibility of "other carbohydrates" and fat was increased. These results led Bouwan to conclude that extrusion of feeds for horses was not economically viable. Hintz *et al.* (1993) also investigated the effect of extruding and pelleting the concentrate portion of a diet for horses. They found that the digestibility of energy and dry matter of the diet containing the extruded feed was significantly greater than that containing the pelleted feed. Crude protein digestibility was significantly greater

for the extruded feed than for the unprocessed feed. By contrast, studies in broiler chicks reported that diets which included extruded barley resulted in a significant depression in feed efficiency, feed AME (apparent metabolisable energy) and a decrease in fat and protein utilisation (Vranjes and Wenk, 1995). Fadel *et al.* (1988) carried out an experiment to investigate the effects of extrusion cooking of barley on ileal and faecal digestibilities of dietary components by pigs. Ileal dry matter was lower and faecal dry matter higher from pigs fed the extruded diet compared to those fed the unprocessed diet. Ileal digestibilities were higher for dry matter, energy, starch and Klason lignin in pigs fed the extruded diet, but there were no significant differences in faecal digestibilities, except for Klason lignin. Studies in early weaned pigs, reported an improvement in daily liveweight gain and feed conversion rates when diets included either extruded barley or maize compared to diets including raw cereals (Medel *et al.*, 1998). Although not directly comparable, an increase in liveweight gain was also noted when mares were fed a complete extruded diet compared to those at pasture (Ott *et al.*, 1999) however, in trials carried out with fattening steers, diets which included 95% extruded maize gave no improvement in liveweight gain or feed conversion ratio when compared to diets containing 85% steam flaked maize (Hale and Theurer, 1972). Inclusion of extruded feeds in diets may therefore be beneficial to monogastrics if the main objective is to increase liveweight gain however, in other species it may be less so.

The effect of extrusion cooking of cereals on digestibility appears to be inconsistent with some studies showing improvements and others not. Extrusion cooking does however appear to alter the chemical composition of cereals and legumes. In the study carried out by Fadel *et al.* (1988) they noted that extrusion cooking of barley increased Klason lignin content and decreased starch content. They also noted that there was an increase in soluble non-starch polysaccharides (NSP) with a concomitant decrease in insoluble NSP. Vranjes and Wenk (1995) also reported increases in soluble fibre when barley was extruded. Extrusion cooking of peas has been shown to

decrease nitrogen solubility (Focant *et al.*, 1990; Chapoutot and Sauvant, 1997). The decrease in nitrogen solubility of extruded cereals and legumes has been shown to be related to the intensity of the extrusion treatment (Chapoutot and Sauvant, 1997), however few papers describe the conditions of extrusion. The differences in extrusion treatment between studies may account for the differences in results obtained.

2.1.3 Effect of starch source and processing method on starch digestibility in equines

From the literature it would appear that both the source of starch and the chosen processing method will impact on the digestibility of cereals and legumes in the digestive tract of animals. Comparatively few studies in equids have investigated the effect of either the botanical source of the starch and/or the effect of processing on apparent starch digestibility within the small intestine. A search of the literature has revealed ten studies which have examined the effect of starch source and/or processing of cereals on their digestibility in equines. In total these studies have included seven starch sources, one concentrate mix and two forage sources, seven processing methods, differing cereal to forage ratios and varying number of meals per day. A brief overview of these studies is outlined in Tables 2.1.3 and 2.1.4. The use of fistulated animals has allowed starch digestibility coefficients in different segments of the digestive tract of equines to be determined (Crawford Jr, 1971; Householder *et al.*, 1977; Arnold *et al.*, 1981; Radicke *et al.*, 1991; Kienzle *et al.*, 1992; Meyer *et al.*, 1993; Meyer *et al.*, 1995). Starch digestibility coefficients have also been determined in different segments of the equine digestive tract in animals that have been euthanased (Hintz *et al.*, 1971b). For ease of presentation in Tables 2.3 and 2.4, apparent starch digestibility coefficients are given for values determined in the small intestine and throughout the entire gastrointestinal tract. It should be noted however, that in the different studies the fistulae were placed at three different sites along the small intestine and therefore digestibility coefficients in Tables 2.1.3 and 2.1.4 are not strictly comparable. It should also be noted that Hintz *et al.* (1971b) measured

"available carbohydrate" rather than starch. In the four early American studies (Crawford Jr, 1971; Hintz *et al.*, 1971b; Householder *et al.*, 1977; Arnold *et al.*, 1981) concentrates were fed in conjunction with either Bermuda-grass hay or alfalfa hay, whereas in the later German studies (Radicke *et al.*, 1991; Kienzle *et al.*, 1992; Meyer *et al.*, 1993; Meyer *et al.*, 1995) concentrates were fed with alfalfa meal unless stated otherwise. All studies used markers to determine starch digestibility.

Table 2.1.3 Effect of starch source, level of intake and frequency of feeding on apparent starch digestibility in equines

Authors	Year	Starch source	Processing/ Intake	Digesta sample obtained *via*	Apparent digestibility of starch in the small intestine (g/kg)	Apparent digestibility of starch in the total tract (g/kg)	No. of horses
Hintz *et al.*	1971	Alfalfa		Slaughter	461	907	4
		Maize (31%)	Ground		540	977	4
		Maize (63%)	Ground		714	991	4
Crawford	1971	Oats	Rolled	Caecal fistula	994	995	3
			Rolled + Hay		974	992	
		Hay	Ground		861	920	
Arnold *et al.*	1981	Maize	Coarse cracked	Ileal fistula	782	970	3
		Oats			911	967	
		Sorghum			943	970	
Hinkle *et al.*	1983	Maize	20% of Diet	Ileal fistula	634	977	4
			40% of Diet		596	989	
			60% of Diet		543	993	
			80% of Diet		658	996	
Massey *et al.*	1985	Conc. Mix	2 meals per day	Ileal fistula	741	970	4
			3 meals per day		769	968	
			4 meals per day		701	971	

Table 2.1.4 Effect of starch source and processing method on starch apparent digestibility in equines

Authors	Year	Starch source	Processing method	Digesta sample obtained *via*	Apparent digestibility of starch in the small intestine (g/kg)	Total Tract Apparent Digestibility %	No. of equine
Householder	1977	Oats	Crimped	Ileal fistula	480	94.6	4
			Micronised		624	93.6	
		Sorghum	Crimped		360	94.1	
			Micronised		567	94.6	
Radicke *et al.*	1991	Maize	Roughly ground	Jejunal fistula	703		4
		Oats			980		
Kienzle *et al.*	1992	Oats	Whole	Jejunal fistula	835		4
			Rolled		852		
			Crushed		981		
		Maize	Whole		289		
			Rolled		299		
			Crushed		706		
Meyer *et al.*	1993	Oats	Whole	Jejunal fistula	835		4
			Rolled		852		
		Maize	Whole		289		
			Crushed		299		
			Ground		456		
			Ground + Amylase		577		
			Ground + Hay		164		
			Popped		901		
		Barley	Rolled (crushed)		214		
Meyer *et al.*	1995	Potato	Fresh	Jejunal fistula	65		5
		Manioc	Rolled		90		4
		Oats	Whole		797		4
			Rolled + Hay		874		4

It would appear from the American studies (Crawford Jr, 1971; Hintz *et al.*, 1971b; Householder *et al.*, 1977; Arnold *et al.*, 1981; Hinkle *et al.*, 1983; Massey *et al.*, 1985) and that of Wolter *et al.* (1982) that botanical source is less important in terms of total tract digestibility as starch was almost completely digested. However, Arnold *et al.* (1981) reported significant differences for pre-caecal starch digestibility when comparing coarsely cracked maize (78.2%), oats (91.1%) and sorghum (94.3%) but reported no significant differences for the same cereals for total tract digestibility (97.0, 96.7, 97.0% respectively). Meyer *et al.* (1993) also measured large differences in pre-caecal starch digestibility between different cereal sources, 85.2%, 29.9% and 21.4 % for rolled (crushed) oats, maize and barley respectively.

Based on the work of Householder *et al.* (1977), Kienzle *et al.* (1992) and Meyer *et al.* (1993, 1995) the way in which cereals are processed has a significant effect on small intestinal digestion of starch. The early work of Householder *et al.* (1977) compared crimping with micronising for both oats and sorghum and reported that micronising improved pre-caecal digestibility for both oats and sorghum but the greatest improvement was seen with sorghum. Householder *et al.* (1977) went on to suggest that for readily digestible cereals such as oats, the benefits of micronising were not sufficient to justify the economic costs and this should be taken into account when deciding what processing method to use. Kienzle *et al.* (1992) also showed that for oats simple processing methods (rolling and crushing) increased pre-ileal digestibility of starch with the biggest increase seen with grinding. However the magnitude of increase was not as great as when maize was processed in the same way. Grinding maize resulted in pre-ileal starch digestibility of 70.6% compared to a value of 28.9% for whole maize. These results also suggest that processing is more beneficial for poorly digested starch sources. The greatest increase in maize starch pre-ileal digestibility was brought about by popping (90.1%) (Meyer *et al.*, 1993; Meyer *et al.*, 1995).

Ratios of concentrates to forage have also been investigated to determine any effect this may have on starch digestibility in the small intestine. The results from these studies have produced conflicting results. Hintz *et al.* (1971b) compared available carbohydrate digestion in three diets; 1.) 100% alfalfa hay; 2.) 31% ground maize + 69% alfalfa and 3.) 63% ground maize + 37% alfalfa and reported apparent digestibilities of available carbohydrate at the end of the small intestine of 46.1%, 54.0% and 71.4% respectively. The apparent digestibilities of the 100% hay diet and 31:69% maize:hay diet were similar but the digestibility of the 69:31 maize:hay diet was significantly higher ($P<0.05$). Meyer *et al.* (1995) also compared two levels of whole oats (unprocessed) in the diet and reported that the diet containing the higher level of whole oats resulted in a decrease in pre-ileal digestibility. Hinkle *et al.* (1983) compared four levels of maize (ground) inclusion (20, 40, 60 and 80%) in the diet and found that although level of inclusion had a small but significant effect on total tract starch digestibility ($^{*}97.7^{a}$, 98.9^{ab}, 99.3^{b}, 99.6^{b} % respectively) it had no significant effect on small intestine starch digestibility (63.4, 59.6, 54.3, 65.8% respectively). Massey *et al.* (1985) then went on to examine the effect of frequency of feeding on starch digestibility and found that precaecal apparent digestibility of starch was similar across treatments and for 2, 3 and 4 meals/day was 74.1, 76.9 and 70.1% respectively.

Although the results of Hinkle *et al.* (1983) demonstrated no effect of level of inclusion on digestibility of starch in the small intestine, at the higher levels of maize inclusion the ponies did not consume the entire meals and the highest levels of starch intake were approximately 0.35% of body weight per meal. Workers at Texas A&M went on to suggest that there may be an upper limit to the capacity for starch digestion in the small intestine of the horse (Potter *et al.*, 1992). In a further study, two horses (described as having "aggressive eating behaviour and good appetites") were fed a fixed amount of hay (1.4 kg alfalfa) and varying amounts of chopped maize at 12 hour

intervals. In each two week experimental period horses were offered maize in increasing amounts beginning at 0.1kg and increasing up to 5.2kg per feed and then in decreasing amounts back to the lowest level, resulting in starch intakes ranging from 0.02 to 0.55% of body weight per meal. As expected total tract starch digestibility was nearly complete, however, the effect of intake on starch digestibility in the small intestine was much more variable. Actual numerical values are not presented and the data are presented in graphical form making direct comparisons with previous work difficult. A polynomial regression ($y = -2.0717 + 0.56282x + 1.8192e\text{-}3x^2 - 4.7795e\text{-}6x^3$) has been fitted to the data, however the R^2 associated with this equation is only 0.571 suggesting that this equation is not necessarily a good fit of the data. Data for the amount of starch reaching the large intestine is also presently graphically with a polynomial regression fitted to it with a R^2 of 0.757. Whilst, this suggests a better relationship the data are still quite variable. Potter *et al.* (1992), however, concluded that the upper limit to starch digestion in the small intestine of equines receiving their daily rations in distinct meals, may be an amount in the range of 0.35 -0.4% of body weight per meal. This would mean that for an average 500kg thoroughbred horse, diet based on a 70:30 concentrate: forage ratio (at 20g DM/kg LW per day) and receiving the concentrate part in a maximum of three meals a day, the concentrate content of any one meal would have to exceed 3kg (with an average starch content of 600g/kg) before small intestine digestibility of starch would be compromised. From the few reports of racing or performance yards feeding regimes it would appear that in practice these levels of feeding starch are rarely achieved. In a recent review of feeding practices in the UK, Harris (1997) gave examples of typical diets used in racing and eventing yards and these give average concentrate: forage ratios of 52:48 for racing and 44:56 for eventing yard (fed at approximately 20g DM/kg bodyweight per day). In earlier studies (Mullen *et al.*, 1979) carried out in the UK, concentrates accounted for 57% of the diet of racing two year old thoroughbreds although total diet was fed at approximately 40g DM /kg LW per day. In studies carried out in racing yards in the USA and Australia (Glade, 1983; Southwood *et al.*, 1993) concentrates

made up 43 and 67% of the diet fed at a total of 30g DM kg^{-1} LW and 25g DM kg^{-1} LW per day respectively. However, with the exception of Mullen *et al.* (1979) none of these reports indicated frequency of feeding. Mullen *et al.* (1979) reported that as concentrate levels were increased the number of meals increased to at least three a day. These reports of feeding regimes in professional yards suggest that in practice, it is unlikely that the capacity of the equine small intestine for starch digestion would be exceeded based on the findings of Potter *et al.* (1992). However, Meyer *et al.* (1995) suggested that starch digestion in the small intestine may be exceeded when 0.02% starch per meal is fed and that some horses may well be suffering from sub-clinical acidosis.

Starch that is not digested in the small intestine is potentially available for fermentation by the micro-flora of the large intestine, resulting in almost complete starch degradation in the horse. Studies investigating the effect of starch fermentation in the hindgut of the horse are rare, despite the widely held view that excessive starch fermentation in the hindgut may lead to excessive gas production, colic and laminitis (Garner *et al.*, 1977; Clarke *et al.*, 1990; Potter *et al.*, 1992). Starch which escapes digestion in the small intestine of equines will enter the caecum where it may alter microbial fermentation parameters resulting in lower pH levels and changes in the molar proportions of acetate, propionate and butyrate (Radicke *et al.*, 1991). Compared to rumen studies in cattle and sheep, few studies have been conducted in equines to examine changes in intra-caecal parameters where known quantities of cereals have been fed in conjunction with a basal forage diet. However, Hintz *et al.* (1971a) offered diets with varying alfalfa: concentrate mix ratios of 100:0, 60:40 and 20:80 respectively. As the concentrate proportion increased, intra-caecal proportions of acetate declined whereas propionate proportions increased. Willard *et al.* (1977) offered caecally fistulated horses either 100% hay or 100% cereal based concentrate diets and reported that intra-caecal pH fell to only 6.75 for the hay diet but to 6.12 for the concentrate diet at 7 and 6 hours post feeding respectively. VFA levels were also

characterised by high acetate proportions on the hay diet and high propionate proportions on the concentrate diet. Similar changes in caecal fermentation parameters in relation to low forage/high concentrate diets for equines have been reported by Stillons *et al.* (1970), Goodson *et al.* (1988) and Radicke *et al.* (1991). In ruminants, where rumen pH falls to between 6.0 and 6.1 cellulolysis may be inhibited due to restriction of microbial fermentative activity (Wolter *et al.*, 1974; Wolter *et al.*, 1975; Malestein *et al.*, 1988). It has been suggested that similar considerations would apply to microbial fermentation in the equine hindgut (Clarke *et al.*, 1990). It has also been suggested that when intra-caecal pH falls below 6.0 equines may be regarded as exhibiting sub-clinical acidosis (Radicke *et al.*, 1991). Whilst the large intestine micro-flora of the horse are able to ferment starch entering the hindgut almost completely the consequences of this may well be impaired fermentative capacity of other carbohydrates in the hindgut.

In the studies referred to in this section various methods of measuring digestibility of starch within the equine digestive tract have been employed. Some studies have used total tract digestibility methods, others have used markers, some were carried out in live animals, others in animals that had been euthanased. Differences in methodology can make comparisons between studies difficult, but as no single method can give definitive answers to all questions regarding digestibility various methods need to be employed. The benefits of different methods for measuring digestive function are discussed in the following section.

2.2 Methods of measuring digestive function in the digestive tract of animals

2.2.1 Measurement of nutrient digestibility

2.2.1.1 *In vivo* apparent digestibility

McDonald *et al.* (1996) have defined the digestibility of feed as that proportion of ingested food which is not excreted in the faeces and which is therefore assumed to be

absorbed by the animal. However, all that appears in the faeces is not entirely composed of undigested feed residues as faeces also contain material derived from enzymes and other substances that have been secreted into the gut during digestion as well as cellular material sloughed from the gut lining (McDonald *et al.*, 1996). Values obtained in digestibility trials are therefore termed as apparent digestibilities. Due to the difficulty in measuring microbial and endogenous material in faeces, apparent digestibility values are generally regarded as acceptable estimated of feedstuff digestibility (McDonald *et al.*, 1996). The apparent digestibility of a feed can be determined by measuring the quantity of feed consumed and the quantity of faeces produced (Cochran and Galyean, 1994; McDonald *et al.*, 1996), (see Equation 2.1.1 below).

$$\text{Apparent Digestibility of DM} = \frac{\text{(DM consumed - DM in faeces)}}{\text{DM consumed}} \qquad \text{Equation 2.1.1}$$

In vivo digestibility studies require a group of animals to minimise variation due to that inherent in individual animals, an adaptation period (usually 14 - 21 days) and a collection period (5 - 10 days) and sufficient quantities of feed to feed all animals for the duration of the trial for if more than one batch of feed is used another source of variation is introduced. These constraints make the *in vivo* determination of digestibility both expensive and time-consuming. However it serves as a control methodology for validating the accuracy of alternatives such as marker and *in sacco* techniques.

2.2.1.2 Marker Studies

It is not always possible to conduct apparent digestibility trials by total faecal collection, due to a lack of equipment and expertise, or due to the nature of the trial, (i.e. measuring the digestibility of pasture in a grazing environment or group feeding

of animals.) When this is the case, another method must be used. Many feedstuffs contain indigestible components and these can be used as "markers" to estimate digestibility. Such markers are known as internal markers and include n-alkanes, lignin and silica. However, external markers are also available to the researcher and include substances such as chromic oxide (Cr_2O_3) and cobalt-EDTA. The concentration of the marker in the feed and in the faeces is determined and the ratio between the two gives an estimate of digestibility. For example to calculate DM digestibility

Apparent digestibility of DM =

$$\frac{(\text{g marker kg}^{-1}\text{ faeces DM} - \text{g marker kg}^{-1}\text{ feed DM})}{\text{g marker kg}^{-1}\text{ faeces DM}}$$

Equation 2.1.2

The use of markers to determine feed digestibility has been reviewed by many workers including (Kotb and Luckey, 1972; Dove and Mayes, 1991; Owens and Hanson, 1992). No one marker is ideal for all feedstuffs and each marker must be validated with *in vivo* data before it can be used routinely for estimating digestibility or faecal output. Owens & Hanson (1992) described the main characteristics of an ideal marker as being:

- not absorbed;
- not affecting or be affected by the digestive tract or it's microbial population;
- has to flow parallel with, be physically similar to or intimately associated with the marked material;
- must have a specific and sensitive method of estimation.

A limitation of the *in vivo* method is that it does not allow an evaluation of where within the digestive tract the nutrient is digested or absorbed. Many researchers have

developed techniques to quantify digestion in different segments of the digestive tract using both *in vivo* and *in vitro* techniques. *In vivo* methodologies include *in sacco* techniques where feedstuffs are incubated within nylon bags in the digestive tract whereas *in vitro* methodologies are based on incubating feedstuffs with digesta in an artificial environment within the laboratory.

2.2.1.3 *In sacco* methods

The most common *in sacco* method in routine use is the *in situ* technique, however, the mobile bag technique is also being developed as a methodology for routine use.

2.2.1.3.1 *In situ* technique

The *in situ* technique was first developed in the late 1930's (Quin *et al.*, 1938) to measure the degradability of feedstuffs in the rumen of sheep, using cylindrical, silk bags. McAnally (1942) also investigated feedstuff degradation in the rumen of sheep but used silk squares, closed using a drawstring to form a bag. Balch & Johnstone (1950) took a more direct approach, and measured cellulose degradation by suspending cotton threads in the rumen and after various time periods assessed the dissolution of the thread. Alexander (1967) carried out a similar experiment to investigate cellulose degradation in the caecum of the horse. In all cases, the material under investigation was inserted into the rumen/caecum *via* a cannula and left immersed in rumen or caecal digesta for a specific length of time.

The *in situ* technique as used in ruminants has been reviewed extensively, covering all aspects of the technique (Lindberg, 1985; Nocek, 1988a; Weiss, 1994; Huntington and Givens, 1995). This method has also been used in equines to study feed degradation in the hindgut (Applegate and Hershberger, 1969; Uden and Van Soest, 1987; Faurie *et al.*, 1992; Lechner-Doll *et al.*, 1992; Stefansdottir *et al.*, 1996b).

Factors that may influence the degradability of a feed sample in situ have been investigated to various extents in ruminant studies but not in equine studies. However, many of the recommendations can be applied to studies in equines as well as in ruminants. Sources of variation within the *in situ* technique include bag characteristics such as pore size and cloth type (Weakley *et al.*, 1983; Uden and Van Soest, 1987; Noziere and Michalet-Doreau, 1996), sample preparation prior to incubation (Weakley *et al.*, 1983; Emanuele and Staples, 1988; Huntington and Givens, 1997b), sample size to surface ratio (Mehrez and Ørskov, 1977; Uden and Van Soest, 1987), diet of host animal (Nocek, 1988a; Weiss, 1994), incubation sequence of bags (Hyslop *et al.*, 1996; Huntington and Givens, 1997a) and post incubation of treatment of bags (Cherney *et al.*, 1990; Huntington and Givens, 1997c). A recent European ring test has indicated the need for standardisation of the *in sacco* methodology between studies to allow for meaningful comparisons (Madsen & Hvelplund, 1994).

In the early *in sacco* studies bags were incubated for a set time and then removed, and no attempts were made to describe the degradation of feeds with time (Huntington and Givens, 1995). Ørskov & McDonald (1979) described a method of describing the time course of degradability for an individual sample. In their method they carried out a sequence of incubations and plotted the dry matter losses from the bags against time. A non-linear model was fitted to the degradation curve according to the equation below.

$$P = a + b * (1 - \exp^{ct}) \qquad \text{Equation 2.1.3}$$

where P = potential degradability

t = incubation time

a = Y axis intercept at time 0. Represents soluble and completely degradable substrate that is rapidly washed out of the bag

b = the difference between the intercept (a) and the asymptote. Represents the insoluble but potentially degradable substrate which is degraded by the micro-organisms according to first order kinetics

c = rate constant of function b

a, b and c are constants fitted by an iterative least squares procedure.

Other models have since been developed which take into account lag phases (Dhanoa, 1988) as well as other non-linear models (Vieira *et al.*, 1997; Lopez *et al.*, 1999). The model of Ørskov and McDonald (1979) is, however, used in most studies. Whilst it is possible to calculate the potential degradability of sample feeds, this does not take into account the fact that feedstuffs may pass out of the rumen before degradation is complete. Ørskov and McDonald (1979) included an estimate of ruminal outflow rates (k) in Equation 2.1.3 to calculate the effective degradability (ED) of a feedstuff in the rumen. Equation 2.1.3 then becomes

$$ED = a + [(b \cdot c)/(c + k)] \qquad \text{Equation 2.1.4}$$

The incorporation of outflow rates allows comparisons between feeds on a common time scale.

Whilst the *in situ* technique has become a standard methodology for determining degradation of feeds in ruminants it has two drawbacks. It requires that animals have fistula within the digestive tract (generally the major site of fermentation) and it only measures degradability within that segment of the tract. Other *in sacco* methodologies such as the mobile bag technique can measure degradability over sequential segments of the digestive tract and does not necessarily require animals fitted with a gastro-intestinal fistula.

2.2.1.3.2 Mobile bag techniques

The use of small bags, which pass through the whole digestive tract, to measure digestion have been cited as early as 1756 (Sauer *et al.*, 1983), it was only more recently, however, that the mobile bag technique was investigated further as a routine method for measuring digestibility. Petry & Handlos (1978) used the mobile bag technique for studying the digestibility of nutrients in feedstuffs by pigs by orally administering feed in small nylon bags. Since then, other workers have used the technique to estimate digestibility in pigs (Sauer *et al.*, 1983; Graham *et al.*, 1985; Cherian *et al.*, 1989; de Lange *et al.*, 1991), ruminants (Hvelplund, 1985; de Boer *et al.*, 1987; Jarosz *et al.*, 1994; Mgheni *et al.*, 1994; Vanhatalo and Keetoja, 1995) and equids (Macheboeuf *et al.*, 1995; Hyslop and Cuddeford, 1996; Moore-Colyer *et al.*, 1997a; Moore-Colyer *et al.*, 1997b).

In the mobile bag method a small amount of milled experimental feed is sealed in a nylon bag and introduced into the digestive tract where it passes through subsequent sections of the gastrointestinal tract before being retrieved. The point of insertion into the digestive tract is dependent on what aspect of digestion is under investigation, as is the point of retrieval of the bag from the digestive tract. For pigs it was found that insertion of bags into the stomach resulted in an over-estimation of protein digestibility due to the prolonged time the bags spent in the stomach (Petry and Handlos, 1978; Sauer *et al.*, 1983). The bags were unable to pass through the pyloric sphincter and only when the pigs had been fasted for 24 h to allow the sphincter to relax could the bags pass through. To overcome this problem, Sauer *et al.* (1983) suggested that bags should be inserted *via* a duodenal cannula after pre-digestion *in vitro*. For ruminants, bags can be inserted *via* a duodenal cannula directly or after they have been incubated in the rumen (Hvelplund, 1985; de Boer *et al.*, 1987; Jarosz *et al.*, 1994; Mgheni *et al.*, 1994). In equids, bags have been inserted directly into the

stomach *via* a naso-gastric tube (Macheboeuf *et al.*, 1995; Araujo *et al.*, 1996a; Araujo *et al.*, 1996b) or *via* a caecal fistula (Hyslop and Cuddeford, 1996). Use of fistulated animals allows bags to be retrieved from different parts of the digestive tract to measure digestion in different segments of the tract. Thus in ruminants, bags have been retrieved from the ileum *via* a cannula either by "observing the passage of the bags towards the ileal cannula" and removing them using curved tweezers as they pass (Hvelplund, 1985) or by inserting a cylindrical magnet into the cannula and placing metal ball bearing within the bags such that the bags adhere to the magnet inserted into the cannula (Jarosz *et al.*, 1994). In caecally-fistulated equids, bags have been retrieved from the caecum, Macheboeuf *et al.* (1995) retrieved bags by hand from the fistula, whereas Hyslop (pers comm.) retrieved bags using a cylindrical magnet placed within the fistula and using flat metal washers in the bags.

Unlike the *in situ* technique, the methodology behind the mobile bag technique has not been thoroughly investigated. Bag size and shape has been investigated (Leibholz, 1991; Hyslop and Cuddeford, 1996; Araujo *et al.*, 1996b) but there is no agreement across studies in different species. In studies in pigs the most common bag size is 2.5 x 4.0 cm whereas, in ruminant studies bags tend to be either 6.0 x 6.0 cm or 3.5 x 5.0 cm and in equine studies bags of either 6.0 x 1.0 cm or 3.5 x 6.5 cm have been used. Pore size of bag cloth has also been investigated (Graham *et al.*, 1985; Cherian *et al.*, 1989; Varvikko and Vanhatalo, 1989; Vanhatalo and Keetoja, 1995) but again there is no consensus across studies, with pore size ranging from 1 - 70 μm. Particle size of samples and sample size have also been investigated (Graham *et al.*, 1985; Cherian *et al.*, 1989; Leibholz, 1991). Other factors that differ across studies include pre-treatment of bags prior to introduction into the digestive tract, number of bags introduced and site of bag retrieval. In pig studies bags undergo a simulated gastric digestion before insertion into the small intestine *via* a duodenal cannula (Graham *et al.*, 1985; Cherian *et al.*, 1989; de Lange *et al.*, 1991; Leibholz, 1991). In ruminant studies, four pre-treatment methods have been used: rumen incubation of

feeds (de Boer *et al.*, 1987; Vanhatalo and Keetoja, 1995; O'Mara *et al.*, 1997); pepsin/HCl incubation only (Jarosz *et al.*, 1994; Mgheni *et al.*, 1994); rumen incubation followed by pepsin/HCl incubation or no pre-treatment (Varvikko and Vanhatalo, 1989). In equine studies no pre-treatment has been recommended as bags are introduced directly into the stomach *via* a naso-gastric tube. The number of bags inserted into the digestive tract at any one time also varies between studies. In pig studies, 2 - 6 bags are inserted into the cannula during the course of a meal, with the procedure taking up to an hour to complete (Graham *et al.*, 1985; Cherian *et al.*, 1989; de Lange *et al.*, 1991). In ruminant studies, the procedure is also time consuming. De Boer *et al.* (1987) inserted one bag every 45 min, Becker *et al.* (1996) inserted one bag every 15 min during a meal (total of six bags per meal) whereas Vanhatalo and Keetoja (1995) inserted four or five bags at any one time, aiming to insert twenty bags per animal per day. Such procedures for bags insertion require meticulous record keeping to record the exact time each individual bag is inserted into the digestive tract. In equine studies, the use of a flexible naso-gastric tube pre-loaded with bags allows for a substantial number of bags to be introduced at any one time. Araujo *et al.* (1996) introduced ten bags at a time using this method, Hyslop *et al.* (1998) introduced 22 bags and Macheboeuf *et al.* (1995) introduced 30 bags.

Few studies have examined the effect of transit time of bags through the digestive tract or the site of bag recovery. Vanhatalo and Keetoja (1995) in studies in ruminants noted that bags recovered in the faeces had a transit time almost double that of bags recovered from the ileum (25 and 12 h respectively) and that degradation of feeds was greater for faecal bags than for ileal bags, particularly for forage feeds. They also noted that the retention time of bags was related to level of feeding and that the shortest retention times were recorded when diets were fed at a high level. Beckers *et al.* (1996) concluded from their studies in ruminants that there was no relationship between transit time and degradability of concentrates. In pig studies, retention time did not influence the extent of barley degradation but did for whole

crop peas (Graham *et al.*, 1985). Degradation of fibrous feeds was greater in bags recovered from equine faeces than for bags recovered in the equine caecum (Moore-Colyer *et al.*, 1997b).

Most of the studies mentioned above have investigated predominantly protein digestion, however, this technique has also been used to measure digestion of other feed components. Digestion of structural carbohydrates (dietary fibre) has been measured in ruminants (Jarosz *et al.*, 1994; Vanhatalo and Keetoja, 1995) and in equids (Longland *et al.*, 1997; Moore-Colyer *et al.*, 1997a; Moore-Colyer *et al.*, 1997b) using mobile bag methodology

The mobile bag technique has great potential as a method to measure degradation of feeds, however it would appear that a common methodology has yet to be adopted. Studies in equines show the most agreement in methodology with similar bag sizes, pore size of cloth, use of naso-gastric tube and site of bag retrieval.

In sacco techniques (both mobile bag and *in situ*) have the advantage of being carried out within the digestive tract of the animal, however, they can be time consuming and only a small number of feeds can be investigated at anyone time. Where many feedstuffs are to be investigated then other techniques can be used such as *in vitro* methods.

2.2.1.4 *In vitro* techniques

In vitro techniques must simulate as closely as possible what happens *in vivo* to be valid methods of predicting *in vivo* digestibility (Weiss, 1994). There are three *in vitro* techniques in routine laboratory use: two-stage incubation (Tilley and Terry, 1963); enzymatic digestion (Jones and Hayward, 1975) and gas production procedures (Menke *et al.*, 1979; Theodorou *et al.*, 1994).

2.2.1.4.1 Tilley & Terry Two Stage Incubation Technique

In vitro laboratory techniques usually involve incubating a known amount of feed sample with digestive fluids (rumen or caecal fluid) and buffers. After a suitable incubation time the remaining feedstuff is weighed and the disappearance calculated. This is then related to digestibility *in vivo*. Tilley & Terry (1963) refined this method by making it a two-stage procedure. In stage one the feed under investigation is incubated with a microbial inoculum (generally rumen fluid) and buffers for 48 h. In stage 2, hydrochloric acid and pepsin are added and left to digest for a further 48 h. The Tilley & Terry procedure was developed as an end-point digestibility method and gives no information of the kinetics of digestion.

2.2.1.4.2 Enzymatic procedures

Digestibility assays using enzymes instead of microbial inocula have also been developed. This is largely due to the increase in availability of fungal enzymes produced commercially. Jones and Hayward (1975) developed an assay which involved the ground forage being treated with pepsin and acid followed by a fungal cellulase. As with the Tilley & Terry technique, enzymatic assays allow only for end-point digestibility measurements. The advantage of enzymatic assays over Tilley & Terry is the reduction in variability of predicted digestibility values by the removal of variation in rumen fluid (due to animal to animal variation and host animal diet). However, they are generally less accurate than assays using rumen fluid and the accuracy of the prediction appears to depend on the feed under investigation and the enzymes used (Nocek, 1988a; Stern *et al.*, 1997). The enzymatic method is therefore used for the relative ranking of feeds rather than for providing actual values of digestibility (Weiss, 1994)

2.2.1.4.3 Gas production Techniques

During rumen fermentation, feedstuffs are degraded by the rumen microorganisms to produce volatile fatty acids (VFA), gases (methane and CO_2) and microbial cells (McDonald *et al.*, 1996). The measurement of gas production using an *in vitro* system can be used to study the extent and rate of digestion of feedstuffs (Hungate, 1966). Gas production techniques are similar to other *in vitro* techniques in that a sample of feedstuff is incubated with digestive fluids and buffers. The gas produced is then measured throughout the incubation period. The amount of gas produced is related to the amount of substrate fermented. Different methods of measuring gas production have been used. The most common methods include the use of either calibrated syringes (Menke *et al.*, 1979), or a pressure transducer (Theodorou *et al.*, 1994) although other methods include measuring water displacement (McAllister *et al.*, 1990). In the method of Menke *et al.* (1979) the production of gas during the incubation causes the barrel of the syringe to rise and the volume of gas is noted at regular intervals. The pressure transducer method (Theodorou *et al.*, 1994) measures the quantity and pressure of gas accumulated in the headspace of sealed serum bottles. This technique allows the kinetics of digestion to be investigated as well as end-point digestibilities. Digestion kinetics are analysed by applying models to the cumulative gas curves obtained.

In vitro techniques have been developed to predict *in vivo* digestibilities. This is normally done through the use of regression equations. More recently studies have investigated *in vitro* techniques to predict *in situ* degradation values (Sileshi *et al.*, 1996; Cone *et al.*, 1998). Where digestion kinetics are calculated by *in vitro* techniques, effective degradabilities can also be calculated. As in the case of *in sacco* methodologies the calculation must incorporate an estimate of digesta passage.

2.2.2 Measurement of digesta passage

Whilst *in sacco* (*in situ* and mobile bag) techniques allow the study of digestion kinetics and calculation of the potential degradability of a feed they do not take into account the effect of particles moving out of the section of the digestive tract in which the bags are incubated. An estimate of passage rate through the digestive tract is required before effective degradabilities can be calculated. The inclusion of a measure of passage rate in such calculations is important, as digestion is not a static process. As digesta move through the gastrointestinal tract, particles are either degraded and the end products absorbed into the blood stream or they flow through into the next section of the tract. Different segments of the digestive tract may well have different flow rates through them and this will affect the extent of degradation within that section of the tract. Penry and Jumars (1987; 1988) were amongst the first to suggest that mammalian digestive tracts could be modelled on chemical reactors. They suggested that there were three types of ideal chemical reactors: batch reactor (BR); plug-flow reactor (PFR) and continuous-flow stirred-tank reactor (CFSTR), and that these were representative of different segments of digestive tracts of animals. As equations describing movement through each of these ideal reactors already existed, Penry and Jumars (1988) suggested that these equations could be used to predict the performance of gut reactor models with respect to digestive reactions. Other researchers have described the digestive tract of different mammals using the terminology of chemical reactors (Hume, 1989; Hume and Sakaguchi, 1991) whilst others have incorporated the mathematical basis of chemical reactors into mathematical models of the digestive tract (Ellis *et al.*, 1994) (see section 2.2.2.1). Hume (1989) suggested that the ruminant digestive tract could be described as a CFSTR (rumen) followed by a PFR (small intestine) in series, whereas hindgut fermenters such as equines could be described as having a PFR (small intestine) with a CFSTR (caecum) in series.

Estimates of digesta passage can be determined for the whole digestive tract or parts thereof by the use of markers. As with markers used to estimate *in vivo* apparent digestibility, passage rate markers must also comply with the criteria suggested by Owens and Hanson (1992) (see section 2.2.1.2). Warner (1981) described markers used in digesta passage studies as recognisable indigestible substances, which are administered either by an oral dose (usually with food) or placed directly in a segment of the digesta tract *via* a fistula. The marker can then subsequently be measured either in the faeces or in digesta in segments of the digestive tract.

As digesta is composed of both a fluid and a solid phase, markers can also be categorised as either liquid (solute) or particulate makers. The most common solute markers are polyethylene glycol (PEG), chromium-ethylenediaminetetra-acetic acid (Cr-EDTA) and cobalt ethylenediaminetetra-acetic acid (Co-EDTA). Although PEG, Cr-EDTA and Co-EDTA are used as solute markers it has been reported that under some conditions, these markers are also associated with digesta dry matter (Faichney, 1975). For particulate digesta passage, a variety of markers have been used and include dietary markers such as lignin, alkanes and neutral detergent fibre, plastic/radio opaque pellets and solutions of rare earth elements (Warner, 1981). On administration these solutions become either physically or chemically associated with the particulate phase. The use of mordanted fibre has also been investigated. However, not all markers meet all the criteria for an ideal marker and problems can occur with marker digestion, migration, biosynthesis, absorption and analysis (Owens and Hanson, 1992).

Markers can be administered in a number of different ways depending on the purpose of the experiment. Likewise, collection of marker and mathematical treatment of the resultant data will also be dependent on the aim of the experiment. Warner (1981) Faichney (1975) and Owens and Hanson (1992) have reviewed the more common combinations of marker administration, collection and mathematical analysis.

When digesta flow is measured the marker is generally administered either by repeated oral doses (marker included in the feed) or *via* a bolus placed directly in a segment of the gastrointestinal tract (i.e. rumen or caecum). This method is known as continuous dosing. Once the marker has achieved a steady state, collection of digesta/faeces can commence.

When measuring digesta flow, a dual marker system is normally employed (both liquid and solid phase markers). Two techniques have been developed to estimate digesta flow through various segments of the gastrointestinal tract.
The exteriorisation of digesta flow using re-entrant cannulae. This allows for the direct measurement of flow rate. Many of these studies have also employed markers, so that sampling from the re-entrant cannula need not be continuous (Galyean and Owens, 1991).

Indigestible markers can be used to calculate rate of flow of digesta between mouth and faeces or between mouth and a cannula in a distal segment or between two cannulae or between a cannula and faeces.

When digesta flow measurements have been completed, administration of the marker can be stopped and collection of the digesta continued. The decline in marker concentration can then be used to determine pool sizes and dilution rates.

The methodologies for digesta flow studies have been developed in ruminants (Huhtanen *et al.*, 1994; Dove and Milne, 1994; Li *et al.*, 1994), pigs (Potkins *et al.*, 1991; Johansen and Bach Knudsen, 1994; Donkoh and Moughan, 1999) and equines (Nyberg *et al.*, 1995; Meyer *et al.*, 1996; de Almeida *et al.*, 1998). The use of re-entrant cannulae in digesta flow studies has been reviewed by MacRae (1975). In the

work undertaken for this thesis digesta flow was not investigated therefore this subject will not be reviewed further here.

When measurements of digestion kinetics are to be made, such as determination of pool size, dilution rates and mean retention time (MRT), markers are generally administered by a pulse dose either orally or directly into a segment of the gastrointestinal tract *via* a fistula. Marker collection includes total faecal collection, faecal sampling, sampling from the same section as marker entry, sampling from a site distal to marker entry or by slaughter where segments of the gastrointestinal tract are tied off and the digesta collected from within the tied off segments.

2.2.2.1 Mathematical interpretation of marker data

Mathematical analysis of the data are largely dependent on what parameters the researcher is trying to derive and how the data was collected. Simple algebraic calculations (Faichney, 1975; Theilemans *et al.*, 1978; Pearson and Merritt, 1991) of MRT can be made when the time of sample collection is known along with knowledge of concentration of the marker in that sample or the amount of marker in the sample. Blaxter *et al.* (1956) calculated mean time for stained particles to pass through the digestive tract of sheep by dividing the sum of times that individual stained particles spent in the tract by the total number of particles. This has the same general form as the equation given by Pearson and Merritt (1991) that states

$$MRT = \Sigma(m_i t_i)/\Sigma m_i \qquad \text{Equation 2.2.1}$$

Where m_i is the amount of marker excreted at time t_i after administration of the marker. Although expressed differently, both methods equated to that of Faichney (1975) who described MRT as

$$MRT = \Sigma t_i M_i \qquad \text{Equation 2.2.2}$$

Where t_i is the time elapsed between dosing and the *i*th defaecation and M_i is the marker excreted in the *i*th defaecation as a fraction of the total amount of marker excreted. Faichney suggested that this equation could also be applied to total digesta collections using re-entrant cannulae. The equation then takes the form of

$$MRT = \Sigma C_i t_i / \Sigma C_i \qquad \text{Equation 2.2.3}$$

Where C_i is the marker concentration at time t_i after dosing.
The above equation assumes a constant time interval between samples and this is generally true when sampling from a cannula. Theilemans *et al.* (1978) suggested that this equation could be modified and used for faecal excretion patterns where the time between subsequent defaecations was not constant. The equation takes the form

$$MRT = \Sigma t_i C_i \Delta t_i / \Sigma C_i \Delta t_i \qquad \text{Equation 2.2.4}$$

Where C_i is the marker concentration at time t_i after dosing and Δt_i is the time elapsed between t_i and the previous sample.

The advantage of Equation 2.2.4 is that it allows MRT to be calculated in situations where total faecal collections are not practical i.e. animals in a grazing situation or where not all faeces are collected.

All of the above equations calculate MRT for the whole of the gastrointestinal tract, however, some workers have used compartmental analysis to calculate MRT for different segments as well as for the whole of the tract.

Faecal excretion data can be plotted to give a bi-exponential curve with a delay followed by a rapid ascending phase followed by a slowly declining phase. The first

to suggest the use of compartmental analysis was Blaxter *et al.* in (1956), who suggested that the digestive tract of ruminants could be modelled as

$$\underset{\text{rumen}}{A} \xrightarrow{k_2} \underset{\text{abomasum}}{B} \xrightarrow{k_1} \underset{\text{duodenum}}{C} \xrightarrow{\tau} \underset{\text{faeces}}{R}$$

Where A, B, C and R represent amounts of a unit of food in the compartments indicated. At t = 0, $A = 1$ and all food is present in the first section, when $t \rightarrow \infty$, $A \rightarrow 0$ and $R \rightarrow 1$. k_1 and k_2 are rate constants and τ is the time delay from C to R. Blaxter *et al.* (1956) mathematically assigned the faster turnover rate to k_1 and the slower turnover rate to k_2, and biologically assigned k_2 to the turnover rate in the rumen and k_1 to the turnover rate of the post-rumen section, although they pointed out that they had no direct experimental evidence for these designations.

Grovum and his co-workers (1973) carried out a series of experiments which further developed the idea of compartmental analysis. Using simple curve peeling procedures Grovum and Williams (1973) calculated rate constants for both the ascending phase and the declining phase of the faecal excretion curve and transit time. In contrast to Blaxter *et al.* (1956) Grovum and Williams (1973) assigned the slower turnover rate to the rumen and designated it k_1. Both models assume transit time is passage through the tubular sections of the digestive tract.

Both the models described above assume that the two compartments are single homogenous pools that follow first order kinetics for outflow/dilution rates. First order kinetics assume that when particles enter the pool they undergo instantaneous mixing and that particles just entering the pool have the same chance of exiting the pool as particles that have spent some time in the pool. This type of digesta passage can be termed time-independent. This has been shown to be not strictly true for the rumen as large particles must undergo a reduction in size before they can exit

(Lechner-Doll *et al.*, 1991; de Vega and Poppi, 1997). If particles must undergo a reduction in size before leaving the rumen, the rumen no longer behaves as a true CFSTR. Matis (1972) suggested that for such particles the probability of particle escape increased with time spent in the rumen. Such passage flow was termed time-dependent. Pond *et al.* (1994) suggested that where a tubular section (PFR) and the fermentation chamber (CFSTR) are in sequence, the non-mixing nature of a PFR would result in a time-delay between dosing and first detection of marker. It would therefore be logical to conclude that where a PFR precedes a CFSTR the passage from the PFR to the CFSTR will also be time-dependent.

Various workers have proposed models that incorporate some form of time-dependency (Matis, 1972; Dhanoa *et al.*, 1985; France *et al.*, 1985; Matis *et al.*, 1989; France *et al.*, 1993). The models of Matis and co-workers (1972, 1989) proposed the use of non-exponential distributions, a mathematical family of which exist and are known as gamma functions. Dhanoa *et al.* (1985) suggested the use of multiple sub-compartments within a mixing compartment whereas France *et al.* (1985) initially suggested distributed time lags and then went on to suggest a diffusion model (1993).

Of the models proposed, those of Matis and co-workers (1972, 1989) and Dhanoa *et al.* (1985) have been shown to be applicable to a large number of ruminant data sets whereas the models of France *et al.* (1985, 1993) no longer use distributed time delays (France *et al.*, 1988) and the incorporation of diffusion mechanisms leads to a model that cannot be fitted to the data.

Few studies have looked at the application of mathematical models to faecal excretion data from equines. However, Rittenhouse *et al.* (1982) applied simple polygonal equations to faecal excretion data obtained from mares and cattle. From the fitted equation faecal production estimates were calculated and then correlated with actual faecal production. Correlation coefficients of 0.991 and 0.955 were obtained for

mares and cattle respectively. By contrast, Corino *et al.* (1995) claimed a correlation coefficient of less than 0.6 when ruminant models were applied to equine data, although actual details of models and data are not given. Corino *et al.* (1995) then proposed a new model based on cumulative faecal excretion curves from both horses and sheep. However, the mathematical basis of this model has yet to be published, thus making it difficult to compare with present models. Although this model has three fitting parameters (k_1, k_2 and TT), the transit time parameter (TT) must be determined experimentally. Therefore, faecal collections must begin at the time of dosing to obtain an accurate estimate of transit time. Any inaccuracies in predicting TT may result in inaccuracies in the remaining two rate parameters. The model of Corino *et al.* (1995) has not, as yet, been applied to data from other studies.

If, as suggested by Hume (1989), the digestive tract of equines can be described as a plug flow reactor followed by a continually stirred tank reactor, then the time dependent models of Ellis *et al.* (1994) may provide a better model to determine MRT in different segments of the digestive tract. This may also be true for other monogastric animals such as pigs. Pond *et al.* (1986) investigated the use of markers to estimate digesta flow in pigs and concluded that a one compartment time dependent model provided the best statistical fit of the data. It should however, be noted that a model is only valid if it has both biological and statistical relevance. A mathematical model can always be derived to fit the data, however if it cannot explain the biology it is not a valid model.

2.3 Summary

Starch is the major storage polysaccharides of many cereal grains and legume seeds and is composed of two fractions, amylose and amylopectin. Most cereal starches are composed of 25% amylose and 75% amylopectin and the crystalline structure of starch is dependent on this ratio. The crystalline structure of starch is disrupted when cereals undergo physical processing. Heat treatment of cereals, such as micronisation

and extrusion result in the gelatinisation of the starch granules which is thought to increase degradation of starch in the digestive tract. Different botanical starch sources are digested to different extents by mammalian α-amylases. In equines, the extent of digestibility of cereal starches in the small intestine is dependent on type of cereal and processing. When starch escapes digestion in the equine small intestine, it may be fermented in the hindgut by the microflora, resulting in a decrease in intra-caecal pH and a concomitant increase in lactate concentrations. Improving starch digestion in the small intestine by processing of cereal starches may help prevent unfavourable changes to the hindgut environment.

In sacco methods have been developed to measure degradation rates of feedstuffs in different segments of the digestive tract. The *in situ* technique is used routinely in ruminant studies to predict protein degradability, however its use in equines has been limited. More recently the mobile bag technique has been developed in pigs, ruminants and equines and shows potential as a means of measuring feed degradation in different segments of the digestive tract.

By incorporating passage rates with *in sacco* results it is possible to calculate the effective degradability (ED) of a feedstuff in a particular segment of the digestive tract. Passage rates studies in equines are limited and tend to focus on mean retention time through the whole digestive tract. However, if *in sacco* methods are to be used routinely in equines to predict ED then predicting MRT in different segments of the digestive tract is important. This can be achieved by applying compartmental models to faecal excretion curves. However, there is a paucity of studies which have undertaken this approach and there is no consensus among workers as to which model is the most appropriate to use for horses.

In sacco methodologies combined with knowledge of digesta passage rates have the potential to determine the site and extent of degradation of feedstuffs in the digestive tract of equines.

2.4 Scope of thesis

The scope of this thesis was therefore, to investigate the effects of processing of grains on intra-caecal fermentation parameters in ponies and to determine the site and extent of starch degradation in ponies using *in sacco* techniques. The objectives therefore were;

To measure *in vivo* apparent digestibility values of a starch-based feedstuff that has undergone physical processing

To develop methodologies for *in sacco* techniques to measure feedstuff degradation kinetics in different segments of the equine digestive tract in order to quantify the effects of physical processing of starch-based feedstuffs

To measure intra-caecal fermentation parameters in ponies receiving either an all forage diet or a diet containing physically processed cereals

To determine rates of digesta passage through the equine digestive tract

Chapter 3. Physical processing of barley and its effect on digestion and passage of nutrients in the digestive tract of ponies.

3.1 Experiment 1a. Physical processing of barley and its effects on intra-caecal fermentation parameters and apparent digestibility *in vivo* in ponies.

3.1.1 Introduction

Cereal grains frequently form a major portion of the concentrate fraction of diets for equines (Frape, 1998). These grains are often physically processed with a view to increasing their digestibility. Starch constitutes the single largest component of cereal grains, but starch plays only a minor role in the diet of wild equids. In addition, the activity of amylase is reported to be low in the small intestine of the horse compared to that of other mammals (Alexander and Chowdhury, 1958; Alexander and Hickson, 1970; Kienzle *et al.*, 1994). Whilst this may limit the extent of precaecal digestion of starch, the *in vivo* apparent digestibility of starch, derived from a number of different cereal types, measured over the whole digestive tract of equines is uniformly high (967-970 g/kg) (Arnold *et al.*, 1981). Starch which escapes digestion in the equine small intestine enters the caecum where it will be rapidly fermented by the amylolytic microflora. However, such activity may alter microbial fermentation parameters resulting in elevated lactate, lower pH and substantial changes in the molar proportions of acetate, propionate and butyrate.

A limited number of studies have examined the effect of physical processing of cereals on the digestibility of starch in the small intestine of equines (Householder, 1978; Kienzle *et al.*, 1992). In general, starch digestibility in the small intestine of equines was improved by physical processing of cereals, although the magnitude of the effect was dependent on both type of cereal and method of processing. However,

these studies have not examined any effects that physical processing of cereals may have on equine hindgut fermentation parameters.

The objectives of this study were:

1) to examine intra-caecal fermentation parameters in ponies offered diets containing rolled, micronised or extruded barley;
2) to determine the *in vivo* apparent digestibilities, digestible energy (DE) and digestible crude protein (DCP) content of the above forms of physically processed barley in ponies

3.1.2 Materials and Methods

3.1.2.1 Animals and housing

Three caecally-fistulated mature Welsh-cross geldings (each weighing approximately 280kg) were used in this trial. All ponies were fitted with a permanent fistula (approximately 130mm x 45mm) in the caecum (Cottrell *et al.*, 1998). The fistula was located in the caecal gas cap, in the base of the caecum, to reduce leakage of caecal contents. The base of the caecum is relatively immobile compared to other parts of the caecum (D. Cottrell, pers. comm.) and is situated next to the ileal-caecal valve.

The ponies were housed in 2 pens measuring 4 metres by 5 metres, which were divided by a central flexible partition. This ensured that feed and faeces were separate for individual ponies but allowed social interaction between ponies. During the adaptation periods ponies were bedded on wood shavings, which were then removed and replaced with rubber floor mats during the collection periods. Automatic drinkers provided clean, fresh water *ad libitum*.

3.1.2.1.1 Caecal parameters and *in vivo* apparent digestibility

3.1.2.1.2 Diets

Ponies were offered 4 kg DM per day of either 100% ground and pelleted hay cubes (HC) or one of three diets consisting of a 50:50 barley:hay cubes mix. The barley in the mixed diets was either rolled (RB), micronised (MB) or extruded (EB) each of which was provided by Dodson and Horrell Ltd, Ringstead, Kettering, UK. Micronising involved passage of whole barley (DM of 820g/kg) under infra-red burners and then through rollers to produce barley flakes. The extruded barley was prepared by grinding whole barley and the resultant meal was then mixed with water to form dough. The dough was then sequentially passed through a single screw extruder and then a die (9.4 mm diameter, with 10 holes open) , and the emergent material was then dried (110°C for 23 min) forming nuggets of *ca.* 1.5cm^3. Diets were offered in two equal meals per day at 09:00 and 17:00 hours where the barley and HC components of the meal were offered simultaneously but in different containers so that feed refusals of each dietary component could be recorded. The HC component of the diet also contained 30g of a mineral-vitamin supplement (see appendix 1 for details, Norvite Feed Supplements, Insch, Aberdeenshire). Diets were assigned to ponies according to a 3 x 4 incomplete Latin Square changeover design (see Table 3.1.2.1) experiment consisting of four 21 day periods. Each period comprised a sixteen day adaptation phase and a five day recording phase.

Table 3.1.2.1 Experimental protocol for diets assigned to ponies according to a 3 x 4 incomplete Latin Square changeover design consisting of four 21 day periods

	Period 1	Period 2	Period 3	Period 4
Pony 1	RB:HC	HC	MB:HC	EB:HC
Pony 2	MB:HC	RB:HC	EB:HC	HC
Pony 3	EB:HC	MB:HC	HC	RB:HC

(See text for abbreviations)

3.1.2.1.3 Caecal pH, lactate and VFA concentrations.

On days 18 - 21 of each period samples of caecal digesta were taken five hours after the 09:00 hour meal as it has been demonstrated that caecal pH is lowest 4-6 hours post-feeding (Garner *et al.*, 1977; Willard *et al.*, 1977). Digesta samples were removed from the caecum *via* suction through an indwelling plastic tube (internal diameter 13 mm) attached to the cap of the caecal cannula. Similarly, on day 20 of each period samples of caecal digesta were withdrawn at 09:00 hours and thereafter hourly until the 17:00 hour meal. Caecal pH was immediately determined on each sample using a Mettler Toledo 320 pH meter (Mettler – Toledo Ltd, 64 Boston Road Beaumont Leys, Leicester LE4 1AW, UK) and 9ml of the sample was preserved with the addition of 1ml of 1.8 molar H_2SO_4. Samples were then stored at –20°C until they were thawed immediately prior to lactate and volatile fatty acid (VFA) analysis.

Lactic acid was quantified as L and D isomers using L and D lactate dehydrogenases (Boehringer test kit no.139084, BCL, Lewes, Sussex, UK) using the method of Merry *et al.* (1995). Acetate, propionate and butyrate concentrations (mmol/l) in the caecal digesta samples were subsequently determined by Gas Chromatography according to the method of Merry *et al.* (1995) using a Chrompack model CP9000 gas chromatograph with flame ionisation detector (FID) detection, split injection and an automatic (model 911) sampler (Chrompack UK Ltd., London, UK). The column used was a wall coated open tubular (WCOT) fused silica capillary column (25mm X

0.32mm internal diameter) coated with free fatty acid phase-chemically bonded (FFAP-CB). The machine was linked to an IBM Personal computer with MOSAIC (Chrompack) integration software. The frozen samples were thawed, stored at 4°C for 2 days to allow for precipitation and then centrifuged at 3500-x *g* for 3 min using a microfuge (Sorvell Ltd., UK). Clear supernatant (1ml) was removed and mixed with 0.2ml of internal standard (15.0mM 2-methylvaleric acid) in a 2ml glass crimp vial (Vials Direct., Macclesfield, UK). The crimped vials were then loaded on the machine and 0.5µl samples were injected into the column.

3.1.2.1.4 *In vivo* apparent digestibilities

During the five day collection phase *in vivo* apparent digestibilities of DM (DMIV), organic matter (OMIV), starch (STCIV), crude protein (CPIV), neutral detergent fibre (NDFIV), acid detergent fibre (ADFIV) and gross energy (GEIV) were determined by total faecal collection. Feed and daily faecal samples were dried in a force draught oven at 60°C and faecal samples were collated according to daily faecal DM output for each pony to represent each 5 day collection phase. Composite samples of the feed offered and the bulked samples of faeces were subsequently analysed for OM, CP, NDF, ADF and GE according to the methods of the Association of Official Analytical Chemists (1990). STC analysis was carried out by the enzymatic method of McCleary *et al.* (1994). Digestible energy (DE) (GE content of diet x GEIV) and digestible CP (DCP) (CP content of diet x CPIV) contents were also calculated for each diet. In addition, *in vivo* apparent digestibilities, DE and DCP contents for each of the physically processed barleys were calculated by difference (see Equation 3.1.1 for example)

$$\text{DMIV of RB} = \frac{[\text{DMIV of RB:HC} - (\text{DMIV of HC} \times \text{HC proportion of diet})]}{\text{RB proportion of diet}}$$

Equation 3.1.1

3.1.2.1.5 Statistical analysis

Observed dry matter intakes (DMI), *in vivo* apparent digestibilities, DE, DCP and intra-caecal fermentation parameters measured five hours following the 09:00 meal were analysed by analysis of variance (ANOVA) where variance due to diet was identified. Due to the repeated nature of the measurements taken hourly between the 09:00 and 17:00 hour meals on day 20 of each period, the data were analysed using a repeated measures ANOVA with an ante-dependence order of 1. All statistical analysis was carried out using Genstat 5 (Lawes Agricultural Trust 1993).

3.1.3 Results

3.1.3.1 Feed composition and dry matter intake

The chemical composition of the four individual feedstuffs is detailed in Table 3.1.3.1. The observed dry matter intakes (DMI) are shown in Table 3.1.3.2. There were no significant differences in the total DMI of the four diets. Due to small feed refusals the barley:HC diets were no longer a 50:50 mix. The dietary proportions were therefore 54:56, 55:45 and 55:45 on a DM basis for RB:HC, MB:HC and EB:HC respectively. Daily starch intakes (DSI) are also given in Table 3.1.3.2 and averaged 4.19 g/kg LW across the three barley diets. Pony live weight (LW) averaged 284 kg across dietary treatments.

Table 3.1.3.1. Chemical composition of the four dietary components, hay cubes (HC), rolled barley (RB), micronised barley (MB) and extruded barley (EB) offered to ponies (g/kg DM)

Constituent	HC	RB	MB	EB
DM (g/kg)	905	880	891	892
OM	917	977	977	969
Starch	0	614	614	621
CP	91	119	130	118
NDF	654	218	203	167
ADF	335	44	58	36
GE (MJ/kg)	18.9	18.6	18.5	18.8

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.

Table 3.1.3.2. Pony liveweight (LW), dry matter intake (DMI), starch intake (g kg^{-1} LW) of ponies fed hay cubes (HC), rolled barley:hay cubes (RB:HC), micronised barley:hay cubes (MB:HC) or extruded barley:hay cubes (EB:HC).

	HC	RB:HC	MB:HC	EB:HC	sed	sig
LW (kg)	289	284	277	285	6.14	NS
DMI (kg/d)						
Barley[1]	0.05^a	1.97^b	1.96^b	1.95^b	0.07	***
HC	3.69^a	1.70^b	1.61^b	1.58^b	0.17	***
TOTAL DMI	3.74	3.67	3.57	3.53	0.18	NS
daily Starch intake (g kg^{-1} LW)	0.00^a	4.16^b	4.25^b	4.16^b	0.23	***

Values in the same row not sharing common superscripts differ significantly ($P<0.05$).
[1] includes mineral and vitamin supplement.

3.1.3.2 Intra-caecal fermentation parameters

Table 3.1.3.3 gives the intra–caecal fermentation parameters measured 5 h following the 09:00 meal on days 18 – 21 of each period. At 5 h post feeding, inclusion of RB significantly ($P<0.05$) reduced pH and molar proportion of acetate whilst significantly increasing ($P<0.05$) lactate concentration and molar proportion of propionate compared with the HC diet. The pH of the caecal digesta from ponies fed RB (6.26) was not significantly lower ($P>0.05$) than that from ponies fed either the MB (6.33) or the EB (6.36) diets. The pH of digesta from ponies fed the MB and EB diets was not significantly different ($P>0.05$) from that recorded in digesta from ponies fed diet HC (6.50). The lactate concentration of the caecal digesta from ponies fed diet RB (0.97 mmol/l) was significantly higher ($P<0.05$) than that in ponies fed diet HC (0.11 mmol/l). Caecal lactate levels in ponies fed either the MB (0.18 mmol/l) or EB (0.26 mmol/l) diets were not significantly different to the levels recorded in ponies fed either RB or HC diets, but were considerably closer to the values recorded for ponies fed diet HC than for those fed diet RB. Total volatile fatty acid concentration and butyrate molar proportion did not differ significantly ($P>0.05$) between diets. Acetate and propionate molar proportions in caecal digesta from ponies fed EB were similar to those of ponies fed RB ($P>0.05$). However, including MB in the diet of ponies increased and decreased their intra-caecal acetate and propionate values significantly ($P<0.05$) relative to those fed diet RB. There were no significant differences ($P>0.05$) in acetate or propionate molar proportions in caecal digesta from ponies fed MB or EB. However, it was only when MB was included in diets that caecal acetate and propionate values were similar ($P>0.05$) to those observed when diet HC was fed.

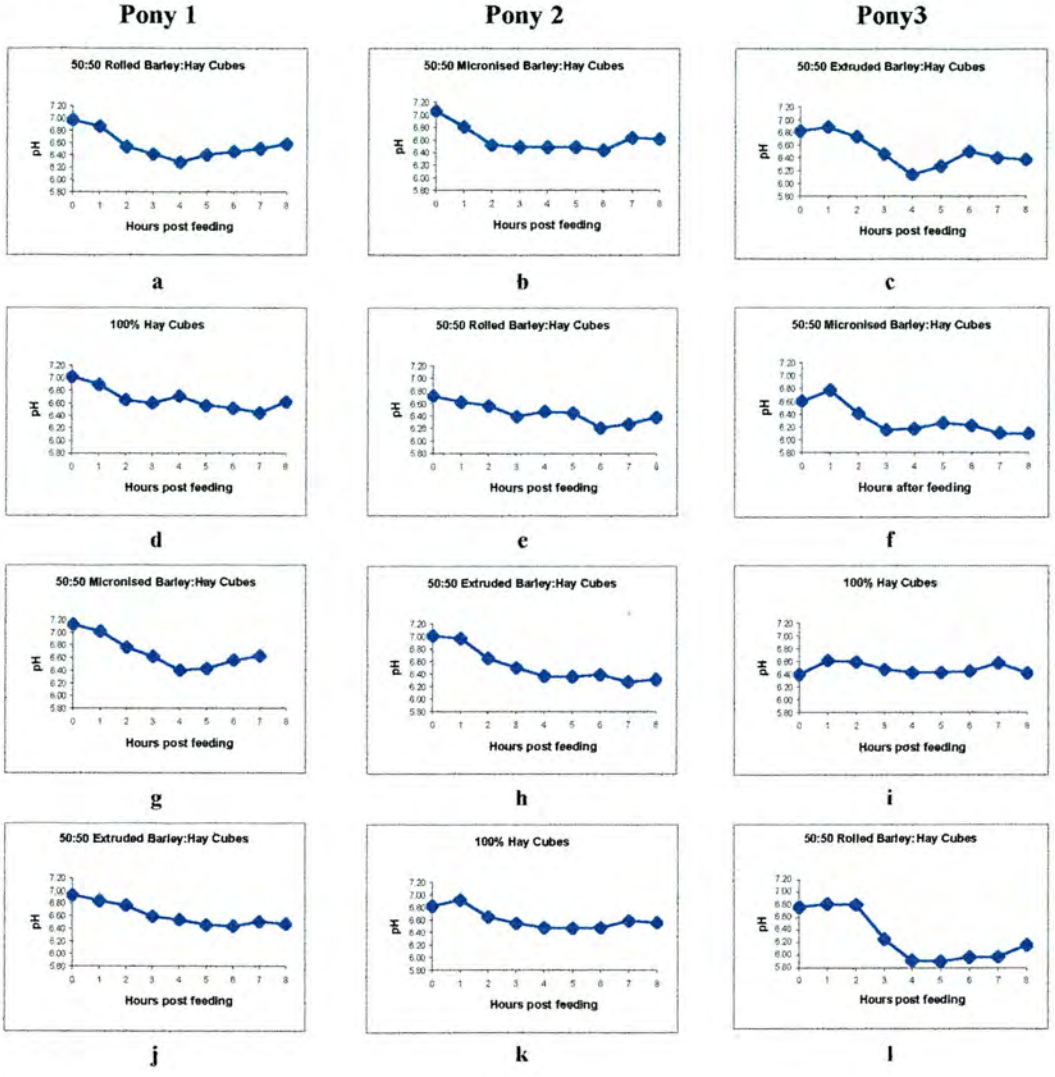
Table 3.1.3.3. Intra-caecal fermentation parameters measured 5 hours following the 09:00 meal in ponies fed hay cubes (HC), rolled barley:hay cubes (RB:HC), micronised barley:hay cubes (MB:HC) and extruded barley:hay cubes (EB:HC).

	HC	RB:HC	MB:HC	EB:HC	sed	sig
pH	6.50^{a}	6.26^{b}	6.33^{ab}	6.36^{ab}	0.097	*
TVFA (mmol/l)	48.5	54.2	49.3	52.9	7.67	NS
Lactate (mmol/l)	0.11^{a}	0.97^{b}	0.18^{ab}	0.26^{ab}	0.411	*
VFA molar proportions (mmol/mol)						
Acetate	767^{a}	630^{b}	716^{ac}	680^{bc}	36.0	*
Propionate	172^{a}	302^{b}	220^{ac}	254^{bc}	32.8	*
Butyrate	61	68	64	66	4.2	NS

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

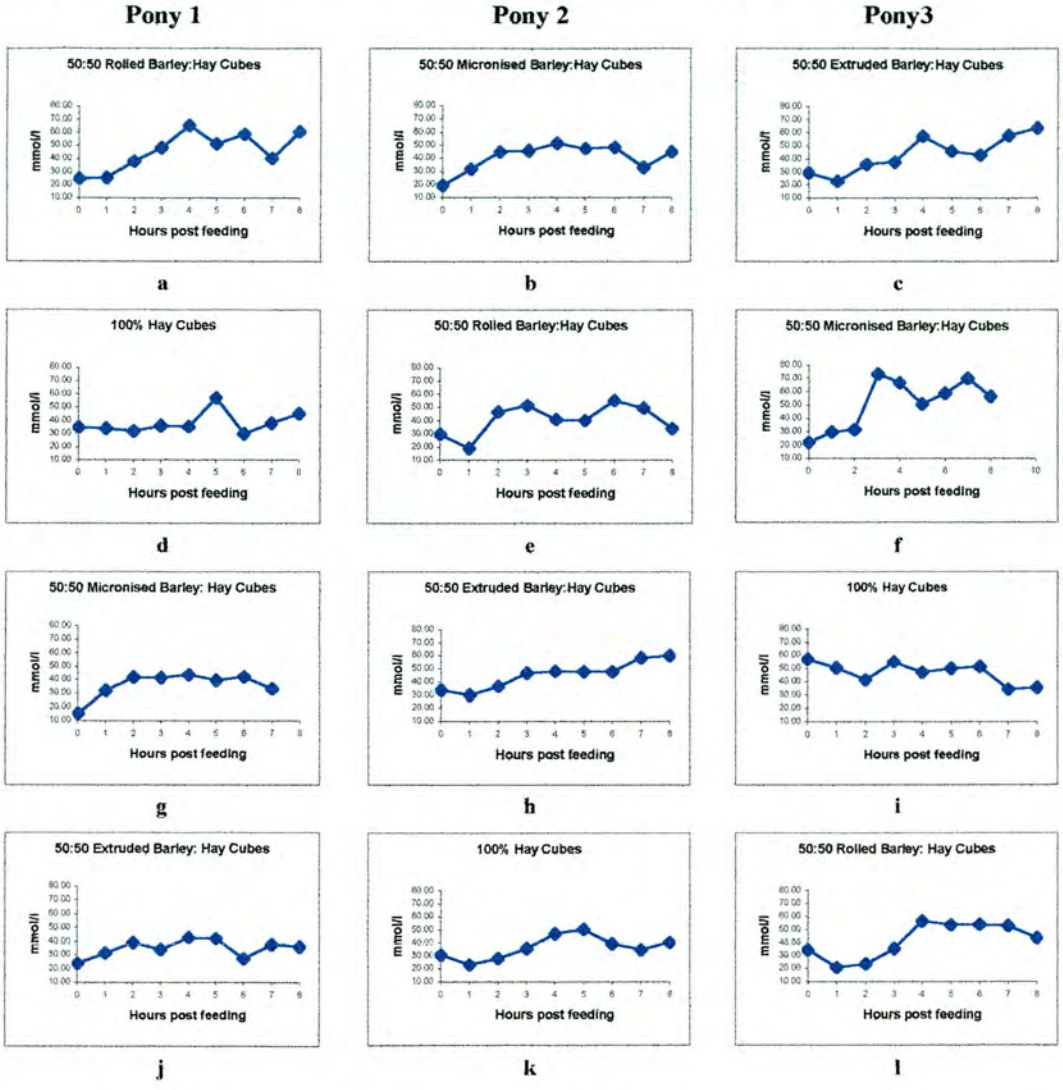
Figures 3.1.3.1 – 3.1.3.6 show the hourly changes in pH, TVFA (mmol/l), lactate (mmol/l), acetate, propionate and butyrate molar proportions (mmol/mol) respectively, measured between the 09:00 and 17:00 hour meals for each pony in each period for all experimental diets. Individual graphs are given for each pony on each diet to demonstrate the large variation between ponies. There was a trend for intra-caecal pH to decline and TVFA levels to increase following the 09:00 h meal in all ponies for approximately three hours after which levels remained relatively stable until the end of the measurement period at 17:00 h. Attempts were made to model the intra-caecal fermentation parameters measured, however due to the 'noise' associated with the data it was only possible to apply a single-node spline (stick regression) model to the pH data (Dhanoa, pers. comm.). This model allowed four parameters to be derived: rate of decline in pH; rate of recovery; time taken to reach minimum pH and minimum pH. These parameters were then subjected to analysis of variance where variance due to diet was identified.

Figure 3.1.3.1 a - l Intra-caecal changes in pH following the 09:00 hour meal in individual ponies offered one of four diets; hay cubes, rolled barley:hay cubes, micronised barley:hay cubes or extruded barley:hay cubes in four different periods



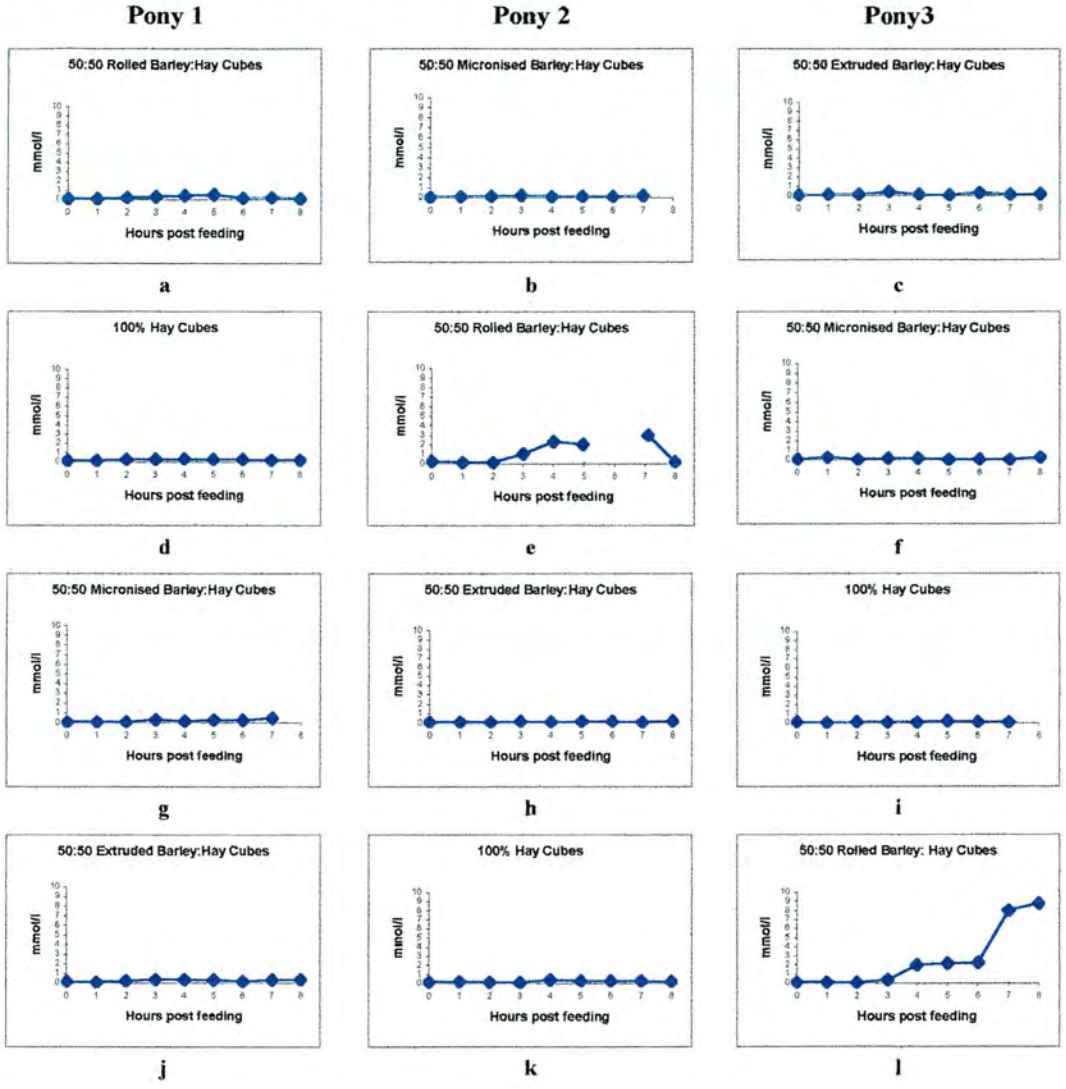
P1 = Period 1, P2 = Period 2, P3 = Period 3, P4 = Period 4

Figure 3.1.3.2 a –l Intra-caecal changes in total volatile fatty acid (TVFA) concentration (mmol/l) following the 09:00 hour meal in individual ponies offered one of four diets; hay cubes, rolled barley:hay cubes, micronised barley:hay cubes or extruded barley:hay cubes in each period



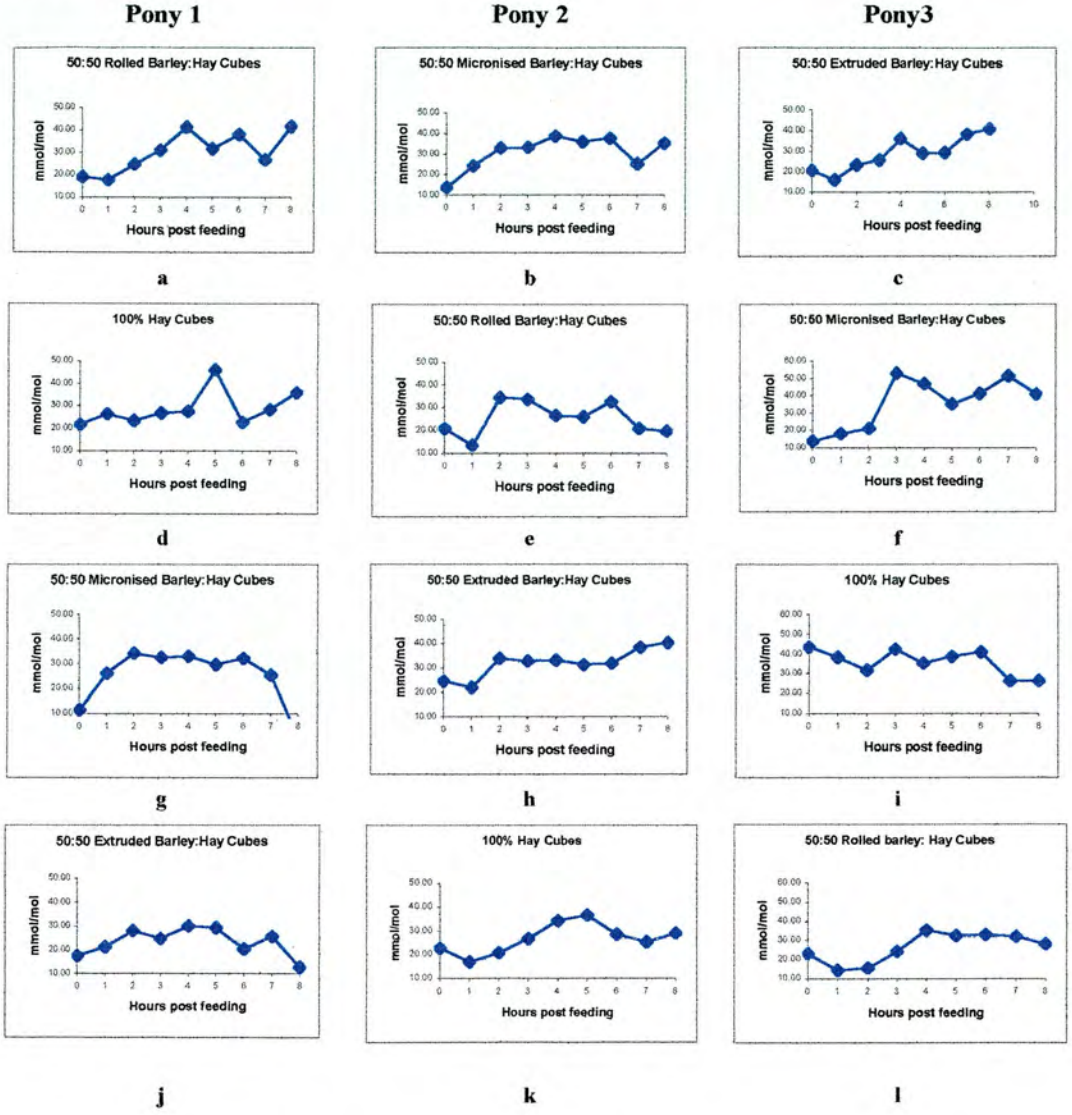
P1 = Period 1, P2 = Period 2, P3 = Period 3, P4 = Period 4

Figure 3.1.3.3 a – l intra-caecal changes in lactate concentration (mmol/l) following the 09:00 hour meal in individual ponies offered one of four diets; hay cubes, rolled barley:hay cubes, micronised barley:hay cubes or extruded barley:hay cubes in each experimental period



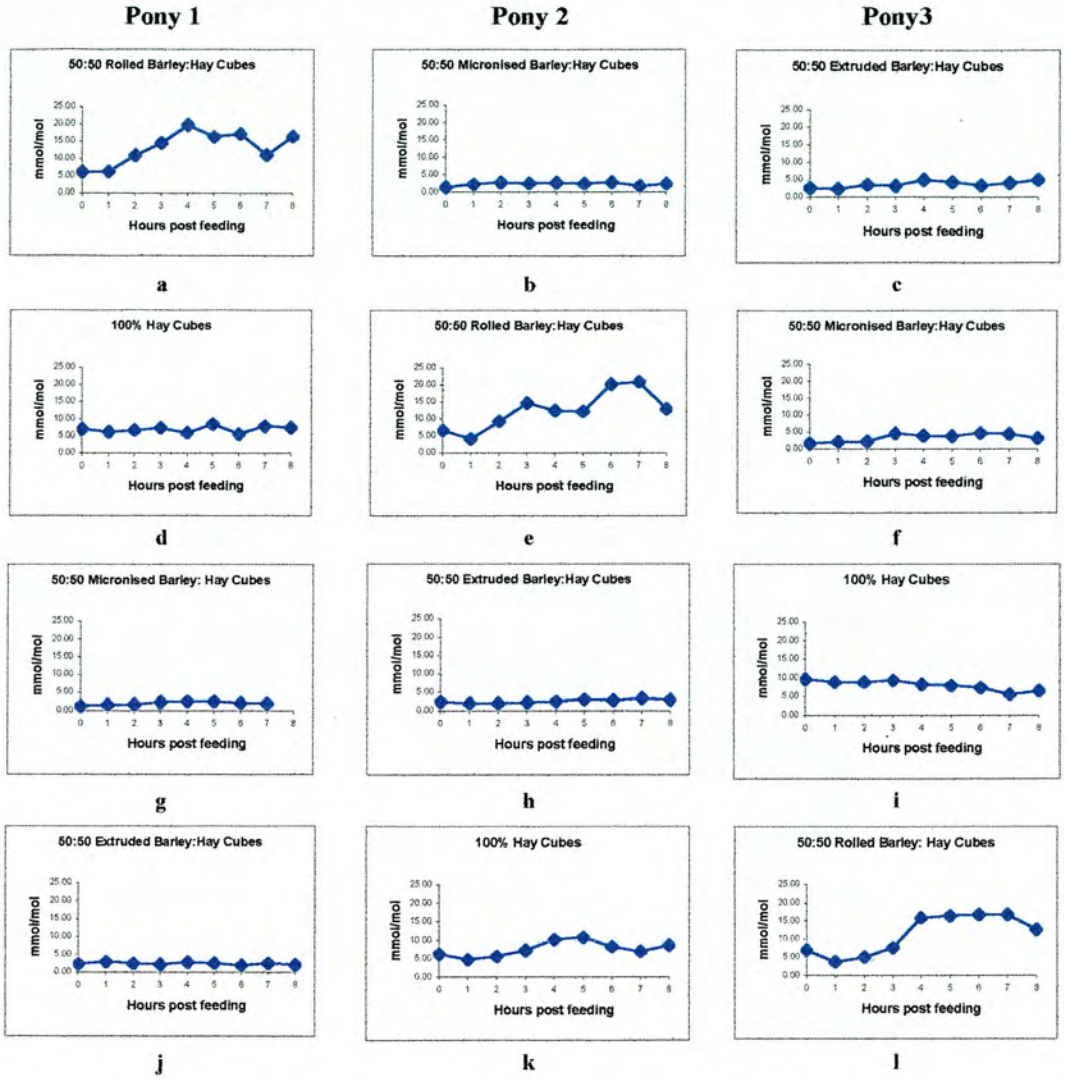
P1 = Period 1, P2 = Period 2, P3 = Period 3, P4 = Period 4

Figure 3.1.3.4 a – l Intra-caecal changes in acetate molar proportion (mmol/mol) following the 09:00 meal in individual ponies offered one of four diets; hay cubes, rolled barley:hay cubes, micronised barley:hay cubes or extruded barley:hay cubes in each period



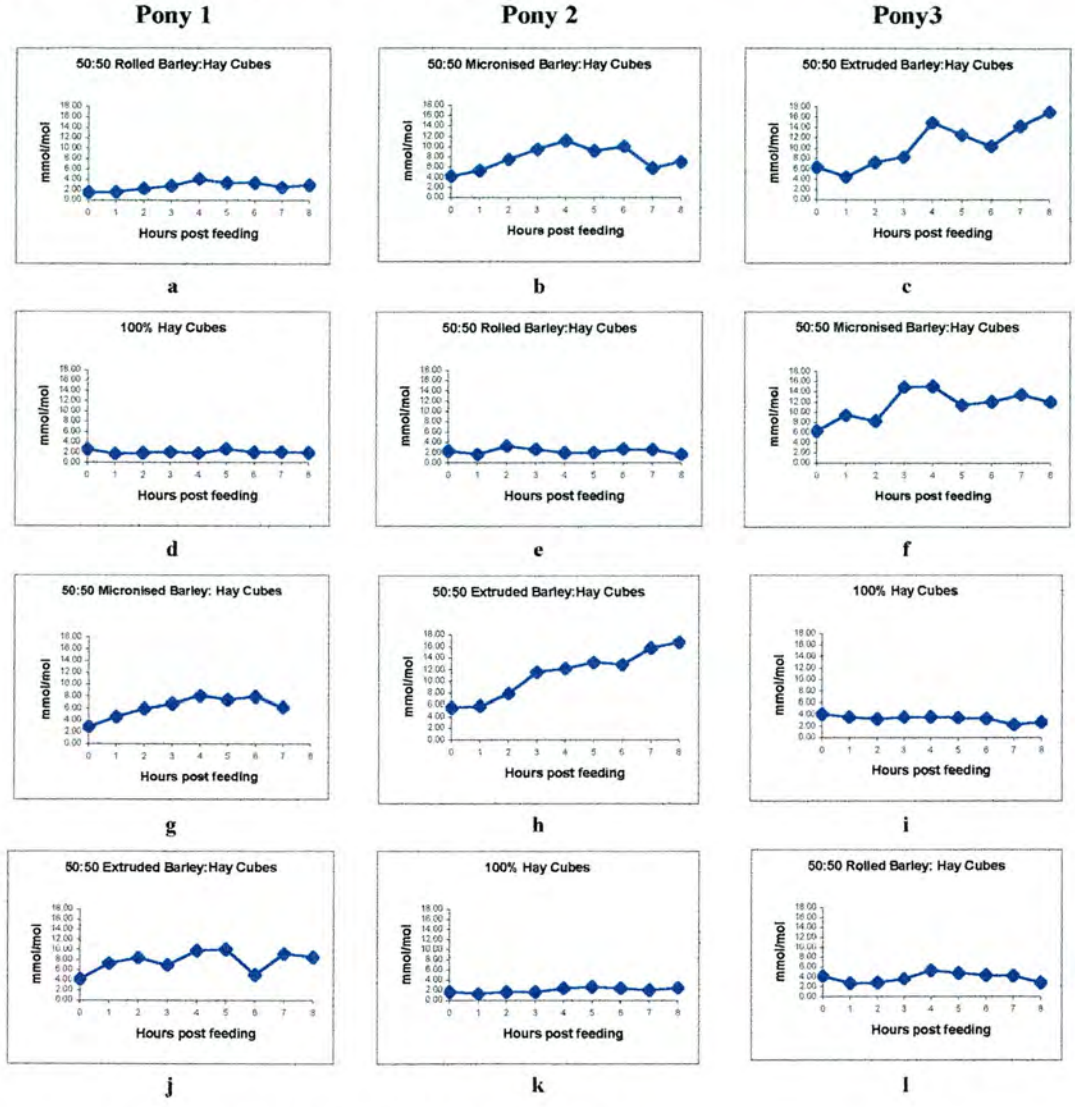
P1 = Period 1, P2 = Period 2, P3 = Period 3, P4 = Period 4

Figure 3.1.3.5 a –l Intra-caecal changes in propionate molar proportion (mmol/mol) following the 09:00 hour meal in individual ponies offered one of four diets; hay cubes, rolled barley:hay cubes, micronised barley:hay cubes or extruded barley:hay cubes in each period.



P1 = Period 1, P2 = Period 2, P3 = Period 3, P4 = Period 4

Figure 3.1.3.6 a – l Intra-caecal changes in butyrate molar proportions (mmol/mol) following the 09:00 hour meal in individual ponies offered one of four diets; hay cubes, rolled barley:hay cubes, micronised barley:hay cubes or extruded barley:hay cubes in each period.



P1 = Period 1, P2 = Period 2, P3 = Period 3, P4 = Period 4

The intra-caecal fermentation parameter data collected for each individual pony was very noisy and was variable between ponies. This made analysis of the data difficult. Attempts were made to fit models to the data, however it was only possible to fit a

The intra-caecal fermentation parameter data collected for each individual pony was very noisy and was variable between ponies. This made analysis of the data difficult. Attempts were made to fit models to the data, however it was only possible to fit a stick regression to the pH data for the barley based diets. The results of this regression analysis are given in Table 3.1.3.4.

Table 3.1.3.4 Derived intra-caecal pH parameters measured hourly following the 09:00 meal in ponies fed rolled barley:hay cubes (RB:HC), micronised barley:hay cubes (MB:HC) and extruded barley:hay cubes (EB:HC).

	RB:HC	MB:HC	EB:HC	sed	sig
Rate of decline	-0.205	-0.176	-0.172	0.1003	NS
Rate of recovery	0.086	0.038	0.014	0.0323	NS
Minimum pH	6.11	6.33	6.41	0.138	NS
Time to minimum (h)	4.86	3.02	4.00	1.072	NS

Although the rate of decline in pH appeared to be faster when the RB diet was fed, this was not significantly different to either the MB or EB diet. The rate of recovery was also faster when the RB diet was fed, however, this was not significantly faster when MB or EB was fed. The time to reach minimum intra-caecal pH was not significantly different across the three barley diets with the shortest time noted for the MB diet (3.02 h) and the longest time noted for the RB diet (4.86). The lowest pH was noted for the RB (6.11) diet but again this was not significantly different to the MB (6.33) or EB (6.41) diet.

A repeated measures ANOVA was carried out to determine if diet affected intra-caecal fermentation parameters for each sampling point, the results of which are given in Appendix 2. Diet had no effect on most of the intra-caecal parameters measured at each sampling point.

In general, the HC diet maintained intra-caecal acetate molar proportions above 730 mmol/mol and those of propionate below 200 mmol/mol throughout the day. However, inclusion of RB in the diet led to lower intra-caecal acetate molar proportions and higher propionate molar proportions when compared to those recorded in ponies fed the HC and MB diets during the 4-8 h period post-feeding. Over the same time period, intra-caecal acetate and propionate values for ponies fed either the MB or EB diet were intermediate between those recorded for ponies fed the RB diet and the HC diet.

3.1.3.3 *In vivo* apparent digestibilities and nutritive values of the four experimental diets

Table 3.1.3.5 contains the *in vivo* apparent digestibilities and nutritive values (DE and DCP) for the total diet. *In vivo* apparent digestibilities and nutritive values of individual barleys were calculated by difference as it has been shown that there are no associative effects of dietary components in equines (Martin-Rosset and Dulphy, 1987) and these values are given in Table 3.1.3.6. With the exception of ADFIV the HC total diet had significantly lower ($P<0.05$) apparent digestibilities and nutritive values than any of the total diets containing barley. DMIV averaged 827 g/kg for the three forms of processed barley whereas DMIV of HC was 439 g/kg. STCIV averaged 967 g/kg across diets containing barley. Although, CPIV of EB (685 g/kg) was lower than that of either RB or MB (709 and 717 g/kg respectively) this difference was not significant. NDF digestibility was significantly higher ($P<0.05$) for EB (722 g/kg) than for HC (405 g/kg). NDFIV of RB (522 g/kg) and MB (575 g/kg) were not significantly different to either HC or EB. DE content was significantly higher ($P<0.001$) for the three forms of processed barley compared to HC (14.5, 14.8, 15.0 and 7.8 MJ/kg DM for RB, MB, EB and HC respectively). DCP

content was also significantly higher (P<0.001) for RB, MB and EB compared to HC (85, 93, 81 and 25 g/kg DM respectively)

Table 3.1.3.5. Total diet *in vivo* apparent digestibilities and nutritive values for hay cubes (HC), rolled barley:hay cubes (RB:HC), micronised barley:hay cubes (MB:HC) and extruded barley:hay cubes (EB:HC) (g/kg unless otherwise stated).

	HC	RB:HC	MB:HC	EB:HC	sed	Sig
DMIV	439[a]	646[b]	660[b]	653[b]	32.9	***
OMIV	445[a]	663[b]	675[b]	669[b]	31.9	***
STCIV	0[a]	967[b]	969[b]	966[b]	6.0	***
CPIV	270[a]	538[b]	564[b]	533[b]	44.3	**
NDFIV	405	433	450	415	50.1	NS
ADFIV	317	227	310	215	82.0	NS
GEIV	413[a]	610[b]	627[b]	628[b]	30.6	***
Nutritive values						
DE (MJ/kg DM)	7.8[a]	11.4[b]	11.7[b]	11.8[b]	0.50	***
DCP (g/kg DM)	25[a]	58[b]	64[b]	56[b]	6.2	***

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

Table 3.1.3.6. *In vivo* apparent digestibilities and nutritive values for hay cubes (HC) when offered alone and rolled barley: (RB), micronised barley (MB) and extruded barley (EB) as calculated by difference from mixed diets (g/kg unless otherwise stated).

	HC	RB	MB	EB	sed	Sig
DMIV	439[a]	825[b]	834[b]	823[b]	48.1	***
OMIV	445[a]	841[b]	846[b]	837[b]	44.4	***
STCIV	0[a]	967[b]	969[b]	966[b]	6.0	***
CPIV	270[a]	709[b]	717[b]	685[b]	59.5	***
NDFIV	405[a]	522[ab]	575[ab]	722[b]	92.5	*
ADFIV	317	187	338	472	187.5	NS
GEIV	413[a]	782[b]	798[b]	799[b]	50.0	***
Nutritive values						
DE (MJ/kg DM)	7.8[a]	14.5[b]	14.8[b]	15.0[b]	0.9	***
DCP (g/kg DM)	25[a]	85[b]	93[b]	81[b]	6.4	***

Values not sharing common superscripts differ significantly (P<0.05).

See text for abbreviations.

3.1.4 Discussion

3.1.4.1 Intra-caecal parameters

When the basal forage diet (HC) was offered without barley supplementation, caecal pH was maintained above 6.5. In conjunction, the intra-caecal VFA profile was characterised by acetate molar proportions above 730 mmol/mol and propionate molar proportions below 200 mmol/mol throughout the measurement period, accompanied by low levels of butyrate and lactate. This observation concurs with results reported by Moore-Colyer *et al.* (2000) for a different batch of the same ground and pelleted hay cubes and for other fibre-based diets. In addition, similar values for intra-caecal pH and VFA parameters have been reported in equines given predominantly forage-based diets (Argenzio *et al.*, 1974; Glinsky *et al.*, 1976, Goodson *et al.*, 1988).

Compared to studies in ruminants, only a limited number of experiments have been conducted in equines where the effects of cereals on intra-caecal fermentation parameters have been studied. In the current study, acetate + propionate values remained constant for the four diets throughout the experimental period, relative to the HC diet. However, the three barley diets elicited a decrease in the molar proportions of acetate with a concomitant increase in propionate, the greatest effect being observed for RB. These results concur with those of Hintz *et al.* (1971a) who offered ponies diets varying in forage (alfalfa):concentrate (maize/soybean) ratios which showed that as the concentrate proportion increased, intra-caecal proportions of acetate decreased whilst propionate increased. Furthermore, Willard *et al.*, (1977), Stillons *et al.*, (1970) Goodson *et al.* (1988) and Radicke *et al.*, (1991) have all published similar changes in caecal fermentation patterns as a result of high levels of concentrate supplementation in diets.

Willard *et al.* (1977) observed that when caecally fistulated horses were offered a 100% hay diet, intra-caecal pH fell to 6.75, 6-7 hours post-feeding, whereas in animals fed a 100% cereal-based concentrate, the corresponding value was 6.12. In the present experiment, the intra-caecal pH of ponies declined during the first three hours post-feeding regardless of diet. Intra-caecal pH of ponies fed the barley diets continued to decline during the latter half of the measurement period, the greatest decline being in ponies fed diet RB. The initial decline in intra-caecal pH observed in this study was assumed to be a result of the feeding schedule employed. The relatively large intervals between meals in this experiment (8 and 16 h) would have resulted in the caecum becoming increasingly empty especially between the evening and morning meals.

In the current study intra-caecal lactate levels in ponies fed the HC diet were low at all time points measured. However, when fed the RB diet, intra-caecal lactate levels were observed that were greater than the corresponding values for HC, MB and EB 4-8 hours post-feeding, and these differences were reflected in the respective intra-caecal pH values. These results are in agreement with the findings of Radicke *et al.* (1991) who found a 70 fold increase in intra-caecal lactate of horses fed a corn diet compared with those fed hay. Furthermore, Willard *et al.* (1977) reported intra-caecal lactate levels in horses fed a 100% concentrate diet to be 25 times greater than those in horses fed a 100% hay diet. High levels of intra-caecal lactate can contribute to hindgut acidosis (Garner *et al.*, 1977), with a loss of mucosal integrity (Clarke *et al.*, 1990) and an increase in absorption of endotoxins (Moore *et al.*, 1979), leading to metabolic disorders such as laminitis and colic. It has been suggested that when intra-caecal pH falls below 6.0 equines may be regarded as exhibiting sub-clinical acidosis (Radicke *et al.*, 1991). In the experiment reported here, average intra-caecal pH declined to approximately 6.2 at its lowest point on the RB:HC diet, however, variation between ponies was such that in one pony caecal pH declined below 6.0, and this pony may well have been acidotic. Indeed, other authors have reported that

acidosis does not occur uniformly in horses that have been given the same amount of cereal starch (Rowe *et al.,* 1994).

In the experiment reported here starch intake averaged 2.1 g/kg LW per meal which is similar to that recommended by Meyer, *et al.*, (1995) to prevent caecal acidosis and hind-gut dysfunction. This value is approximately half of the maxima of 4.0g starch/kg LW per meal recommended by Potter *et al.*, (1992). However, in the current study, when 2.1g/kg LW per meal of starch was consumed as RB significant unfavourable changes occurred in intra-caecal fermentation parameters, which were not observed when this amount of EB or MB starch was fed. In conclusion, these results suggest that if barley is to be fed to horses then it should preferably be micronised rather than being rolled. However, if circumstances dictate that rolled barley must be fed, then it should be at levels considerably lower than 2.1 g/kg LW to ensure that particularly susceptible animals are safeguarded from the effects of lactate acidosis. This is particularly important when acidotic animals succumb to laminitis, as once the disease has been contracted the animal is many times more likely to contract the condition on subsequent occasions.

3.1.4.2 Nutritive value and *in vivo* apparent digestibility of energy and protein

In vivo apparent digestibilities and nutritive values of the HC diet are similar to values for HC reported by Moore-Colyer *et al.* (2000) and for grass hays offered to equines reported by Fonnesbeck (1968) and Cymbaluk (1990). Inclusion of RB, MB or EB in the diet increased the digestibilities and nutritive values of the total diets, however no significant differences could be seen between total diets containing different forms of physically processed barley. Consequently, total tract *in vivo* apparent digestibilities and nutritive values for each of the barleys as calculated by difference were also not significantly different between the rolled, micronised or extruded forms.

The observation that method of physical processing (rolling, micronisation or extrusion) did not alter *in vivo* apparent digestibilities and nutritive values of barley measured over the total digestive tract of equines is in broad agreement with reports from the literature. Neither crimping nor micronisation altered total tract digestibilities of starch in oats and sorghum when offered to horses (Householder, 1978; Potter *et al.*, 1992). In contrast however, the same authors reported that micronisation improved the pre-caecal digestibility of starch in both grains and this may have also occurred in the current study. Similar conclusions have been drawn with regard to the effects of physical processing on pre-caecal starch digestion of oats and maize (Kienzle *et al.*, 1992; Meyer *et al.*, 1995).

In vivo apparent digestibilities of the starch fraction of the three barleys ranged from 966 - 969 g/kg, which is in agreement with the observations of Arnold *et al.*, (1981) in relation to total tract digestibility of maize, oats and sorghum starch (967 - 970 g/kg). The OMIV of the three forms of physically processed barley is similar to that of 830 g/kg quoted for barley in the French Horse Feed Evaluation System (Martin-Rosset *et al.*, 1994). DE content of the three types of barley averaged 14.8 MJ/kg DM and is intermediate between the values of 15.0 MJ/kg DM published by NRC (1989) and that of 14.5 MJ/kg DM quoted by Frape (1998). Similarly, the DCP contents for the three forms of physically processed barley averaged 86 g/kg DM which is slightly lower than the value of 92 g/kg DM given by Frape (1998).

Digestibility of physically processed barley in the small intestine of ponies or intra-caecal rates of barley degradation were not measured in the experiment reported here. However, it is possible that changes to intra-caecal fermentation parameters were less marked in this experiment when MB and EB were fed compared with RB because micronisation and extrusion altered either digestibility in the small intestine or altered degradation rates or both. No conclusions can be drawn in this area but further research is clearly warranted to establish any effects that physical processing methods

may have on the site and rate of barley degradation within different segments of the equine digestive tract.

3.1.5 Conclusion

Where equines receive a large proportion of their diet as cereal starch, such as racing thoroughbreds, then changes in hindgut fermentation patterns may be a particular problem. From the results of the study reported here, it is recommended that in order to minimise changes in the hindgut environment, micronised or extruded barley should be fed in preference to barley which had merely been rolled.

To summarise:

1. Starch intake should not exceed 2.1 g/kg LW per meal.
2. Starch intake of greater than 2.1 g/kg LW per meal can lead to hindgut dysfunction such as acidosis, laminitis and colic.
3. Processing of cereals may lead to a lessening of this effect.
4. Micronisation appears to be the most effective processing method in maintaining a hindgut environment similar to that when an all-forage diet is fed.

3.2 Experiment 1b. Measurement of the mean retention time in different segments of the equine digestive tract using faecal excretion curves and caecal outflow rates.

3.2.1 Introduction

Digestion of a feedstuff is a product of both the degradation rate and the time it remains within the digestive tract. Therefore the time digesta remains in a particular segment of the digestive tract will impact on the extent of degradation. Rates of degradation in different segments of the digestive tract can be determined by *in sacco* methods (see chapters 4, 5 and 6). Mean retention time (MRT) of digesta is determined by the use of external markers (see section 2.2.2).

Information on the passage of digesta through the equine digestive tract is limited in comparison to similar information for ruminants. Pearson and Merritt (1991) calculated total tract mean retention time (TMRT) of digesta passage in ponies and donkeys using a non-compartmental algebraic model as did Wolter *et al.* (1974) who calculated the TMRT in ponies fed forage based diets. Mathematical modelling of faecal excretion curves, in order to estimate passage rate through different segments of the gastro-intestinal tract, has been applied successfully to ruminant data (Blaxter *et al.*, 1956; Grovum and Williams, 1973; Faichney, 1975; Dhanoa *et al.*, 1985; Ellis *et al.*, 1994) but few attempts have been made to apply these same models to rate of passage data obtained with equids. It would appear from the literature that the established two compartment models used in ruminant studies have rarely been applied to equine data. Bertone *et al.* (1989) applied the curve peeling method (as used by Grovum and Williams) to faecal excretion data obtained from horses prior to large colon resection surgery and post surgery and concluded that this two compartmental model could be applied to equine faecal excretion data. Corino *et al.* (1992; 1993; 1995) also applied the Grovum and William model but concluded that it was not a good model for equine data. They went on to develop a two interacting

compartment model for equine faecal excretion curves based on cumulative excretion curves. However, this is based on a different mathematical premise to the models currently used in ruminant work and the maths have yet to be fully described. Consequently it was not included in the study reported here.

Digesta passage has been determined in equids by a variety of marker methods. Alexander (1946) used carbon granules mixed into the ration to determine passage rate through the digestive tract of horses and concluded that for horses fed a ration of oats and bran, carbon granules first appeared in the faeces within 22.6 (± 1.32) hours and continued to appear up to 47.4 (± 2.26) hours after feeding. Vander Noot *et al.* (1967) determined maximal faecal excretion of hay measured, using chromic oxide as the marker, as being between 36 and 48 hours with complete recovery of chromic oxide by 96 hours. A few studies have investigated the effect of fibre length on passage rate in equines. Wolter *et al.* (1974) measured TMRT (using coloured particles) of hay which was either long, ground or ground and pelleted, and obtained values of 37, 26 and 31 hours respectively. Hintz and Loy (1966) also found that pelleting increased passage rate in horses when measured using yellow Styrofoam particles. Pearson and Merritt (1991) measured TMRT using Cr-mordanted fibre in both ponies and donkeys and found that TMRT was longer in donkeys than ponies and that TMRT was longer when both species were fed a straw diet compared to a hay diet. Information on diets containing cereals is however limited. Hintz *et al.* (1994) compared the passage rate of a cereal based diet where the cereal portion was either pelleted, extruded or unprocessed and concluded that processing of the cereal portion of the diet did not affect passage rate.

The objectives of this experiment were to:

1. determine if two compartment models are applicable to faecal excretion curves obtained from ponies;
2. apply mathematical models to the faecal excretion data obtained to determine if rate of passage of digesta is time-independent or time-dependent through the whole digestive tract and the post-ileal segment of the digestive tract;
3. measure caecal outflow rates to determine if outflow rate is time-independent or time-dependent from the caecum of ponies;
4. determine mean retention time of four dietary ingredients through the digestive tract of ponies.

3.2.2 Materials and Methods

Two markers were used to measure passage rates through the digestive tract. HC, RB, MB and EB used in Experiment 1a were labelled with chromium (Cr) as described by Uden *et al.* (1980), and were used to determine caecal outflow rates and passage through the post ileal section of the digestive tract. The four feeds were also labelled with ytterbium (Yb), as described by Teeter *et al.* (1984) to act as digesta passage markers for the whole of the digestive tract.

Ponies were housed and allocated to their diets as described in experiment 1a. Marker studies were carried out on days 17 - 21 of each period. On day 17 markers were introduced either directly through the caecal fistula (Cr- feeds) to measure post-ileal passage rate or were voluntarily consumed as a pulse dose prior to the 09:00 meal (Yb- feeds) for determination of total tract passage rate.

Cr-mordanted sample (according to diet fed) was placed directly in the caecum *via* the caecal cannula. Caecal sampling began 45min after administering the chromium mordanted feed. Digesta samples were removed from the caecum as described in section 3.1.2.1.2. Samples were taken every 30min for 90min. The ytterbium marker

was administered orally, 135min after the Cr- feeds were introduced through the caecal cannula. The ponies then received their 09:00 h meal. Caecal samples were then taken every 45min for a further 450min. Faecal sampling was initiated 6h after the introduction of the ytterbium marker and continued throughout the remaining recording phase. All samples were weighed, subsamples taken, labelled and dried in a forced draught oven at 60°C for at least 48 h.

Dried caecal and faecal samples were then ground through a 1.0mm screen before further analysis of markers. Approximately 250 mg of dried ground samples was weighed accurately into a digestion vessel (CEM, Bucks, UK) and 10ml of nitric acid (HNO_3) was added. Vessels were then sealed and placed in a laboratory microwave (MDS2000, CEM, Bucks, UK). Samples were then digested using a pre-programmed three-phase programme. At the end of the programme samples were air cooled for five minutes, after which the vessels were vented. The digested solution was then filtered through Whatman No. 541 filter paper into a 50ml volumetric flask and made up to 50 ml with distilled H_2O.

The Cr concentration of the digested solution was determined by atomic absorption spectroscopy at a wavelength of 357.9nm whereas Yb was determined by atomic emission at a wavelength of 398.8nm.

3.2.2.1 Mathematical models and statistical analysis

Mean retention time (MRT) through the whole digestive tract (Yb data) and through the post-ileal section (Cr data) where calculated using three algebraic models. These were

Model 1: $MRT = \Sigma t_i M_I$ Faichney (Equation 8) (1975)

Where t_i is the time elapsed between the dosing and the ith defecation and M_i is the marker excreted in the ith defecation as a fraction of the total amount of marker excreted.

Model 2: $MRT = \Sigma t_i C_i \Delta t_i / \Sigma C_i \Delta t_i$ Theilemans *et al.* (1978)

Where C_i is the marker concentration at time t_i after dosing and Δt_i is the time elapsed between t_i and the previous sample.

Model 3: $MRT = \Sigma C_i t_i / \Sigma C_i$ Faichney (Equation 9) (1975)

Where C_i is the marker concentration at time t_i after dosing.

Model 1 is the standard model to determine MRT and is considered the model to which all others should be compared. Models 2 and 3 were based on marker concentrations rather than absolute amounts and are generally used were total collection of faeces is not feasible. Model 2 takes into account the fact that sample timing is not regular: i.e. animals do not defecate at regular time intervals. Model 3 however assumes that time between sampling is regular.

MRT were also calculated using six compartmental models. These models included three time-independent and three time-dependent models (see Appendix 2 for model details):

Model 4	Grovum and Williams (1973)		time-independent
Model 5	Dhanoa *et al.* (1985)		time-independent
Model 6	Pond *et al.* (1988)	(G1G1)	time-independent
Model 7	Pond *et al.* (1988)	(G2G1)	time-dependent
Model 8	Pond *et al.* (1988)	(G3G1)	time dependent
Model 9	Pond *et al.* (1988)	(G4G1)	time-dependent

All six models incorporated a fast compartment, slow compartment and a time delay (TD).

$$MRT = FMRT + SMRT + TD$$

Where FMRT is the MRT in the fast compartment and SMRT is MRT in the slow compartment.
In this case, the fast compartment is assumed to be representative of the caecum, the slow compartment the large intestine and the time delay represents the tubular segments of the tract including the small intestine, the small colon and the rectum.

The models of Grovum and Williams (1973) and Dhanoa *et al.* (1985) were fitted to faecal excretion curves using programmes written for Genstat 5 and supplied by MS Dhanoa. The models of Pond *et al.* (1988) were fitted using programmes developed by Moore *et al.* (1992) in SAS.

Caecal outflow rates were calculated from chromium content of caecal digesta samples using Genstat 5. Equation 3.2.4 described the caecal outflow curves

$$C_i = C_o \exp^{-kt} \qquad \text{Equation 3.2.4}$$

Where C_i is the concentration remaining at time t
C_o is the concentration immediately following pulse dosing
And k is the rate parameter. Caecal MRT is calculated as 1/k.

Caecal volumes were calculated using Equation. 3.2.5

$$\text{Volume} = \text{initial dose}/C_o \qquad \text{Equation 3.2.5}$$

A "goodness of fit" of model was determined by the correlation coefficient (R^2) and from the error mean squares (EMS). A high R^2 and low EMS are generally indicative of the model fitting well. Runs of residuals (i.e. differences between observed values and fitted values) were compared to indicate if the models were over- or under-fitting parts of the excretion curves. Having examined the R^2 and EMS of the models, the most appropriate model was then used to determine differences in compartmental MRT between feeds and between ponies. Analysis of variance (ANOVA) was carried out on this data with variance due to feed and pony identified

ANOVA was also carried out on MRT calculated for each model (algebraic and compartmental) and each feed. Variance due to feed, model and feed x model interaction were identified. All ANOVA were carried out using Genstat 5 (Lawes Agricultural Trust, 1993).

3.2.3 Results

3.2.3.1 Faecal excretion curves

Recovery of markers was almost complete and in keeping with other studies: 98% of Cr was recovered whereas 97% of Yb was recovered. For each marker 12 marker excretion curves could be fitted to each model. The number of samples within each data set varied however, due to different faecal excretion rates. This presented some problems in fitting some of the compartmental models to certain data sets, particularly models 4, 5 and 7.

A "goodness of fit" of model was determined by examining R^2 and EMS and the results are given in Table 3.2.3.1. When models were fitted to chromium faecal excretion curves the models of Grovum and Williams (model 4) and of Dhanoa *et al.* (model 5) resulted in the lowest R^2 along with the G2G1 model (model 7) (0.763, 0.798 and 0.830 respectively). The G1G1 model (model 6) along with the G3G1

(model 8) and G4G1 (model 9) resulted in the highest R^2 (0.941, 0.939 and 0.935 respectively) for Cr faecal excretion curves. The EMS were lowest for the G1G1 model (model 6) and highest for the G2G1 model (model 7). It was therefore concluded that the time independent G1G1 model of Pond *et al.* (model 6)gave the best fit to the Cr faecal excretion curves.

When the same models were fitted to the Yb faecal excretion curves the same pattern emerged with the Grovum and Williams (0.638), Dhanoa *et al.* (0.873) and G2G1 model (0.866) resulting in the lowest R^2 and the G1G1 (0.920), G3G1 (0.940) and G4G1 (0.946) models fitting with the highest R^2. The EMS were similar for the G3G1 and G4G1 models. The greatest EMS were found for the Grovum and Williams model. The G3G1 and G4G1 models fitted the Yb faecal excretion data with similar R^2 and EMS, however as the G4G1 model fitted with the higher R^2 and lower EMS it was chosen to compare differences between feeds. Figures 3.2.3.1 and 3.2.3.2 depict one example of the chosen model (i.e. actual *vs.* fitted data) for Cr and Yb data respectively.

Table 3.2.3.1 Goodness of fit parameters, R^2 and error mean squares (EMS) for six models fitted to 12 different equine faecal excretion curves obtained using either chrome-mordanted (Cr) or ytterbium marked (Yb) feeds.

	Time independent			Time dependent				
	Model 4	**Model 5**	**Model 6**	**Model 7**	**Model 8**	**Model 9**	**sed**	**sig**
Cr marker								
R^2	0.763^{a}	0.798^{a}	0.941^{b}	0.830^{ab}	0.939^{b}	0.935^{b}	0.0613	*
EMS	183	169	69	250	72	73	131.9	NS
Yb marker								
R^2	0.638^{a}	0.873^{b}	0.920^{b}	0.866^{b}	0.940^{b}	0.946^{b}	0.0593	***
EMS	138^{a}	65^{b}	54^{b}	73^{b}	37^{b}	36^{b}	27.5	*

Values in the same row not sharing common superscripts differ significantly ($P<0.05$). Model 4 was based on Grovum and Williams (1974), model 5 was Dhanoa *et al.* (1985), models 6,7,8 and 9 are based on the GnG1 models of Pond *et al.* (1988) where model 6 is G1G1, model 7 is G2G1, model 8 is G3G1 and model 9 is G4G1.

Figure 3.2.3.1 Fitted faecal excretion curves for a G1G1 time independent model to actual faecal excretion data for pony 1 when a pulse dose of chromium-mordanted rolled barley was placed directly into the caecum *via* a fistula.

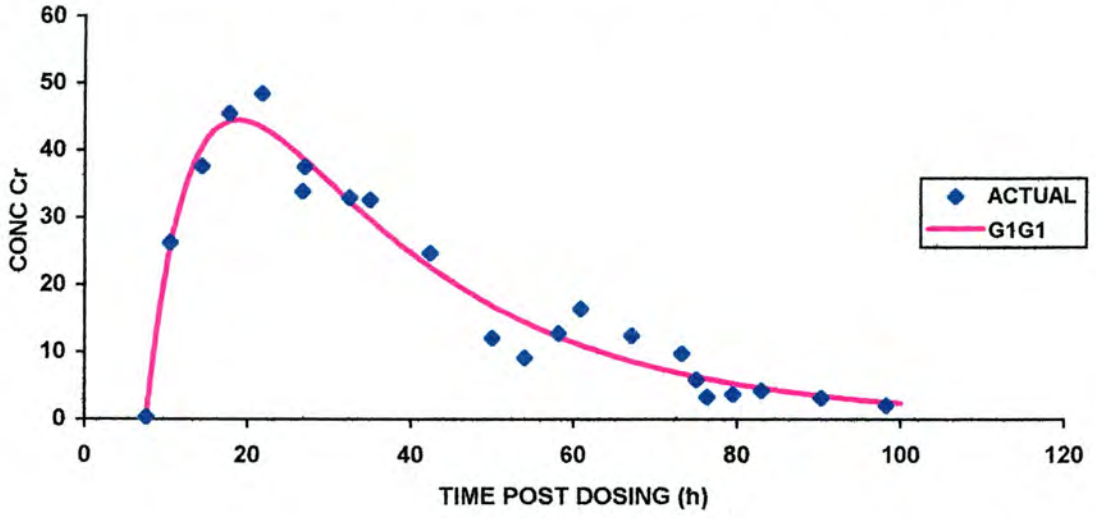
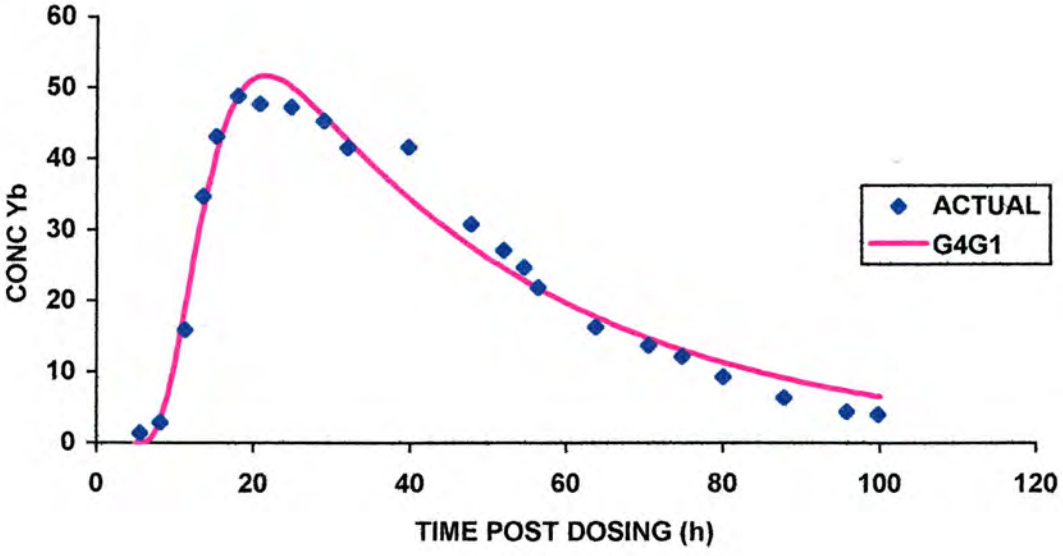


Figure 3.2.3.2 Fitted faecal excretion curves for a G4G1 time dependent model to actual faecal excretion data for pony 1 fed a pulse dose of ytterbium marked rolled barley.



3.2.3.2 Mean Retention Times

3.2.3.2.1 Algebraic *vs.* compartmental analysis

The calculated mean retention time of digesta through the post-ileal segment of the equine digestive tract measured using Cr-mordanted feeds are given in Table 3.2.3.2 whereas calculated mean retention times of digesta through the whole of the equine digestive tract measured using Yb-marked feeds are given in Table 3.2.3.3. In each table mean retention times have been calculated using three algebraic and six compartmental models.

Table 3.2.3.2 Mean retention times (MRT) through the post-ileal segment of the equine digestive tract when ponies were fed either hay cubes only diet (HC) or 50:50 rolled barley:HC (RB:HC), or micronised barley:HC (MB:HC) or extruded barley:HC (EB:HC) calculated using either simple algebraic formulae or time independent models or time dependent models

								Sig	
method	**model**	**HC**	**RB:HC**	**MB:HC**	**EB:HC**	**sed**	**F**	**M**	**FxM**
algebraic	Faichney eqn 8	32.13	33.18	39.52	38.04	4.158	***	NS	*
	Faichney eqn 9	28.45	30.38	39.03	37.71				
	Thielemans *et al.*,	31.72	33.42	40.01	39.89				
Time independent	Grovum & Williams	30.10	33.05	39.73	43.23				
	Dhanoa *et al.*,	29.96	34.18	41.80	42.57				
Time dependent	G1G1	34.00	35.18	41.64	37.49				
	G2G1	32.50	36.41	40.46	38.39				
	G3G1	33.33	35.98	41.07	38.55				
	G4G1	33.35	36.05	41.36	40.75				

Models used were Faichney 1975 (equation 8), Faichney 1975 (equation 9), Theilemans *et al.*, 1978, Grovum and Williams 1974, Dhanoa *et al.* 1985 and the gamma function models of Pond *et al.* 1988.

There was no effect of model on MRT calculated using the Cr data but model did significantly affect ($P<0.05$) calculation of MRT when using Yb data. For both sets

of data there was a significant feed x model interaction ($P<0.05$). Model 3 underestimated TMRT compared to model 1 for all feeds, whereas model 2 underestimated TMRT for HC but overestimated for RB, MB and EB for both markers. Although for the compartmental models (models 4 -9) the tendency was for models to overestimate TMRT compared to model 1 this difference was only significant for model 7 when calculated using Yb data.

Table 3.2.3.3 Mean retention times (MRT) through the whole of the equine digestive tract when ponies were fed either hay cubes only diet (HC) or 50:50 rolled barley:HC (RB:HC), or micronised barley:HC (MB:HC) or extruded barley:HC (EB:HC) calculated using either simple algebraic formulae or time independent models or time dependent models

							Sig		
method	**model**	**HC**	**RB:HC**	**MB:HC**	**EB:HC**	**sed**	**F**	**M**	**FxM**
algebraic	Faichney eqn 8	33.07	38.05	42.50	40.05	4.302	**	*	*
	Faichney eqn 9	29.60	35.47	42.45	39.73				
	Thielemans *et al.*,	32.91	38.23	42.96	42.58				
Time independent	Grovum & Williams	31.50	39.15	40.37	41.04				
	Dhanoa *et al.*,	33.00	41.58	47.99	43.57				
Time dependent	G1G1	33.05	41.84	45.75	45.20				
	G2G1	42.90	42.93	47.83	43.56				
	G3G1	31.96	43.33	44.80	44.13				
	G4G1	31.95	41.26	45.61	43.90				

Models used were Faichney 1975 (equation 8), Faichney 1975 (equation 9), Theilemans *et al.*, 1978, Grovum and Williams 1974, Dhanoa *et al.* 1985 and the gamma function models of Pond *et al.* 1988 for the G1Gn models.

3.2.3.3 Mean retention time of feeds

Figures 3.2.3.3 and 3.2.3.4 depict the average fitted faecal excretion curves for all four feeds for Cr and Yb markers respectively. In Table 3.2.3.4 the calculated MRT in different segments of the equine digestive tract are given for the G1G1 model (Cr

data) and the G4G1 model (Yb data). Both models calculate MRT for a "fast" compartment (FMRT), a "slow compartment" (SMRT) as well as a time delay (TD). Initial assumptions were that the fast compartment was representative of the caecum, the slow compartment the large colon and the time delay represents passage through the tubular segments of the digestive tract: i.e. small intestine, small colon and rectum.

Table 3.2.3.4 Calculated mean retention time (MRT) for the fast compartment (FMRT), slow compartment (SMRT), time delay (TD) and total MRT (TMRT) from either chromium (Cr, pre-caecal segment) or Ytterbium (Yb, whole digestive tract) faecal excretion curves measured in ponies fed either 100% hay cubes (HC), 50:50 rolled barley:hay cubes (RB:HC) 50:50 micronised barley:hay cubes (MB:HC) or 50:50 extruded barley:hay cubes (EB:HC)

	HC	RB:HC	MB:HC	EB:HC	sed	sig
Cr data (h)						
FMRT	2.6[a]	11.3[ab]	11.5[ab]	20.8[b]	6.33	*
SMRT	21.6	14.1	20.6	14.9	7.91	NS
TD	3.9	5.3	6.4	4.9	2.49	NS
TMRT	32.9	36.1	42.4	43.8	5.21	NS
Yb data (h)						
FMRT	14.6	14.9	16.6	21.3	8.34	NS
SMRT	28.0	22.1	23.9	16.8	11.40	NS
TD	8.7	10.7	10.3	8.1	2.30	NS
TMRT	46.1	42.3	46.9	43.0	10.20	NS

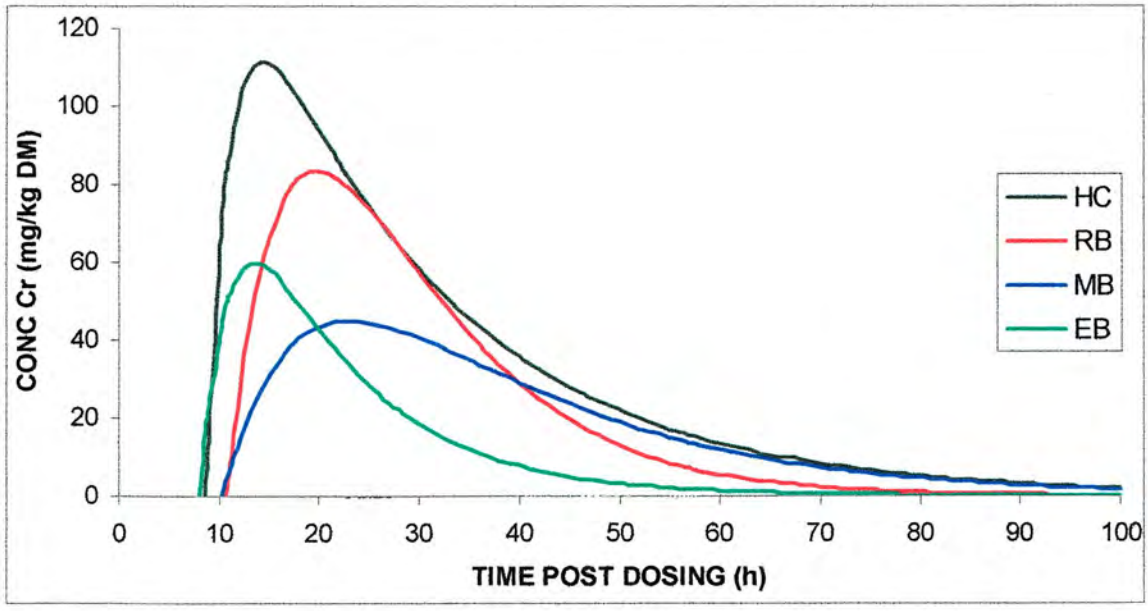
Values on the same row not sharing common superscripts differ signifcantly

Table 3.2.3.4 Calculated mean retention time (MRT) for the fast compartment (FMRT), slow compartment (SMRT), time delay (TD) and total MRT (TMRT) from either chromium (Cr, pre-caecal segment) or Ytterbium (Yb, whole digestive tract) faecal excretion curves measured in ponies

	Pony 1	Pony 2	Pony 3	sed	sig
Cr data (h)					
FMRT	11.9	5.4	17.4	5.48	NS
SMRT	19.1	16.5	17.6	6.85	NS
TD	7.4^{a}	8.0^{a}	13.0^{b}	1.99	*
TMRT	38.4^{a}	29.9^{a}	48.0^{b}	4.51	**
Yb data (h)					
FMRT	12.0	11.1	27.5	7.22	NS
SMRT	25.1	17.7	25.4	9.88	NS
TD	3.3	3.9	7.8	2.16	NS
TMRT	40.4^{ab}	32.7^{a}	60.7^{b}	8.83	*

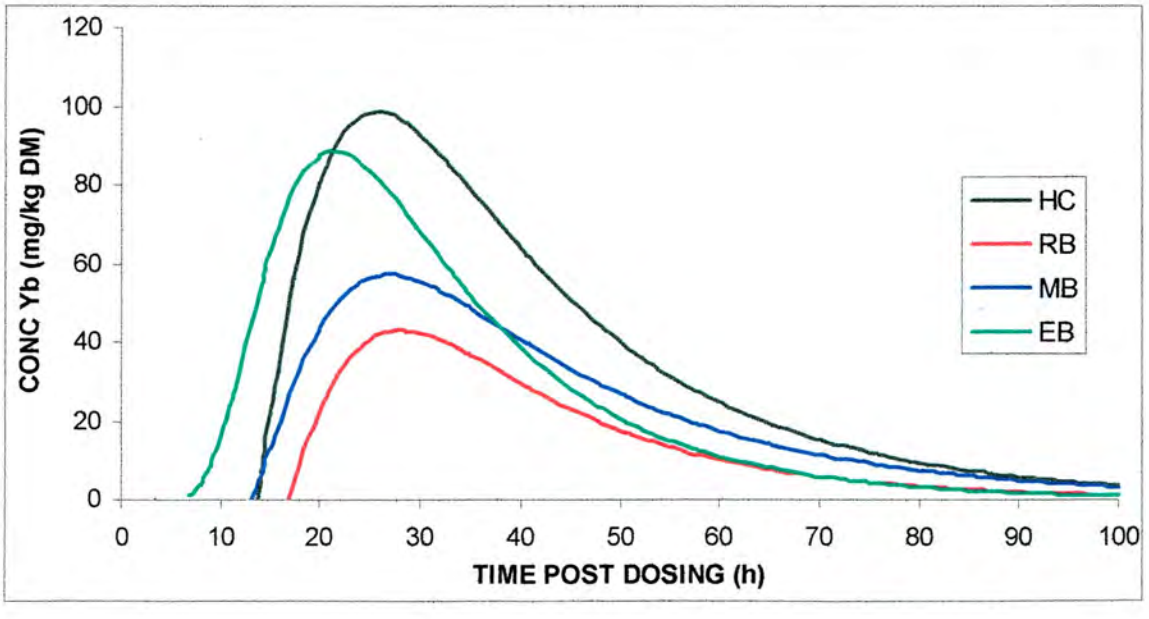
Values on the same row not sharing common superscripts differ significantly

Figure 3.2.3.3 Fitted faecal excretion curves for ponies receiving a pulse dose of chromium-mordanted feedstuffs directly into the caecum *via* a fistula. Feeds were hay cubes (HC), rolled barley (RB), micronised barley (MB) or extruded barley (EB)



All diets had similar MRT (32.9 - 43.8 h) when calculated using the Cr-mordanted feeds despite there being a significant difference ($P<0.05$) in FMRT between HC (2.6 h) and EB:HC (20.8). SMRT ranged from 14.1 - 21.6 h and TD from 8.1 - 10.7 h across all diets. When the same parameters were calculated for the Yb-labelled feeds no differences between the diets were detected. MRT ranged between 42.3 - 46.9 h, FMRT ranged between 14.6 - 21.3 h, SMRT ranged between16.8 -28.0 h and TD ranged between 3.9 and 6.4 h. For both Cr- marked EB and Yb-marked EB, MRT in the "fast" compartment was longer than that of the "slow" compartment. Differences between diets may, however have been masked by the large variation between ponies and this is presented in Table 3.2.3.5. When measured using Cr data, pony 3 had a significantly longer ($P<0.05$) TD than either pony 1 or 2 and consequently a significantly longer ($P<0.01$) MRT. A significantly longer ($P<0.05$) MRT was also calculated for pony 3 when measured using Yb data. MRT was also longer in the "fast" compartment than in the "slow" compartment for pony 3.

Figure 3.2.3.4 Fitted faecal excretion curves for ponies receiving a pulse dose of ytterbium marked feedstuff orally. Feeds were hay cubes (HC), rolled barley (RB), micronised barley (MB) or extruded barley (EB)



3.2.3.4 Caecal outflow curves

Caecal outflow rates along with calculated MRT are given in Table 3.2.3.6 along with calculated caecal DM volume. R^2 was also calculated for each caecal outflow curve to test for "goodness of fit". These ranged from 0.908 to 0.971 and are also given in Table 3.2.3.6. Examples of caecal outflow curves for HC and RB are shown in Figures 3.2.3.5 and 3.2.3.6 respectively.

Table 3.2.3.6 Dry matter (DM) volume of caecum, caecal outflow rates and mean retention time (MRT) for diets containing hay cubes only (HC) or 50:50 rolled barley:HC (RB:HC), micronised barley:HC (MB:HC) or extruded barley:HC (EB:HC)

	HC	RB:HC	MB:HC	EB:HC	sed	sig
Vol. DM (kg)	0.259	0.399	0.260	0.450	0.2017	NS
k (h)	0.733	0.414	0.420	0.301	0.1959	NS
MRT (h)	1.60	3.80	7.40	5.10	4.46	NS
R^2	0.971	0.925	0.927	0.908	0.0454	NS

Figure 3.2.3.5 Fitted caecal outflow curve to observed caecal chromium (Cr) content for pony 3 when a pulse dose of Cr -mordanted hay cubes was placed directly into the caecum *via* a fistula

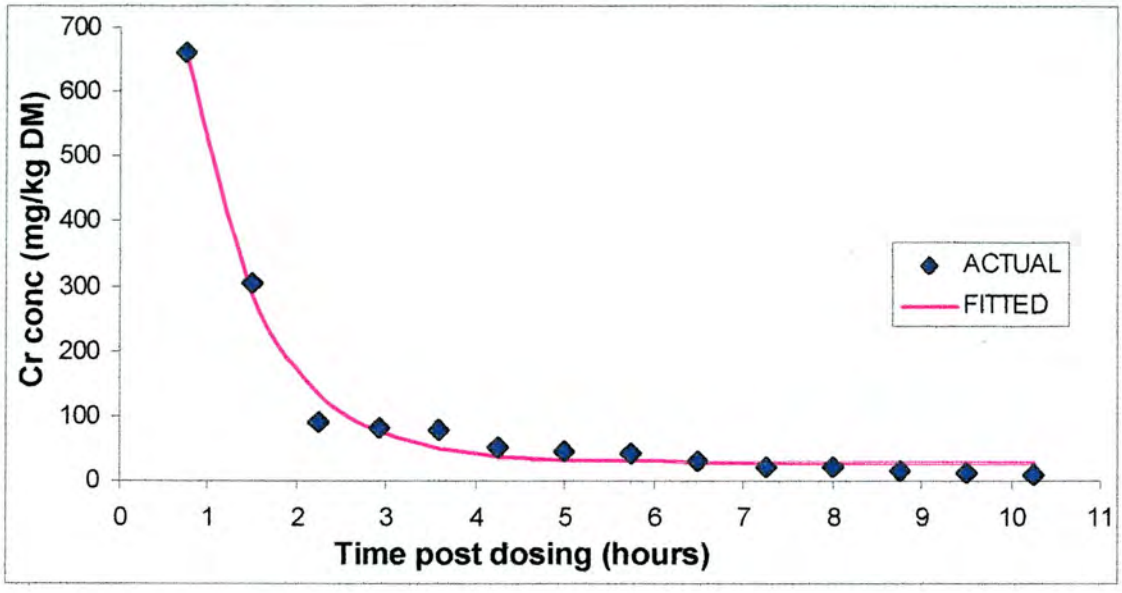
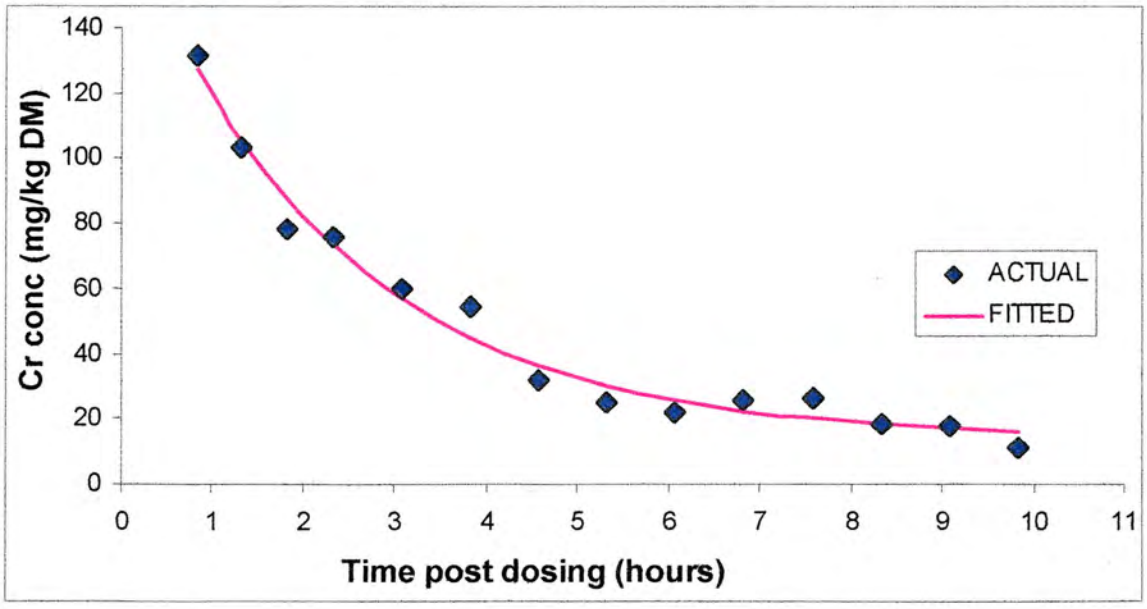


Figure 3.2.3.6 Fitted caecal outflow curve to observed caecal chromium content for pony 3 when a pulse dose of Cr - mordanted rolled barley was placed directly into the caecum *via* a fistula



Caecal outflows were faster when ponies were fed HC in comparison to the barley diets but these differences were not significant ($P>0.05$), consequently there were no significant differences ($P>0.05$) between diets for mean retention time within the caecum. Caecal DM volume averaged 0.342 kg across diets. Despite the fact that the k value for HC (0.733) is more than double that of EB (0.301) no significant difference was detected. MRT of MB was 7.40 h but this was not significantly different to EB, RB or HC (5.10, 3.80 and 1.60 h respectively) despite being approximately 1.5, 2 and 5 times as great, respectively. The large pony to pony variation may have masked any differences between diets. R^2 averaged 0.933 across diets indicating that a simple exponential equation adequately describes outflow rates from the equine caecum.

3.2.4 Discussion

3.2.4.1 Compartmental analysis

In contrast to ruminant studies, few rate of passage studies have been carried out in equines (Alexander, 1946; Hintz and Loy, 1966; Vander Noot *et al.*, 1967; Wolter *et al.*, 1974; Clemens and Stevens, 1980; Bertone *et al.*, 1989; Pearson and Merritt, 1991; Corino *et al.*, 1995). Few of these studies have calculated mean retention times (Wolter *et al.*, 1974;, Pearson and Merritt, 1991), and fewer still have attempted to apply compartmental models (Bertone *et al.*, 1989; Corino *et al.*, 1995).

MRT can be calculated using simple algebraic models without resorting to more complex mathematical models. The three algebraic models used here all give the similar answers although model 3 tended to underestimate MRT when compared to model 1 (Faichney, 1975; equation 8). Which of these three models should be used in passage rate studies will depend on the method of faecal collection. If total faecal collection is possible then model 1 should be used as it is considered the standard

model. If however total collection is not possible then model 2 (Theilemans *et al.*, 1978) should be used in preference to model 3 (Faichney, 1975; equation 9) as model 2 takes into account the irregularity of faecal excretion.

Six, two compartmental models were also used to calculate MRT giving a total of nine models to calculate MRT. There were significant differences between models in calculation of MRT when calculated using Yb data. This is in contrast to ruminant studies where it has been shown that model does not influence calculation of MRT (Poore *et al.*, 1991; Moore *et al.*, 1992). When MRT was calculated for the post-ileal segment of the equine digestive tract (using Cr data) the model employed was not significant.

The compartmental models were categorised either as time-independent or time-dependent. The time-independent models assume that mixing compartments behave as a "continuous flow stirred tank reactor "(CFSTR) and that first order kinetics apply. Time-dependent models assume that digesta passage does not follow first order kinetics and that digesta passage is altered either by passing through a "plug flow type reactor" (a tubular segment of the digestive tract) or that digesta is selectively retained within a sector of the digestive tract.

The equine digestive tract can be described as being a plug-flow reactor (stomach and small intestine) followed by two constantly stirred flow tank reactors in series (caecum and colon) and finally another plug-flow reactor (small colon and rectum). The relative proportions of these segments will influence the impact each will have on digesta passage. The small colon and rectum account for a relatively small proportion (7%) of the equine digestive tract (Argenzio, 1993) and whilst passage through this section may be time-dependent its influence on total MRT will be small. The stomach and small intestine account for almost 39% of the digestive tract (Argenzio, 1993) and passage through this segment may well influence total MRT. In this study,

a time-dependent model (G4G1) gave the best fit to total tract faecal excretion curves whereas a time- independent model (G1G1) gave the best fit to post-ileal faecal excretion curves. This suggests that when a tubular segment, which represents a large proportion of the gut, precedes the mixing compartment(s) the choice of model to interpret data is important. In a study examining digesta passage in pigs, Pond *et al.* (1986), noted that a time-dependent one compartment model gave a much better statistical fit than a two compartment time-independent model. In pigs the stomach and small intestine account for approximately 63% of the digestive tract capacity (Argenzio, 1993) i.e. a large tubular section precedes the fermentation section. This too suggests that time- dependent models may be more appropriate for monogastric studies.

3.2.4.2 Mean retention times

In this experiment no significant differences were detected between diets due to the large variation between ponies. MRT through the whole of the equine digestive tract for ponies fed 100% HC was 46.1 which is considerably longer than published results for MRT for forages (26 -37 h) (Wolter *et al.*, 1974; Pearson and Merritt, 1991). Hintz *et al.* (1993) investigated the effect of processing of cereals on passage rate and found no differences between pelleted, extruded or unprocessed cereal. In the experiment reported here there were no differences in TMRT when barley was either rolled, micronised or extruded. However, MRT for EB was longer in the "fast" compartment than in the "slow" compartment regardless of marker. It is not clear however, if this effect was due to the extrusion of barley or was an artefact due to one pony. For both markers, pony 3 had an MRT in the "fast" compartment either similar or longer than that in the "slow" compartment. This could be attributed to the lack of faecal samples from this pony.

The results of this study are in contrast to other studies where different diets have been shown to have different MRT in equines. Pearson and Merritt (1991) reported faster MRT when ponies and donkeys were fed hay compared to straw. Other studies have shown a decrease in MRT when the diet is pelleted compared to non-pelleted (Hintz and Loy, 1966; Wolter *et al.*, 1974).

3.2.4.3 Caecal outflows

For the models used in this experiment the fast compartment was assumed to be representative of the caecum. However, when model FMRT are compared with actual measured caecal outflows they are quite different. Whilst it is logical to assume that the two are not directly comparable, it would also be logical to assume a correlation between them if they are actually measuring the same thing. In this experiment there was poor correlation ($R^2 = 0.39$) between calculated FMRT and caecal outflow measurements, thus suggesting in this experiment that FMRT may include more than just the caecum or that the caecal outflow measurements are measuring more than caecal outflow.

Due to the nature of the experiment, the ponies were fed a restricted quantity of feed in two meals each day. This type of feeding regime will have a large influence on the caecum, which accounts for 16 % of the capacity of the equine digestive tract (Argenzio, 1993). The relatively large intervals between meals in this experiment (8 and 16 h) will result in the caecum becoming increasingly empty especially between the evening and morning meals. At the time of dosing in this experiment the caecum would be relatively empty. A few hours after dosing the ponies were fed, resulting in a large influx of feed particles into the caecum and consequent dilution of the marker. The values derived for caecal outflow in this experiment may well be a combination of dilution and outflow.

The compartments derived in the mathematical models are mathematical concepts and need to be carefully interpreted before giving them a biological designation. In this experiment the fast compartment was thought to be solely representative of the caecum but this may not be the case. Argenzio *et al.* (1974) dosed ponies with both a liquid marker and a particle marker in the caecum and found that both markers moved rapidly from the caecum into the ventral colon, with a peak concentration occurring by 12 hours. It may well be therefore, that the fast compartment represents the caecum and the ventral colon together.

In this experiment passage through the post-ileal segment of the equine digestive tract was measured as well as passage through the whole digestive tract, yet differences between the two were not easily discernible. Whilst, in the main, passage rates measured through the whole tract are longer than those measured through the post-ileal segment, it would be unwise to conclude that this difference is represented entirely by passage through the stomach and small intestine. This is because chromium and ytterbium are not strictly comparable as markers (Prigge *et al.*, 1981). Moore *et al.* (1992) noted that TMRT of hay and a complete pelleted feed was longer when measured with Cr- labelled than with Yb - labelled feed. Similar conclusions were also reached by Beauchemin and Buchanan-Smith (1989), Mader *et al.* (1984) and Coleman *et al.*, (1984). Nonetheless, the difference between MRT measured through the total tract and the post-ileal segment was on average 3 h (across all diets) and this is in keeping with published values for passage through the stomach and small intestine (Frape, 1998).

3.2.5 Conclusion

In this study, inclusion of processed cereals in the diet did not alter rate of passage compared to the basal diet of HC. However, only three ponies were used in this study and variation between ponies was large, making it impossible to carry out rigorous

statistical testing. In future studies more animals would be required to minimise between animal variation.

Mathematical models normally applied to ruminant data were successfully applied to equine data in this study, however again only a limited number of animals and diets were used. In this study digesta passage through the whole equine digestive tract was best described by a time-dependent model whereas digesta passage through the post-ileal section was best described by a time-independent model. Again however, only a limited number of ponies and diets were used. Further studies would be required, using a larger number of ponies to minimise between-animal variation, before firm conclusions about digesta passage in equine could be concluded.

To summarise:

1. Mathematical models of the function of the digestive tract can be successfully applied to equids.
2. Digesta passage through the whole digestive tract is best described by a time-dependent model, whereas passage through the post-ileal tract is best described by a time-independent model.
3. Between animal variance in equids can be high. Further studies should aim to use as many ponies as practicable to yield statistically robust results.

Chapter 4. The effect of physical processing on *in situ* degradation of cereals and legumes in the caecum of ponies

4.1 Introduction

The nylon bag "*in situ*" technique is used routinely as a means of estimating rumen microbial degradation of protein in rationing systems developed for ruminants (Jarrige, 1987). The technique requires animals to be fistulated and for this reason *in situ* studies in equids have been limited. However, the technique has been used in equids to relate *in situ* degradation of feed constituents to *in vivo* apparent digestibilities (Applegate and Hershberger, 1969; Miraglia *et al.*, 1988; Faurie *et al.*, 1992), to compare caecal digestion with ruminal digestion (Koller *et al.*, 1978; Uden and Van Soest, 1987; Lechner-Doll *et al.*, 1992), and to compare degradation profiles of feedstuffs (Stefansdottir *et al.*, 1996) although, to date, most *in situ* studies in equids have been used to investigate the degradation of fibrous feedstuffs which are more likely to undergo microbial fermentation than enzymatic digestion. However, the *in situ* method also offers the possibility of being a suitable method to determine the effect of physical processing on the kinetics of starch degradation.

Comprehensive studies on the procedural aspects (see section 2.2.1.3) of the *in situ* technique have led to its adoption as a routine method for determining nutritive value of feeds in ruminants. Whilst many of the details of methodology have not been thoroughly investigated in equines it is reasonable to assume that factors such as bag pore size, pre-treatment of feedstuffs, particle size, sample size to surface area ratio and post- incubation treatment of samples will all have a bearing on the outcome. However, some aspects may assume different importance in species which differ in their digestive physiology. One such aspect is the effect of incubation sequence in relation to animal feeding (see section 2.2.1.3). Huntington and Givens (1997) concluded that incubation sequence had no significant effect on the effective degradability of feeds incubated *in situ* in the rumen of cattle or sheep. However,

Hyslop *et al.* (1996) concluded that the sequence in which feeds were incubated in the caeca of ponies significantly affected ($P<0.05$) the degradation profiles and effective degradability values of feed components of a commercial concentrate feed for horses.

Oats are the traditional cereal grain concentrate fed to horses in Britain. In recent times, however, horse feed manufacturers have moved away from the use of oats as the sole cereal ingredient in their formulations and now often include barley, maize and peas which have undergone some form of physical processing. The objectives of Experiment 2 were to compare degradation profiles and calculate effective degradabilities for unprocessed barley (UB), micronised barley (MB), extruded barley (EB) and dehydrated grass (DHG). The barleys were from the same batches used in Experiment 1a (see section 3.1). In Experiment 3 the aim was to compare extrusion with micronisation in maize and peas. In both experiments the effect of incubation sequence on dry matter (DM) disappearance from *in situ* bags and on DM degradation parameters was also investigated.

4.2 Material and Methods

4.2.1 Experiment 2: Effect of physical processing on *in situ* degradation of barley in the caecum of ponies

4.2.1.1 Animal Management:

The three caecally fistulated ponies described in section 3.2.1 were used in this study and were housed as previously described (section 3.2.1). All ponies were given *ad libitum* access to hay and hayracks were filled twice daily at 09:00 and 17:00h. Ponies also received a mineral-vitamin supplement (60g) daily (see Appendix 1 for composition) in approximately 250g ground hay cubes. Fresh water was always available.

4.2.1.2 Preparation of Feed Samples:

The four feeds were unprocessed barley(UB), micronised barley (MB), extruded barley (EB) and dehydrated grass (DHG). UB, MB and EB were supplied by Dodson and Horrell Ltd (Ringstead, Kettering, UK) and DHG (which is sold under the trade name 'Readi-grass') was supplied by Spillers Speciality Feeds, (Milton Keynes, UK). All feedstuffs were milled to pass through a 1.0mm mesh screen and then sieved across a 45μm screen. Particles of < 45μm would not be included in the samples as these particles would be lost through the bag pores. Percentage of sample less than 45 μm was 0% for all feedstuffs. Fresh feed (4.2 ± 0.3g) was weighed into each bag for incubation. Immediately prior to incubation in the caecum the bags were soaked in water for 1min in order to prevent their floating in the caecum and to ensure rapid mixing of samples with caecal contents.

4.2.1.3 Preparation of Bags:

Incubation bags measured 6.5cm x 20cm and were made of a monofilamentous polyester fibre (Sericol Ltd., Westwood Road, Broadstairs, Kent, CT10 2PA) with a pore size of 41μm. Sample weight to surface area was approximately 16mg/cm^2 as recommended by Huntingdon and Givens (1995). The bags were double stitched and had a rounded base to: a) avoid feed being trapped in the corners; b) facilitate easy removal of sample residues after incubation and c) avoid the physical loss of fine particles during incubation and subsequent washing. All bags were numbered for ease of identification. Each bag was securely tied using a plastic bag tie and a rubber castration ring and suspended on 20cm of dacron string. The dacron string was sheathed with plastic tubing to prevent entanglement of bag strings and to ensure the bags were submerged within the caecal digesta. This arrangement also allowed free movement of bags within the digesta and facilitated mixing of samples with the caecal contents. The strings were passed through a central pore of a rubber bung and securely tied with a large knot. The bung was designed to fit securely into the

cannula, which was then closed using a specially designed cap to maintain anaerobic conditions within the caecum.

4.2.1.4 Incubation Sequence

The complete exchange method (Paine and Crawshaw, 1982) was used where two bags were placed in the caecum at one time. Two bags containing different feedstuffs were incubated *in situ* in the caecum of each pony for a fixed period of time, at the end of which the bags were removed and two new bags inserted. Two feedstuffs were incubated at any one time resulting in four feedstuffs being tested over four weeks.

The bags were incubated in the caecum for fixed time periods according to two sequences designed to be a forward and reverse sequence. However, it was not possible to have a complete reversal of the first sequence due to the timing of incubations. Sequence 1 was 0, 2, 4, 6, 12, 8, 24, 48 h and sequence 2 was 48, 24, 8, 4, 12, 6, 2, 0 h. Each sequence was designed to fit into one working week and the actual sequence is detailed in Table 4.2.1.

Table 4.2.1 Timetable for *in situ* incubation of bags in the caecum of ponies for two incubation sequences (1 and 2).

Day	Time	Duration (h)
Sequence 1		
Monday	09:00 - 11:00	2
Monday	11:00 - 15:00	4
Monday	15:00 - 21:00	6
Monday - Tuesday	21:00 - 09:00	12
Tuesday	09:00 - 17:00	8
Tuesday - Wednesday	17:00 - 17:00	24
Wednesday - Friday	17:00 - 17:00	48
Sequence 2		
Monday - Wednesday	09:00 - 09:00	48
Wednesday - Thursday	09:00 - 09:00	24
Thursday	09:00 - 17:00	8
Thursday	17:00 - 21:00	4
Thursday - Friday	21:00 - 09:00	12
Friday	09:00 - 15:00	6
Friday	15:00 - 17:00	2

4.2.1.5 Post Incubation Processing

When bags were removed from the caecum they were rinsed under running tap water to remove adherent digesta from the exterior of the bag. The bags were then washed in an automatic washing machine (Indesit 824) on a cold-water programme that lasted approximately 45minutes and comprised of 4 rinses with a final spin (400rpm). Each rinse involved 4 minutes when bags were immersed in water with no agitation and 3.5 minutes washing with agitation. The bags were then dried in a forced draught oven at 60°C for at least 48h.

DM and STC losses from zero time bags were determined by subjecting at least five bags of each feedstuff to the washing and drying process described above, without prior incubation of bags in the caecum of ponies. Zero time washing losses were only determined once per feedstuff and represented part of the rapidly soluble component of the feeds.

4.2.1.6 Analysis of Residues

After the washing procedure dry matter (DM) disappearance was calculated for each bag (DM disappearance = DM in bag prior to incubation – dried residue remaining in bag/ DM in bag prior to incubation). Dried residues were then bulked across replicates for each pony for subsequent starch (STC) analysis according to the method described in section 3.1.2.2.3.

4.2.1.7 Modelling Degradation Profiles

The degradation kinetics of feeds were described by a single exponential equation

$$P = a + b\,(1 - e^{-ct}) \qquad \text{(Equation 4.2.1: (Ørskov and McDonald, 1979))}$$

where P = is the amount degraded at time t

a = rapidly soluble fraction

b = the amount which in time will degrade

c = the fractional rate constant at which the fraction described by b will be degraded per hour.

a + b = the total degradability (asymptote) of the feedstuff which cannot exceed 1000 g/kg

1 - (a + b) = the undegradable portion of the sample

a, b and c are constants fitted by an iterative least squares procedure. These constants were then used with an outflow rate constant (k) to estimate the effective degradability (ED) of the feedstuffs.

$$ED = a + ((b*c)/(c+k)) \qquad \text{(Equation 4.2.2: (Ørskov and McDonald, 1979))}$$

where a, b and c are defined as in Equation 4.2.1

and k = 1/mean retention time

4.2.1.8 Statistical Analysis

The model of Ørskov and McDonald (1979) (Equation 4.2.1) was fitted to the data using NOWAY (Rowett Research Institute, Aberdeen) and degradation parameters derived. From the derived parameters effective degradabilities (ED) for mean retention times of 3, 6, 10 and 40 h were calculated. These mean retention times were chosen to reflect respectively:

1.) passage through the small intestine of equines (k = 0.33) – (Frape, 1998)

2.) outflow rates from the caecum of equines as measured by Howell and Cupps (1950) (k=0.167)

3.) A combination of passage through the small intestine and the caecum of equines (k = 0.10)

4.) Passage through the whole digestive tract (k = 0.025) based on previous studies carried out within this laboratory (Hyslop, pers. comm.).

Genstat 5 (Lawes Agricultural Trust, 1990) was used to carry out analysis of variance on observed DM losses at each incubation time, degradation parameters and effective degradabilities of DM and STC. Variance due to pony, feed, incubation sequence and feed x incubation sequence was identified for DM losses, degradation parameters and ED whereas only variance due to pony and feed was identified for STC losses, degradation parameters and ED.

4.2.2 Experiment 3. Effect of physical processing on *in situ* degradation of maize and peas in the caecum of ponies.

4.2.2.1 Materials and Methods

Two caecally-fistulated Welsh-cross geldings (weighing approximately 280kg) used in the previous experiment were used in this experiment. DM and STC degradation profiles and parameters were derived for unprocessed maize (UM), micronised maize

(MM), extruded maize (EM), unprocessed pea (UP), micronised pea (MP) and extruded pea (EP). Calculated effective degradabilities of DM and STC were also determined for each feedstuff.

Animal housing, management and feeding schedule, along with incubation bags, pre-incubation feed preparation, incubation sequence, post -incubations procedures and analysis of residues were as described for the previous experiment. DM and STC disappearances were fitted and ED values calculated according to the equations of Ørskov and McDonald (1979) described previously. Statistical analysis of all degradation parameters was by analysis of variance as described in the previous experiment (4.2.1.8)

4.3 RESULTS:

4.3.1 Experiment 2

4.3.1.1 Feed Composition and Intake

The chemical composition of the hay offered *ad libitum* and barleys incubated in the caecum are give in Table 4.3.1.1. The composition of hay and DHG concur with published values (MAFF, 1990), however there are differences between composition of the barleys observed here and published values for STC content. The published values ranged from 516 - 636 g/kg but all three barleys used in this experiment had higher STC content (645 -684 g/kg). Intakes of hay were between 6 - 8kg (fresh weight) per pony per day.

Table 4.3.1.1: Chemical composition of the basal diet (hay) fed to the ponies and the processed feeds: unprocessed barley (UB); micronised barley (MB) or extruded barley (EB), which were incubated in nylon bags in the caeca of ponies

	UB	MB	EB	DHG	HAY
DM (g/kg)	879	890	903	903	888
OM	985	993	996	857	953
Starch	645	684	645	5	0
CP	119	133	121	111	53
NDF	218	212	186	629	650
ADF	55	53	54	310	343

4.3.1.2 DM Degradation

All incubations were completed successfully. The average zero time DM disappearances from incubation bags for UB, MB, EB and DHG were 452, 493, 257 and 289 g/kg respectively. Observed DM disappearances at each of the incubation times can be found in Table 4.3.1.2. Significantly lower ($P<0.05$) DM disappearances were observed with incubation sequence 2 at the 4, 12, 24 and 48 incubation times for the DHG. There were also significantly lower ($P<0.05$) observed DM disappearances from UB and EB after 4 and 48 hours incubation respectively for incubation sequence 2.

Table 4.3.1.2 Observed dry matter (DM) disappearances (g/kg) of unprocessed barley (UB), micronised barley (MB), extruded barley (EB) and dehydrated grass (DHG) when incubated in nylon bags in the caeca of ponies for two different incubation sequences (IS)(1&2)

	IS	UB	MB	EB	DHG	sed	sig
2 h incubation	1	674^{ab}	704^{a}	677^{ab}	328^{c}	26.5	*
	2	627^{b}	684^{ab}	641^{b}	303^{c}		
4 h incubation	1	**746^{a}**	735^{ab}	771^{c}	**338^{d}**	10.5	*
	2	**719^{b}**	723^{b}	753^{ac}	**310^{e}**		
6 h incubation	1	788^{a}	770^{a}	771^{a}	336^{b}	13.9	***
	2	788^{a}	773^{a}	786^{a}	316^{b}		
8 h incubation	1	813^{a}	795^{a}	807^{a}	348^{b}	15.0	***
	2	807^{a}	789^{a}	793^{a}	321^{b}		
12 h incubation	1	849^{a}	808^{b}	809^{b}	**348^{c}**	6.9	*
	2	839^{a}	802^{b}	812^{b}	**324^{d}**		
24 h incubation	1	871^{a}	842^{bc}	839^{c}	**358^{d}**	7.9	*
	1	859^{ab}	845^{bc}	834^{c}	**341^{e}**		
48 h incubation	1	884^{a}	870^{b}	**871^{b}**	**368^{d}**	3.8	*
	1	883^{a}	870^{b}	**861^{c}**	**352^{e}**		

a,b,c,d,e values within incubation time, not sharing common superscripts differ significantly (P<0.05). Differences between incubation sequences 1 and 2 within each feedstuff occur only where values are given in bold

The DM degradation profiles of the four feedstuffs in Figure 4.3.1.1 show that DHG had a different degradation profile to those of UB, MB or EB but that the three barleys had similar profiles. The differences in the profiles are reflected in the DM degradation parameters, which are given along with the feed * incubation sequence interaction values in Table 4.3.1.3. No significant differences (P>0.05) in any degradation parameters were seen between the two incubation sequences for any of the three experimental barleys. For the DHG feed, the $a+b$ value along with the calculated ED values at k=0.025 were significantly lower (P<0.05) when incubation sequence 2 was used.

DHG had significantly lower (P<0.05) values for each degradation parameter compared to UB, MB or EB, except for the fractional rate constant c which was only

significantly lower ($P<0.05$) than that of UB. There was no difference between UB, MB or EB for parameter *c*. For the rapidly soluble fraction *a*, MB had a significantly higher ($P<0.05$) value compared to that of UB in IS 2, but was not different from EB. There was no difference between UB and EB for parameter *a*. For parameter *b*, UB had a significantly greater ($P<0.05$) value than MB. EB was not significantly different from either UB or MB with regard to *b*. UB had a significantly greater $a + b$ (potential degradability) value compared to EB in IS 1. MB was not significantly different from either UB or EB for $a + b$.

Figure 4.3.1.1 Fitted dry matter (DM) degradation profiles (based on two incubation sequences) for unprocessed barley (UB), micronised barley (MB), extruded barley (EB) and dehydrated grass (DHG) incubated in nylon bags in the caeca of ponies.

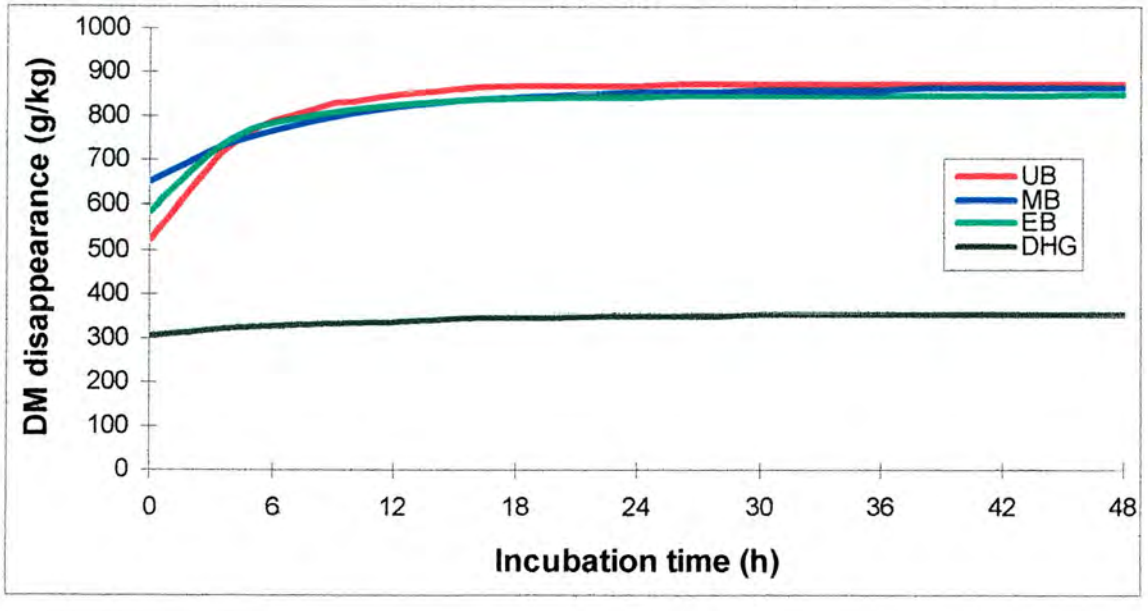


Table 4.3.1.3 Derived degradation parameters and calculated effective degradability (ED) values[¥] (g/kg) for unprocessed barley (UB), micronised barley (MB), extruded barley (EB) and de-hydrated grass (DHG) when incubated in nylon bags in the caeca of ponies for 2 different incubation sequences (IS) (1&2).

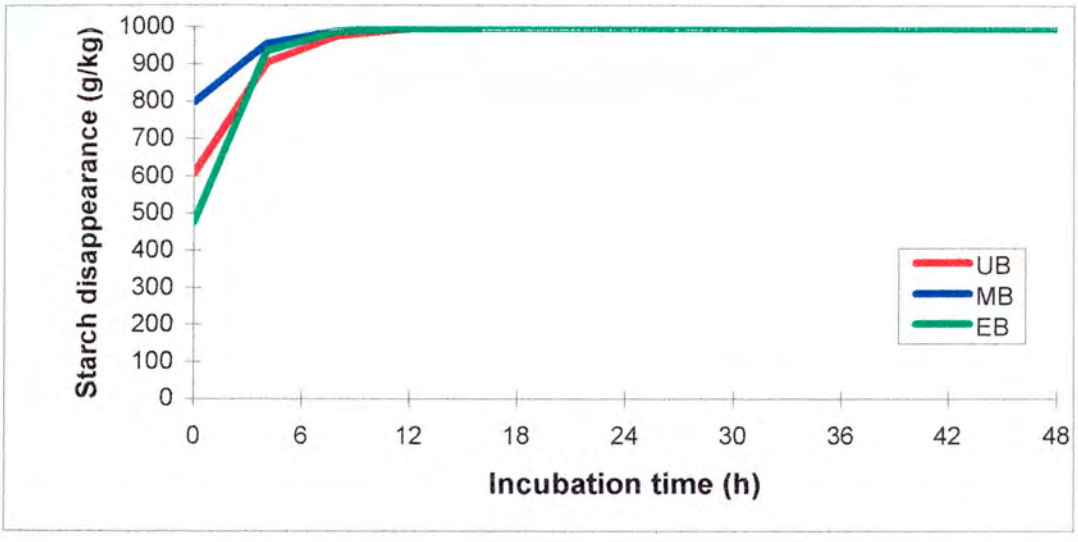
	IS	UB	MB	EB	DHG	sed	sig
a	1	578^{ab}	657^{a}	591^{ab}	325^{cd}		
value	2	463^{bc}	634^{a}	574^{ab}	284^{d}	74.3	*
b	1	301^{ab}	208^{b}	258^{b}	45^{c}		
value	2	407^{a}	227^{b}	281^{ab}	60^{c}	68.1	*
c	1	0.199^{ab}	0.132^{ab}	0.216^{ab}	0.065^{b}		
value	2	0.249^{a}	0.132^{ab}	0.196^{ab}	0.149^{ab}	0.0706	*
a + *b*	1	878^{a}	865^{ab}	850^{b}	**370^{c}**		
value	2	869^{ab}	862^{ab}	854^{b}	**344^{d}**	10.2	*
ED[1]	1	690^{ab}	714^{b}	695^{ab}	332^{c}		
k = 0.33	2	639^{a}	699^{ab}	682^{ab}	301^{c}		
ED[2]	1	741^{a}	746^{a}	739^{a}	337^{b}		
k = 0.167	2	707^{a}	735^{a}	726^{a}	309^{b}	21.1	***
ED[3]	1	777^{a}	771^{a}	769^{a}	342^{b}		
k=0.10	2	753^{a}	764^{a}	757^{a}	315^{b}	14.4	***
ED[4]	1	844^{a}	829^{b}	823^{b}	**357^{c}**		
k=0.025	2	832^{ab}	826^{b}	817^{b}	**332^{d}**	7.4	*

a,b,c,d,e Values not sharing common superscripts differ significantly ($P<0.05$). Differences between incubation sequences within each feedstuff occur only where values are given in bold.
[1]MRT = 3h (small intestine only), [2]MRT = 6h (caecum only), [3]MRT = 10h (small intestine + caecum), [4]MRT = 40h (total tract).
[¥] According to the model of Ørskov and McDonald (1979)

4.3.1.3 Starch degradation

As the DHG contained minimal amounts of starch (see Table 4.3.1.1) residues were not included in the starch analysis. The starch degradation profiles of UB, MB and EB are shown in Figure 4.3.1.2 and degradation coefficients along with calculated ED values are given in Table 4.3.1.4.

Figure 4.3.1.2 Fitted starch degradation profiles for unprocessed barley (UB), micronised barley (MB) and extruded barley (MB) incubated in nylon bags in the caeca of ponies



There were no significant differences between UB, MB and EB for any of the starch degradation parameters. MB had a significantly higher ($P<0.05$) ED value at MRT equal to 3, 6 and 10 (905, 937 and 956g/kg respectively) compared to UB (809, 874 and 913g/kg respectively) and EB (800, 875 and 915g/kg respectively).

Table 4.3.1.4. Derived degradation parameters and calculated effective degradabilities[¥] (ED) for starch disappearances (g/kg) for unprocessed barley (UB), micronised barley (MB) and extruded barley (EB) incubated in nylon bags in the caeca of ponies

	UB	MB	EB	sed	Sig
a value	607	799	476	133.5	NS
b value	393	199	520	132.3	NS
c value	0.354	0.389	0.519	0.0903	NS
a+*b* value	1000	997	996	1.9	NS
ED[1] k=0.33	809[a]	905[b]	800[a]	35.5	*
ED[2]k=0.167	874[a]	937[b]	875[a]	20.0	*
ED[3]k=0.10	913[a]	956[b]	915[a]	12.9	*
ED[4]k=0.025	974	985	973	4.6	NS

Values bearing different superscripts on the same row differ significantly.
[1]MRT = 3h (small intestine only), [2]MRT = 6h (caecum only) [3]MRT = 10h (small intestine + caecum)
[4]MRT = 40h (total tract).
[¥] According to the model of Ørskov and McDonald (1979)

4.3.2 Experiment 3

Intakes of hay were similar to those in Experiment 2 and ranged between 6 - 8kg (fresh weight) per pony per day. The chemical composition of the hay offered *ad libitum* and feedstuffs incubated in the caecum are given in Table 4.3.2.1. The composition of the hay and the three peas was similar to published values, however the composition of the three maize samples differed from published values (MAFF, 1990). Starch values ranged from 754 - 775 g/kg for the three maize samples which are higher than published values of 661 -755 g/kg.

Table 4.3.2.1: Chemical composition of the basal diet (hay) fed to ponies and the processed feeds: unprocessed maize (UM); micronised maize (MM); extruded maize (EM); unprocessed pea (UP); micronised pea (MP) and extruded pea (EP), incubated in nylon bags in the caeca of ponies

	UM	MM	EM	UP	MP	EP	HAY
DM (g/kg)	889	893	898	897	906	905	926
OM	989	993	987	972	972	973	949
Starch	775	763	754	433	465	463	
CP	92	77	83	228	238	239	70
NDF	108	68	84	180	100	120	728
ADF	17	17	21	71	58	67	369

4.3.2.1 DM Degradation

All incubations were completed successfully. Observed DM disappearances for each incubation period for both forward and reverse sequences are given in Table 4.3.2.2. Observed DM disappearances were significantly lower ($P<0.05$) for the reverse incubation sequence at the 2 h incubation with MM and significantly higher ($P<0.05$) at the 8 h incubation for UP for the reverse incubation sequence.

DM degradation profiles are shown in Figure 4.3.2.1, whilst degradation parameters and calculated ED values are given in Table 4.3.2.3. It can be seen from Figure 4.3.2.1 that differences in DM degradation profiles exist between UM, MM and EM and that these three profiles are different to those of UP, MP and EP, which in turn are very similar to each other. Effect of sequence was only significant ($P<0.05$) for the $a+b$ parameter in the case of UM and for the rate constant c for MP and EP. Processing of peas did not significantly ($P>0.05$) affect ED values compared to UP but processing in each case did significantly increase ($P<0.05$) ED values compared to UM. Calculated ED values were significantly greater for peas compared to maize ($P<0.05$) for each outflow rate. However, UM had significantly lower ($P<0.05$) ED

than MM or EM for each outflow rate. MP also had higher ED values than UP but the effect was only significant ($P<0.05$) in sequence 1 for k values = 0.33, 0.167 and 0.10.

Table 4.3.2.2 Observed dry matter (DM) disappearances (g/kg) for unprocessed maize (UM), micronised maize (MM), extruded maize (EM), unprocessed peas (UP), micronised peas (MP) and extruded peas (EP) when incubated in nylon bags in the caeca of ponies for two different incubation sequences (IS) (1 & 2)

	IS	UM	MM	EM	UP	MP	EP	sed	sig
2 h incubation	1	368^{a}	$\mathbf{618^{b}}$	582^{bc}	687^{bd}	787^{e}	767^{de}	44.0	*
	2	353^{a}	$\mathbf{510^{c}}$	559^{bc}	679^{bd}	753^{de}	691^{bde}		
4 h incubation	1	401^{a}	631^{b}	604^{b}	725^{c}	799^{d}	796^{d}	31.8	*
	2	411^{a}	604^{b}	601^{b}	742^{cd}	793^{cd}	743^{cd}		
6 h incubation	1	442^{a}	647^{b}	647^{b}	763^{c}	857^{e}	770^{cd}	37.4	*
	2	459^{a}	670^{b}	667^{b}	806^{cde}	868^{e}	850^{de}		
8 h incubation	1	469^{a}	683^{b}	678^{b}	$\mathbf{849^{c}}$	916^{de}	857^{cde}	28.1	*
	2	526^{a}	722^{b}	663^{b}	$\mathbf{917^{de}}$	920^{e}	870^{cde}		
12 incubation	1	520^{a}	743^{b}	689^{b}	934^{cd}	910^{cd}	868^{c}	31.0	*
	2	559^{a}	714^{b}	707^{b}	949^{d}	946^{d}	888^{cd}		
24 h incubation	1	623^{a}	831^{bc}	747^{b}	973^{d}	983^{d}	972^{d}	41.3	*
	2	688^{a}	847^{c}	765^{bc}	973^{d}	965^{d}	950^{d}		
48 h incubation	1	819^{ab}	911^{c}	909^{c}	996^{d}	996^{d}	979^{d}	25.3	*
	2	768^{a}	895^{c}	873^{bc}	998^{d}	992^{d}	992^{d}		

a,b,c,d,e values, within incubation time, not sharing common superscripts differ significantly ($P<0.05$)
Differences incubation sequences within each feedstuff occur only where values are given in bold

Figure 4.3.2.1 Fitted dry matter (DM) degradation profiles for unprocessed maize (UM), micronised maize (MM), extruded maize (EM), unprocessed peas (UP), micronised peas (MP) and extruded peas (EP) incubated in nylon bags in the caeca of ponies

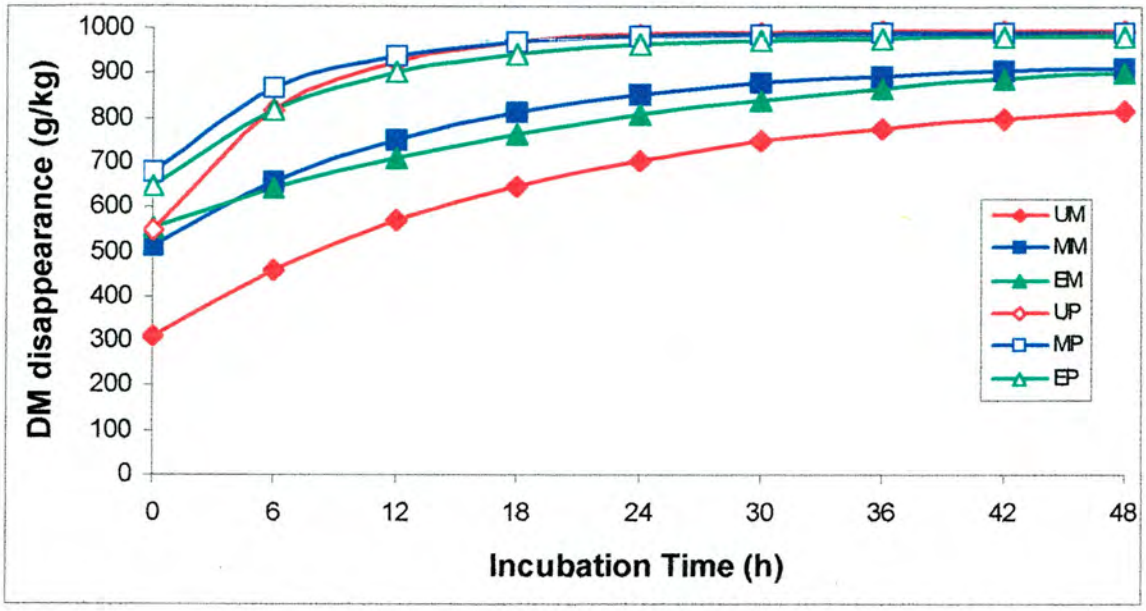


Table 4.3.2.4: Derived starch degradation parameters and calculated effective degradabilities[¥] (ED) for starch disappearances (g/kg) for unprocessed maize (UM), micronised maize (MM), extruded maize (EM), unprocessed peas (UP), micronised peas (MP) and extruded peas (EP) incubated in nylon bags in the caeca of ponies

	UM	MM	EM	UP	MP	EP	sed	Sig
a value	322^{a}	578^{b}	572^{b}	777^{c}	814^{c}	795^{c}	63.1	*
b value	673^{a}	399^{b}	428^{b}	223^{c}	186^{c}	205^{c}	53.7	*
c value	0.034^{a}	0.080^{bc}	0.040^{ab}	0.144^{d}	0.168^{d}	0.090^{c}	0.0174	*
a+*b*	995	977	1000	1000	1000	1000	14.1	NS
ED[1] k=0.33	386^{a}	657^{b}	618^{b}	844^{c}	876^{c}	839^{c}	46.1	*
ED[2]k=0.167	437^{a}	709^{b}	655^{b}	880^{c}	907^{c}	867^{c}	37.6	**
ED[3] k=0.10	495^{a}	757^{b}	694^{b}	909^{c}	930^{c}	892^{c}	31.7	**
ED[4] k=0.025	711^{a}	882^{b}	834^{b}	967^{c}	975^{c}	955^{c}	22.0	**

Values not sharing common superscripts differ significantly ($P<0.05$)
[1]MRT = 3h (small intestine only) [2]MRT = 6h (caecum only) [3]MRT = 10h (small intestine + caecum)
[4]MRT = 40h (total tract)
[¥]According to the model of Ørskov and McDonald (1979)

Figure 4.3.2.2 Fitted starch degradation profiles for unprocessed maize (UM), micronised maize (MM), extruded maize (EM), unprocessed peas (UP), micronised peas (MP) and extruded peas (EP) incubated in nylon bags in the caeca of ponies

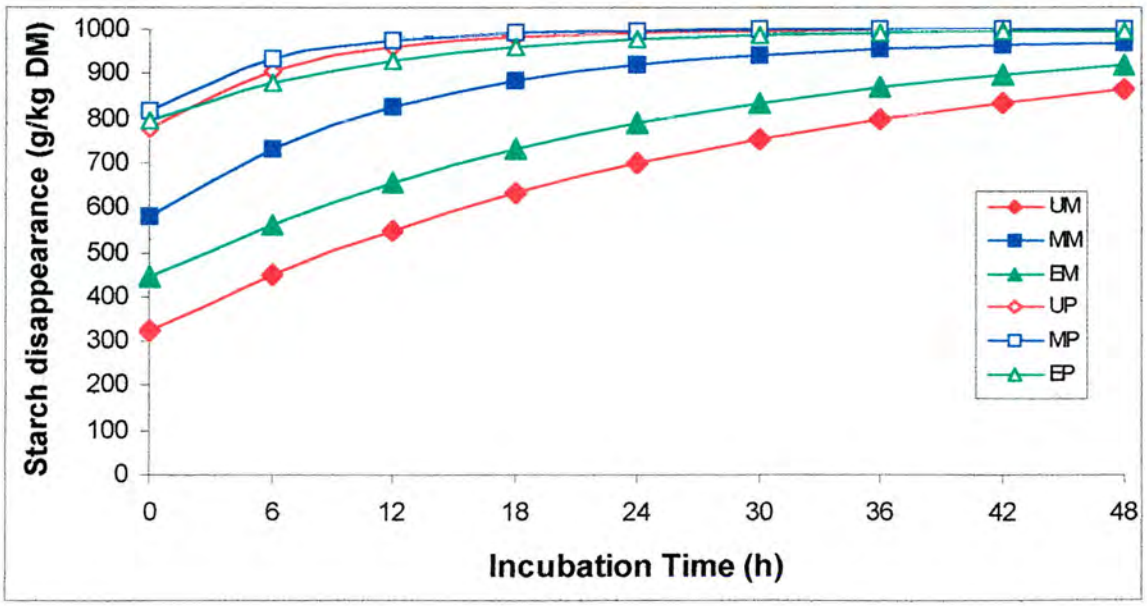


Table 4.3.2.3 Derived dry matter degradation parameters and calculated effective degradability (ED) values[¥] (g/kg) for unprocessed maize (UM), micronised maize (MM), extruded maize (EM), unprocessed pea (UP), micronised pea (MP) and extruded pea (EP) when incubated in nylon bags in the caeca of ponies for two different incubation sequences (IS) (1 & 2).

	IS	UM	MM	EM	UP	MP	EP	sed	sig
a value	1	331^{ab}	569^{cde}	561^{cde}	567^{cde}	719^{g}	705^{fg}	60.3	*
	2	291^{a}	450^{bc}	543^{cde}	522^{cd}	637^{efg}	584^{def}		
b value	1	609^{a}	391^{bcd}	413^{bcde}	431^{bcd}	279^{de}	286^{de}	67.9	*
	2	491^{ab}	439^{bc}	413^{bcde}	473^{abc}	347^{cde}	391^{cde}		
c value	1	0.032^{a}	0.045^{ab}	0.034^{a}	0.128^{cde}	$\mathbf{0.118^{cd}}$	$\mathbf{0.086^{abc}}$	0.0272	*
	2	0.073^{abc}	0.100^{bd}	0.046^{ab}	0.179^{e}	$\mathbf{0.182^{e}}$	$\mathbf{0.148^{d}}$		
a+b value	1	$\mathbf{940^{abd}}$	960^{bd}	974^{bd}	999^{b}	998^{b}	991^{b}	41.1	*
	2	$\mathbf{782^{c}}$	889^{ad}	957^{bd}	995^{b}	984^{b}	976^{bd}		
ED[1]k=0.33	1	383^{a}	616^{bc}	598^{b}	687^{cd}	791^{e}	762^{de}	34.9	*
	2	378^{a}	552^{b}	590^{b}	687^{cd}	759^{de}	709^{d}		
ED[2]k=0.167	1	426^{a}	653^{b}	628^{b}	754^{c}	833^{e}	799^{cde}	25.3	*
	2	437^{a}	615^{b}	625^{b}	766^{cd}	818^{de}	772^{cd}		
ED[3] k=0.10	1	475^{a}	691^{b}	662^{b}	808^{c}	868^{e}	833^{cde}	20.3	*
	2	495^{a}	670^{b}	661^{b}	825^{cde}	861^{de}	821^{cd}		
ED[4]k=0.025	1	666^{a}	820^{b}	793^{b}	927^{c}	948^{c}	921^{c}	17.2	***
	2	654^{a}	802^{b}	784^{b}	937^{c}	942^{c}	920^{c}		

a,b,c,d,e Values not sharing common superscripts differ significantly ($P<0.05$). Differences between incubation sequences within each feedstuff occur only where values are given in bold.
[1]MRT = 3h (small intestine only) [2]MRT = 6h (caecum only) [3]MRT = 10h (small intestine + caecum)
[4]MRT = 40h (total tract)
[¥] According to the model of Ørskov and McDonald(1979)

4.3.2.2 Starch Degradation

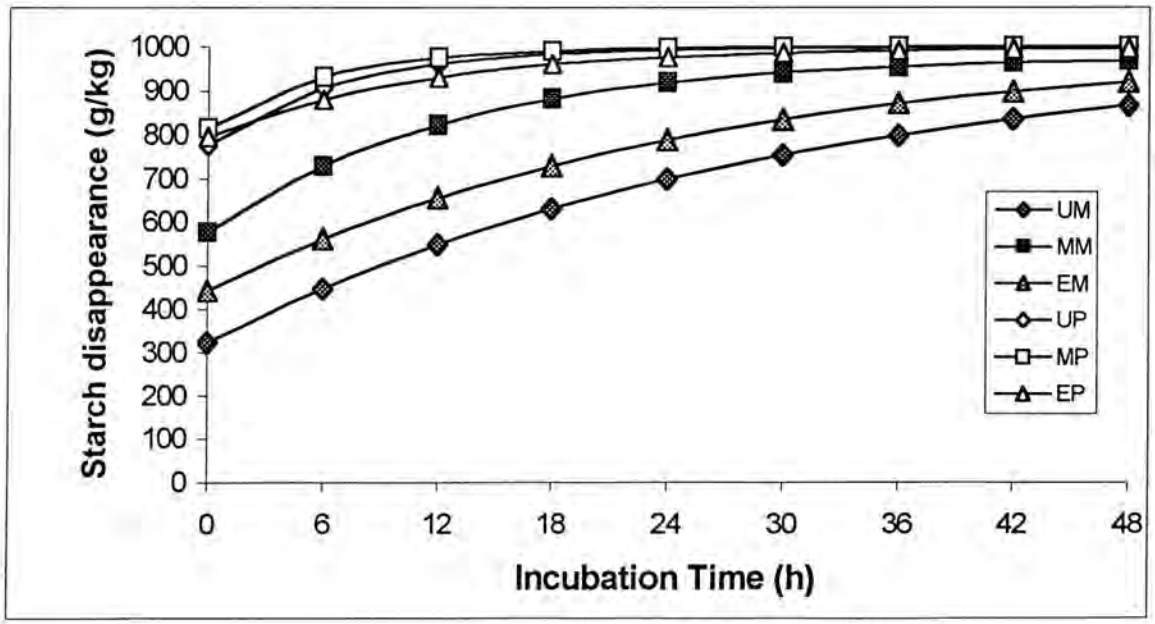
Starch degradation profiles are shown in Figure 4.3.2.2 where it can be seen that UP, MP and EP had very similar starch degradation profiles but those of UM, MM and EM were quite different. Starch degradation parameters of the feeds and the corresponding effective degradabilities are given in Table 4.3.2.4.

Table 4.3.2.4: Derived starch degradation parameters and calculated effective degradabilities[¥] (ED) for starch disappearances (g/kg) for unprocessed maize (UM), micronised maize (MM), extruded maize (EM), unprocessed peas (UP), micronised peas (MP) and extruded peas (EP) incubated in nylon bags in the caeca of ponies

	UM	MM	EM	UP	MP	EP	sed	Sig
a value	322^{a}	578^{b}	572^{b}	777^{c}	814^{c}	795^{c}	63.1	*
b value	673^{a}	399^{b}	428^{b}	223^{c}	186^{c}	205^{c}	53.7	*
c value	0.034^{a}	0.080^{bc}	0.040^{ab}	0.144^{d}	0.168^{d}	0.090^{c}	0.0174	*
a+b	995	977	1000	1000	1000	1000	14.1	NS
ED[1] k=0.33	386^{a}	657^{b}	618^{b}	844^{c}	876^{c}	839^{c}	46.1	*
ED[2]k=0.167	437^{a}	709^{b}	655^{b}	880^{c}	907^{c}	867^{c}	37.6	**
ED[3] k=0.10	495^{a}	757^{b}	694^{b}	909^{c}	930^{c}	892^{c}	31.7	**
ED[4] k=0.025	711^{a}	882^{b}	834^{b}	967^{c}	975^{c}	955^{c}	22.0	**

Values not sharing common superscripts differ significantly (P<0.05)
[1]MRT = 3h (small intestine only) [2]MRT = 6h (caecum only) [3]MRT = 10h (small intestine + caecum)
[4]MRT = 40h (total tract)
[¥]According to the model of Ørskov and McDonald (1979)

Figure 4.3.2.2 Fitted starch degradation profiles for unprocessed maize (UM), micronised maize (MM), extruded maize (EM), unprocessed peas (UP), micronised peas (MP) and extruded peas (EP) incubated in nylon bags in the caeca of ponies



For starch degradation parameters, with the exception of $a+b$, there were significant differences between maize and peas. UM had an a value of 322 g/kg which was significantly lower ($P<0.05$) than MM (578 g/kg), however EM (442 g/kg) was not significantly different ($P>0.05$) to either. MM had a significantly lower ($P<0.05$) b value (399 g/kg) than either UM (673 g/kg) or EM (558 g/kg). The rate constant c was significantly higher ($P<0.05$) for MM compared to UM but was not significantly different to EM. Physical processing significantly increased ($P<0.05$) calculated ED values for each outflow rate for maize. In peas, processing had no significant effect on the degradation parameters, with the exception of the rate constant c. Extrusion resulted in a significantly lower c value compared to either unprocessed or micronised peas. Micronising increased calculated ED values for each outflow rate compared to unprocessed peas, whereas extrusion decreased ED values, however neither was significant ($P>0.05$).

4.4 Discussion

4.4.1 Incubation sequence

A central objective of these experiments was to investigate the effect of incubation sequence on DM disappearance and DM degradation parameters of different feedstuffs. Hyslop *et al.* (1996) and Stefansdottir (1996a) have shown that incubation sequence is important when fibrous feedstuffs are incubated in the caecum of ponies. In Experiment 2, three starch-based feeds and one fibre-based feed were investigated. In Experiment 3, six starch-based feeds were studied. In Experiment 2, incubation sequence had a significant effect ($P<0.05$) on DM disappearances at incubation times of 4 and 48 h for UB and EB respectively. However, for DHG, incubation sequence had an effect for incubation times of 4, 12, 24 and 48 h. There was no effect of incubation sequence on calculated ED values for UB, MB and EB but there was for DHG. In Experiment 3 incubation sequence had a significant effect ($P<0.05$) on DM

disappearance at 8 h incubation time for UP only. There was also a significant effect ($P<0.05$) of incubation sequence on the degradation rate parameter *c* for MP and EP and on the $a+b$ parameter for UM, although this did not significantly affect calculated ED values.

These results indicate that for rapidly degradable feedstuffs, such as cereal grains and legume seeds incubated in the caecum of ponies, the sequence of sample incubation has a minimal effect on DM disappearance and subsequent degradation parameters. For slowly degradable feedstuffs however, incubation sequence can have a significant effect on DM disappearance and degradation parameters. This is in contrast to reported studies in ruminants where no significant effect of incubation sequence has been reported for any class of feedstuff (Huntingdon and Givens, 1995; 1997).

Hyslop *et al.* (1999) suggested that the effect of incubation sequence on DM disappearance and degradation parameters was due to the variation within the caecal digesta pool. This variation may be due to a combination of intra-caecal variables, including passage of digesta through the caecum and fermentative activity of microbes in the caecum. The equine caecum is comparatively small compared to the rumen of cattle and sheep. The caecum accounts for 16 % of the equine digestive tract whereas the rumen accounts for 70% of that in cattle (Argenzio, 1993). Howell and Cupps (1950) measured digesta passage through the caecum of a horse and found that by six hours the test meal had passed through the caecum. In Experiment 1b (see section 3.2.3.3) outflow from the caecum was measured at 30% per hour ($k = 0.3$). Both the results of Howell and Cupps (1950) and Experiment 1b indicate that passage through the caecum is considerably faster than in the rumen, where passage rates are generally in the range 2 - 8% per hour ($k = 0.02$ to 0.08). It may also be relevant that in the equine, caecal volumes can vary greatly compared to the relatively stable volumes encountered in the rumen. Thus, Goodson *et al.* (1988) reported a two-fold change in caecal volume when a 100% alfalfa diet was offered whereas Argenzio *et*

al. (1974) reported a four-fold change in caecal volume in ponies between pre-feeding and 8 h post-feeding a complete pelleted diet twice a day. Such large fluctuations in caecal volume may result in large changes in microbial populations and activity, as well as influencing gut motility and intra-caecal mixing of digesta. These variables make the equine caecum a much less stable environment than that of the rumen of sheep or cattle. As a consequence *in situ* degradation of feedstuffs, particularly that of a slowly degradable feedstuff, may be more variable in equines compared to sheep or cattle. In practical terms, therefore, it would seem appropriate to use two different incubation sequences when carrying out *in situ* incubations in the caecum of equines, especially where residues from bags are to be combined to carry out subsequent chemical analyses.

4.4.2 Calculating Effective Degradabilities

The model of Ørskov and McDonald (1979) allows for the calculation of the potential degradability of a feedstuff (see Equation 4.1). However the incorporation of an estimate of digesta passage rate allows for the calculation of the effective degradability of a particular feedstuff in a particular segment of the digestive tract. This allows for comparison of feedstuffs on a common time scale. For effective degradabilities calculated in different segments of the digestive tract to be valid, appropriate models and estimates of digesta passage for each segment of the digestive tract must be established.

As mentioned previously, digesta passage through the caecum is much more rapid than in the rumen, therefore estimates of digesta passage (k values) commonly used in ruminant studies are not appropriate for equine studies. Few studies have investigated digesta passage rates in the caecum of equines (Howell and Cupps, 1950). The difficulty in determining caecal outflow rates in Experiment 1A (see section 3.2.3.3), highlights the need for a more rigorous methodology to determine passage rates in the hindgut of the equine.

4.4.3 Dry matter (DM) degradation

The *in situ* degradation of starch-based feedstuffs by ruminants has been reported in a number of studies (Herrera-saldana *et al.*, 1990; Cerneau and Michalet-Doreau, 1991; Pauly *et al.*, 1992; Wahlain *et al.*, 1992; Malcolm and Kiesling, 1993; de Smet *et al.*, 1995; Arieli *et al.*, 1995; Batajoo and Shaver, 1998). Most of these have compared differences between types of cereal. Thus Cerneau and Michalet-Doreau (1991) incubated unprocessed barley, maize and peas *in situ* in the rumen of cattle and calculated ED (k = 0.06) for DM of 82.8, 58.0 and 88.6% respectively which compare well with the ED (k = 0.05) calculated in the present study of 80.9, 57.1 and 88.4% for UB, UM and UP respectively.

Although reports of *in situ* incubation of processed and unprocessed starch sources in ruminants are rare, the following may be of relevance: Arieli *et al.* (1995) incubated a variety of cereals that had undergone either expansion or extrusion *in situ* and these authors concluded that thermal processing decreased ruminal degradation rate in all cereals tested (wheat, barley, maize and sorghum). In the experiments reported here the degradation rates for extruded starch sources tended to be lower than for their unprocessed counterparts, but this trend was not significant. Arieli *et al.* (1995) noted that although degradation rates were reduced, calculated ED values were not modified by heat treatment and suggested that this was due to the increase in the rapidly soluble component of the heat processed cereals. Wahlain *et al.* (1992) also reported an increase in the rapidly soluble fraction for extruded peas when incubated in the rumen of cattle (steers and bulls) but no modification of calculated ED values. The results presented here, (summarised in Table 4.4.2.1 below) demonstrate an increase in the rapidly soluble fraction for the starch component of extruded maize and peas but not for barely, however extrusion increased the rapidly soluble fraction for the DM component of all three feeds compared to their unprocessed counterparts. Both micronisation and extrusion reduced the DM degradation for all three feedstuffs,

however degradation rates were increased for the starch component with the exception of EP.

Table 4.4.2.1 The effect of processing on degradation parameters derived from *in situ* incubations in the caeca of ponies

	Unprocessed		Micronised		Extruded	
	DM	Starch	DM	Starch	DM	Starch
Degradation rate parameter, *c*						
Barley	0.224	0.354	0.132	0.389	0.206	0.519
Maize	0.105	0.034	0.072	0.080	0.038	0.040
Peas	0.153	0.144	0.150	0.168	0.117	0.090
Rapidly soluble parameter, *a*						
Barley	520	607	646	799	582	476
Maize	311	322	509	578	552	572
Peas	545	777	678	814	645	795
Slowly degradable parameter, *b*						
Barley	354	393	218	199	269	520
Maize	550	673	415	399	413	428
Peas	452	223	313	186	339	205

4.4.4 Starch degradation

The potential degradability ($a + b$) of a dietary component is the fraction of the component that would be degraded if time was infinite. The potential degradability of starch in UB, MB and EB was 1000, 998 and 996 g/kg respectively which, although greater than, do concur with the results of the *in vivo* digestibility trial (see section 3.1.3) where total tract apparent digestibility of rolled, micronised or extruded barley was 967, 969 and 966 g/kg respectively. Calculated ED (MRT = 40 h) were 974, 985 and 973 g/kg for UB, MB and EB respectively which again, were not dissimilar to *in vivo* values where MRT was estimated to be 41, 45 and 44 h for UB, MB and EB respectively. These results suggest that the *in situ* technique would be a useful

technique to predict *in vivo* digestibilities, however with only three feeds in this study no meaningful correlations can be calculated.

Micronising significantly increased ($P<0.05$) the *a* fraction and the degradation rate for all feeds compared to their unprocessed counterparts. Extrusion increased the rate of starch degradation for barley and maize and increased the *a* fraction for maize and peas compared to unprocessed feedstuffs.

Wahlain *et al.* (1992) reported that the rapidly soluble fraction of peas was increased by extrusion, as was the rate parameter and the calculated ED value. Arieli *et al.* (1995) reported slight alterations in starch degradation parameters and ED values of barley and maize due to extrusion but these changes were not significant. In the studies of Arieli *et al.* (1995) and Wahlain *et al.* (1992) *in situ* incubations were carried out in the rumen of steers. Differences in degradation of substrates have been observed between rumen and caecal micro-organisms (Alexander, 1952; Hyslop *et al.*, 1997). In a comparative study, Hyslop *et al.* (1997) incubated unmolassed sugar beet pulp and hay in the rumen of steers and the caecum of ponies using the same incubation sequence. Degradation rates were higher for feedstuffs incubated in the caecum of ponies. These results concur with the earlier work of Alexander (1952) who showed that cotton threads suspended in the caecum of ponies were degraded quicker than similar threads suspended in the rumen of cattle. Such differences in substrate degradation may well account for the significant values observed in the experiments reported here compared to studies in the literature where no significant values have been reported.

The aim of physically processing feedstuff is to increase starch susceptibility to enzymatic hydrolysis by disrupting the starch granule. However, disruption of the starch granule is achieved in different ways by different processing methods. In the micronisation process, starch is gelatinised by a hydrothermal process. The grain is

first moistened and allowed to absorb water until it reaches a moisture content of 18 – 20%. Upon heating under the gas burners, the water vapour pressure within the grain rises dramatically resulting in the rupturing of the starch granules. The grain is then flaked while still in the "plastic state". By flaking the grain, the starch granules are prevented from re-forming as the grain cools. In the extrusion process the disruption of the starch granule is caused by a combination of shear forces and gelatinisation. During the extrusion process, the grain is first ground to a flour and then steam is added to form a dough, which is then passed through the extruded barrel. Each barrel contains either a single or twin screw. As the dough passes along the screw the starch granules are exposed to shear forces which mechanically disrupt the structure, as well as being exposed to different temperatures and pressures in different sections of the barrel. At the end of the barrel the "cooked" dough is forced through a die at extreme pressure (400 – 1000 psi) whereupon the extruded product is dried in a forced draught oven. The important variables to be considered when cereal grains are being extruded are, temperature, moisture content and screw speed (Chiang and Johnson, 1977; Lund, 1984). Extrusion is also known to result in the formation of starch-lipid complexes which are less susceptible to enzymatic hydrolysis (Chinnaswamy and Hanna, 1990; Bhatnagar and Hanna, 1994; Lin *et al.*, 1997). The different processing methods result in differences in susceptibility to enzymatic hydrolysis.

4.4.5 Effect of physical processing on starch degradation

In the experiments described in this chapter the *in situ* technique was used as a quasi-*in vivo* assay method to investigate the effect of physical processing on starch degradation. Effective degradabilities were calculated for each of the feedstuffs under study at different mean retention times (MRT) which were assumed to be indicative of passage rate through different segments of the equine digestive tract based on literature results and previous experience within this laboratory (Hyslop pers. comm.). The effective degradability of barley and maize starch was increased by micronising for MRT of 3, 6 and 10 h. In particular ED calculated at MRT = 3 h demonstrated a

12% increase in starch degradation for micronised barley compared to the corresponding unprocessed material and an increase of 70% for micronised maize compared to its unprocessed counterpart. Such increases in starch degradation in the small intestine would result in less starch reaching the hindgut. In effect this results in a 50% decrease in the amount of starch reaching the hindgut when micronised cereals, as opposed to unprocessed cereals, are fed. In Experiment 1a (see section 3.1.3.2) a more stable hindgut environment was maintained when ponies received a diet containing micronised barley compared to rolled barley and this would suggest that less starch does indeed reach the hindgut when the cereal portion of the diet is micronised.

If the capacity of the small intestine to digest starch is exceeded then the undigested starch enters the hindgut where it will be fermented resulting in acidosis or colic. The results for maize show an even greater potential for hindgut dysfunction than barley. Whereas only 20% of the starch in unprocessed barley reaches the hindgut after 3h, the corresponding values for maize are 40% and 60% from micronised and unprocessed maize respectively. Thus, an equine receiving the cereal component of their diet as maize is more likely to exhibit signs of sub-clinical acidosis than an equine receiving a barley based diet.

There is no information in the literature on the benefit of peas in diets for equines, yet the results here suggest that unprocessed peas may well be one of the "safest" forms of starch to feed to equines. Processing has little effect on pea starch which appeared to be more readily digestible than cereal starches in this study. With less starch passing through to the hindgut there is less opportunity for starch induced dysfunction to occur.

4.5 Conclusion

The *in situ* technique may well be a useful method which can allow degradation rates and profiles of different feeds to be compared on a common time basis. Certainly, the similarity of results between *in vivo* and *in situ* data suggests that the *in situ* method has potential for use in equine studies. However, some aspects of the procedure for studies in equines require further research, in particular the effect of incubation sequence. In the study reported here incubation sequence had little effect on degradation parameters for rapidly degradable feeds but slowly degraded feeds were affected. In practical terms, it would seem appropriate to use two different incubation sequences to minimise the effect of incubation sequence.
Where effective degradabilities of feedstuffs, incubated in discrete segments of the digestive tract, are to be calculated estimates of passage rate should be appropriate to that segment.

The objective of thermal processing of feedstuffs is to increase starch availability and it would appear from the results reported here that there is an interaction between method of processing and feedstuff being processed. Although micronising increased the effective degradability of starch from barley, maize and peas, this effect was only significant for maize and barley. Extrusion also increased the effective degradability of starch for maize but either did not alter or slightly decreased the effective degradability of starch for barley and peas respectively. It would appear that micronising is more likely to increase starch availability compared to extrusion, however for readily digested feeds such as peas the increase in starch availability is minimal.

To Summarise:

1. The *in situ* technique appears to have potential for use in equine studies, evidenced by similarities to both the *in vivo* data reported within this thesis and ruminant *in situ* data in the literature;

2. Incubation sequence appeared to have an effect on *in situ* data obtained from equines, especially where slowly degraded feeds were under study. In the absence of other data, it is therefore recommended that both forward and reverse incubation sequences are employed in equine studies to minimise this effect;

3. Appropriate estimates of digesta passage should be used to calculate effective degradabilities

4. Different processing methods result in differences in susceptibility to enzymatic hydrolysis;

5. Micronisation had a greater and more consistent effect in improvement of degradability of maize, barley and peas compared to extrusion.. The effects of extrusion were variable and not always desirable;

6. Thermal processing of peas generally did not improve their degradability but degradability of peas was generally greater than either barley or maize.

Chapter 5. Purified starch sources and their degradation in the pre-caecal segment of the digestive tract of ponies.

5.1 Experiment 4. Development of the mobile bag technique to determine the degradation kinetics of purified starch sources in the pre-caecal segment of the equine digestive tract

5.1.1 Introduction

The mobile bag technique (MBT) has been developed to measure digestibility in the small intestine of pigs (Leibholz, 1991), ruminants (Hvelplund, 1985; de Boer *et al.*, 1987; Antoniewicz *et al.*, 1992; Frydrych, 1992; Jarosz *et al.*, 1994; Vanhatalo and Keetoja, 1995) and in the pre-caecal segment of the digestive tract of equids (Macheboeuf *et al.*, 1995; Longland *et al.*, 1997; Moore-Colyer *et al.*, 1997a; Moore-Colyer *et al.*, 1997b). Furthermore, the MBT has been used to used to determine digestibility over the total digestive tract in pigs (Petry and Handlos, 1978; Sauer *et al.*, 1983; Graham *et al.*, 1985) and in equids (Hyslop and Cuddeford, 1996; Araujo *et al.*, 1996a; Hyslop *et al.*, 1998). Unlike the *in situ* technique, the methodology behind the mobile bag has not been thoroughly investigated, and there is no single methodology recommended for use in all species. However, some aspects of the technique such as the pore size of cloth from which the bags are made (Graham *et al.*, 1985; Cherian *et al.*, 1989; Varvikko and Vanhatalo, 1989; Vanhatalo and Keetoja, 1995), bag size and shape (Cherian *et al.*, 1989; Leibholz, 1991; Hyslop and Cuddeford, 1996; Araujo *et al.*, 1996b), sample size (Graham *et al.*, 1985; Leibholz, 1991), particle size (Cherian *et al.*, 1989; Leibholz, 1991) and site of recovery (Hvelplund, 1985; Graham *et al.*, 1985; Vanhatalo and Keetoja, 1995; Beckers *et al.*, 1996) have been studied to varying degrees, and have resulted in useful information

which can be used and modified for future investigations. However, although, the methodology behind the mobile bag technique is less well developed than the *in situ* technique it is assumed that similar principles apply.

Only one study using ponies has looked at the degradation kinetics of mobile bag data, whereby residues from bags with common total tract transit times were pooled and analysed for DM and NDF (Hyslop *et al.*, 1998). Degradation profiles were then fitted to the pooled residues for DM and NDF disappearances using the models of Ørskov and McDonald (1979) and Dhanoa (1988) respectively and effective degradability values were calculated. Their results showed that the MBT could be used successfully to model degradation kinetics of fibre over the whole of the equine digestive tract.

The experiment reported here develops the mobile bag technique further for use in equines and examines the potential of degradation models developed for use in ruminants to be applied to data obtained from mobile bags recovered in the caeca of ponies.

In this experiment only purified starch sources were used, as DM measurement of bag residues was equivalent to starch content without the need for: a) relatively complicated starch analysis *per se*. and b) pooling of bag residues. Of the six starch sources used, two were pure native starches (wheat and pea) and four were chemically cross-linked. The chemical modification of starch makes it less susceptible to adverse environmental conditions such as pH and shear stresses. Cross-linking is present in native starches and occurs when hydrostatic bonds form between chains of amylose and amylopectin. These natural cross-linkages are not stable at high temperatures and when heated the chains dissociate and retrogradation of the starch molecule occurs. To prevent retrogradation, purified starch can be treated in a number of ways to stabilise the cross-linkages. The most common of these is to replace the hydrostatic

bonds with a stronger bond such as phosphate. Not all of the hydrostatic bonds are replaced with stronger bonds and the level of replacement will determine the use of that chemically modified starch. Within industry these levels of chemical modification are described as low, medium, high and very high and are generally classified according to the viscosity of the modified starch.

5.1.2 Materials and methods

5.1.2.1 Animal Management

Two of the caecally-fistulated ponies described in section 3.1.2.1 were used in this study and were housed as previously described. Rubber matting was used to cover the floor of the pen. The ponies received daily, on a fresh weight basis, 3 kg grass nuts (GN), 1kg micronised flaked barley (MFB) and 60mg min-vit supplement (see appendix 1) in two equal meals at 09:30 h and 17:30 h. Hay was available *ad libitum* between 17:30 h and 09:00 h.

5.1.2.2 Preparation of Feed Samples

Six commercially produced modified starch sources were supplied by ABR Ltd. (Norwich, UK). The starches were:

1. ABRA starch — a pure wheat starch product with no modifications;
2. V21 — wheat starch with low levels of cross- linking;
3. V1 — wheat starch with medium levels of cross-linking;
4. V33 — wheat starch with high levels of cross-linking;
5. V65 — wheat starch with very high levels of cross-linking;
6. PEA starch — pure pea starch with no modifications.

All samples had been sieved to remove particles of less than 32μm. All samples were 98% starch.

The modified starches were produced by treating the purified ABRA starch with sodium trimetaphosphate to produce different levels of cross-linking. Pea starch was the material remaining after removal of protein from pea flour.

5.1.2.3 Preparation of bags for mobile bag studies

Bags were made in the laboratory from a monofilamentous polyester fibre (Sericol Ltd., Westwood Road, Broadstairs, Kent, CT10 2PA) with a pore size of 7μm. This is a smaller pore size compared to bags used in Experiments 2 & 3, however in preliminary trials it was found that using material with a pore size of greater than 7μm resulted in complete disappearance of starch from bags when incubated in the pre-caecal segment of the digestive tract of ponies. Rectangles of material, either 6.5 x 3.5 cm or 4.5 x 3.5 cm were folded along their length and heat sealed, using a heat sealer, to form a tube. When the heat seal was set, the resultant tube was turned inside out, (so that the seam was on the inside) and then heat sealed across one end. Two small steel washers were then sealed into a small compartment at one end. The final dimensions of the bags were 6 x 1 cm or 4 x 1 cm for large and small bags respectively. Bags were numbered for ease of identification. Starch sample (300mg and 165mg) was weighed accurately into large bags and the small bags respectively. The bags were then closed along the open end by heat sealing.

5.1.2.4 Bag administration and recovery

Bags were administered directly into the stomach of each pony *via* a naso-gastric tube (i.d 1.3 cm). On day 1 (Monday) prior to the 09:30 meal 12 large and 12 small bags (two bags per starch sample per bag size) were pre-loaded into the stomach end of the naso-gastric tube. The tube was then introduced into the stomach and bags flushed into the stomach by pumping *ca* 0.75 l water through the tube using a hand operated pump. Immediately following this procedure a specially designed magnetic capture

device (Harry Brash, Dept. of Medical Physics, Edinburgh University) was placed in the caecal fistula and held in place with a specially designed cap, thus allowing anaerobic conditions to be maintained within the caecum. The animals then received their 09:30 h meal. Bags were administered *via* naso-gastric tube on three alternate working days (Monday, Wednesday and Friday) per calendar week for three weeks.

Within the magnetic capture device was a light emitting diode (LED) that flashed red when activated. This was visible through the fistula cap and was activated when a bag containing a washer became attached to the magnet. The magnets were checked every 10 minutes for flashing lights. When the LED was activated the magnetic capture device was removed from the caecum, bags removed from the device and the LED deactivated. The magnetic capture device was then returned to the caecum and secured in place. The bag number and time of recovery were noted. After eight hours the magnetic capture device was removed from the caecum. On days 3 (Wednesday) and 5 (Friday) the procedure was repeated

5.1.2.5 Post Incubation Processing

On recovery from the caecum, bags were rinsed under running cold tap water to remove adherent digesta material from the exterior of the bag. The recovered bags were then placed in a fridge (4°C) until they were washed in an automatic washing machine (Indesit 824) on a cold-water programme (as in section 4.2.1.5)

Dry matter disappearance from zero time bags was determined by subjecting at least four bags of each design per starch sample to the same automatic washing machine programme. Zero time washing losses were determined once per feedstuff.

Washed bags were placed in a forced draught oven at 60°C for at least 48 h before DM determination. As the samples were pure starch products, only dry matter (DM) disappearances were determined for each bag.

5.1.2.6 Modelling Degradation Profiles

The model of Dhanoa (1988) was used to derive degradation parameters. Effective degradabilities were calculated using both time independent (Ørskov and McDonald, 1979) and time dependent models (Ellis *et al.*, 1994). Effective degradabilities (ED) incorporating time dependency functions were calculated using the Equation 5.1 (Ellis *et al.*, 1994)

$$ED = a + [(b*c)/(c + (\lambda*C))] \qquad \text{(Equation 5.1)}$$

where a, b and c are defined as in Equation 4.1

$\lambda = n/MRT$

where n = the order of the model i.e. 2, 3,or 4 and MRT = mean retention time

and C = the constant associated with a Gamma n function i.e.:

0.59635	= constant associated with a Gamma 2 function
0.47454	= constant associated with a Gamma 3 function
0.4085686	= constant associated with a Gamma 4 function

5.1.2.7 Statistical Analysis

DM disappearance (DMD) and mean transit time (MTT) for zero time bags and bags retrieved from the caecal cannula, were subjected to an analysis of variance where variance due to starch type and replicate (week) was identified.

MLP (Maximum Likelihood Programme, Lawes Agriculture Trust,1993) was used to fit degradation parameters to DMD which were pooled across ponies. Degradation parameters were subjected to an analysis of variance where variance due to starch type and week were identified. Effective degradabilities (ED) were calculated according to either a time independent or one of four time dependent models. Mean retention times of 2, 3, 4, 5 and 7 h were used to calculate effective degradabilities, as this represented the range of times mobile bags resided in the pre-caecal segment of the equine gut (based on previous work of this laboratory; Hyslop pers. com.). Calculated ED were then subjected to a further analysis of variance where variance due to model and starch type were identified.

Genstat 5 (Lawes Agricultural Trust, 1993) was used to carry out all analysis of variance tests. Where F ratios were significant , a multiple range test (least significance difference) was used to identify individual differences between starch types, model and starch type-model interactions.

5.1.3 Results

The chemical composition of both the basal diet and the hay offered to the ponies is given in Table 5.1.3.1. There were no refusals of basal diet. Hay intakes ranged from 3 - 4 kg per pony.

Table 5.1.3.1 Chemical composition (g/kg DM) of the basal diet of grass nuts (GN), micronised flaked barley (MFB) and hay fed to ponies during the experimental period.

	GN	MFB	Hay
DM (g/kg)	888	878	867
OM	899	978	924
Starch	48	621	34
CP	139	130	56
NDF	521	198	828
ADF	334	40	494

5.1.3.1 Single time point disappearances

DMD from zero-time (WM) bags and from bags that had travelled through the pre-caecal segment of the GI tract of ponies (PC) together with the MTT of the PC bags are given in Table 5.1.3.2. There were significant differences in DMD ($P<0.05$) between starches for WM bags with the largest DMD being recorded for ABRA (630 g/kg) and the lowest for PEA (31g/kg). PEA had significantly lower ($P<0.05$) DMD (609g/kg) in the pre-caecal segment of the equine digestive tract compared with ABRA (791g/kg), V1 (807g/kg), V21 (770g/kg) or V33 (810g/kg) but was not significantly different to V65 (711g/kg). Starch type did not affect bag transit time through small intestine. Overall mean transit time was 2.32 h

Table 5.1.3.2. Dry matter disappearances from bags containing different starch types (ABRA, V1, V21, V33, V65 and PEA) that had been either cold water washed only (WM) or travelled through the pre-caecal segment of the digestive tract of ponies (PC) calculated according to the mean transit time (MTT) of bags.

	ABRA	V1	V21	V33	V65	PEA	sed	sig
WM (g/kg)	630[a]	558[b]	594[c]	610[ac]	545[b]	31[d]	15.5	*
PC (g/kg)	791[a]	807[a]	770[a]	810[a]	711[ab]	609[b]	64.8	*
MTT (h)	2.23	2.16	2.32	2.30	2.14	2.76	0.236	NS

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

5.1.3.2 Degradation Profiles

The transit times of bags from the stomach until capture on the magnet ranged from 1.0 - 7.5 h, thus in effect yielding a range of incubation times in the pre-caecal segment of the equine digestive tract. Degradation profiles were constructed using the model of Dhanoa (1988). Figure 5.1.3.1 shows an example of a degradation profile fitted to individual bag DM disappearances for one replicate (week) of one starch source (ABRA), and this serves to illustrate how closely the observed data fitted the model. Average DM degradation profiles for the six starch sources are depicted in Figure 5.1.3.2. Degradation parameters (*a*, *b*, *c*, *lag*, *a*+*b*) are given in Table 5.1.3.3.

Table 5.1.3.3 Derived degradation parameters for dry matter (DM) disappearances of different starch types (ABRA, V1, V21, V33, V65 and PEA) from nylon bags which have travelled through the precaecal segment of the digestive tract of ponies[1]

	ABRA	V1	V21	V33	V65	PEA	sed	sig
Degradation coefficients								
a	388^{ab}	564^{a}	465^{a}	581^{a}	106^{c}	258^{bc}	121.6	*
b	571^{ab}	409^{a}	478^{a}	407^{a}	842^{b}	623^{ab}	147.1	*
c	1.073	0.553	1.155	0.776	0.714	0.561	0.3439	NS
lag (h)	0.80	1.10	0.96	0.64	0.14	0.79	0.483	NS
a+b	958	972	943	988	948	881	65.3	NS

Values on the same row not sharing common superscripts differ significantly (P<0.05) [1]Degradation parameters derived using the model of Dhanoa (1988)

There were significant differences between starches for degradation parameters (P<0.05) *a* and *b* but not for *c*, *a+b* or *lag*. ABRA had a significantly (P<0.05) greater value for *a* than V65. V1, V21 and V33 had significantly smaller (P<0.05) *b* values than V65. PEA had a significantly smaller (P<0.05) value for parameter *a* but not for parameter *b* compared to V1, V21 and V33

V65 and PEA had significantly lower (P<0.05) ED values than the other four starches for all MRT. In this experiment effective degradabilities were calculated for both time independent and time-dependent models and the results are given below in Table 5.1.3.4.

The effect of model was dependent on the starch source. For the gamma 4 model (λ = 4/MRT), effective degradabilities for V65 and PEA were significantly lower (P<0.05) than the time-independent model (k = 1/MRT) at MRT = 5 and 7 h. The gamma 4 model also gave significantly lower (P<0.05) ED for V65 when calculated for a MRT = 3 h compared to the time independent model.

Figure 5.1.3.1 Dry matter (DM) degradation profile fitted to observed losses of ABRA starch from mobile bags that had travelled through the pre-caecal segment of the digestive tract of ponies

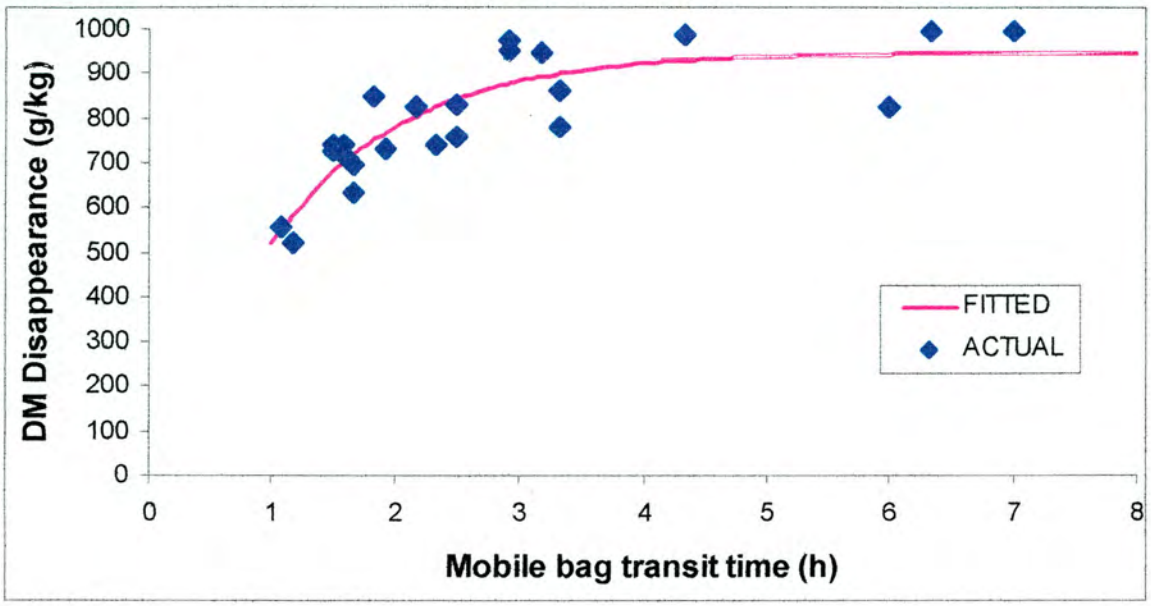


Figure 5.1.3.2 Fitted dry matter (DM) degradation profiles of six purified starch sources (ABRA, VI, V21, V33, V65, PEA) which had been incubated in porous nylon bags during their passage through the pre-caecal segment of the GI tract of ponies.

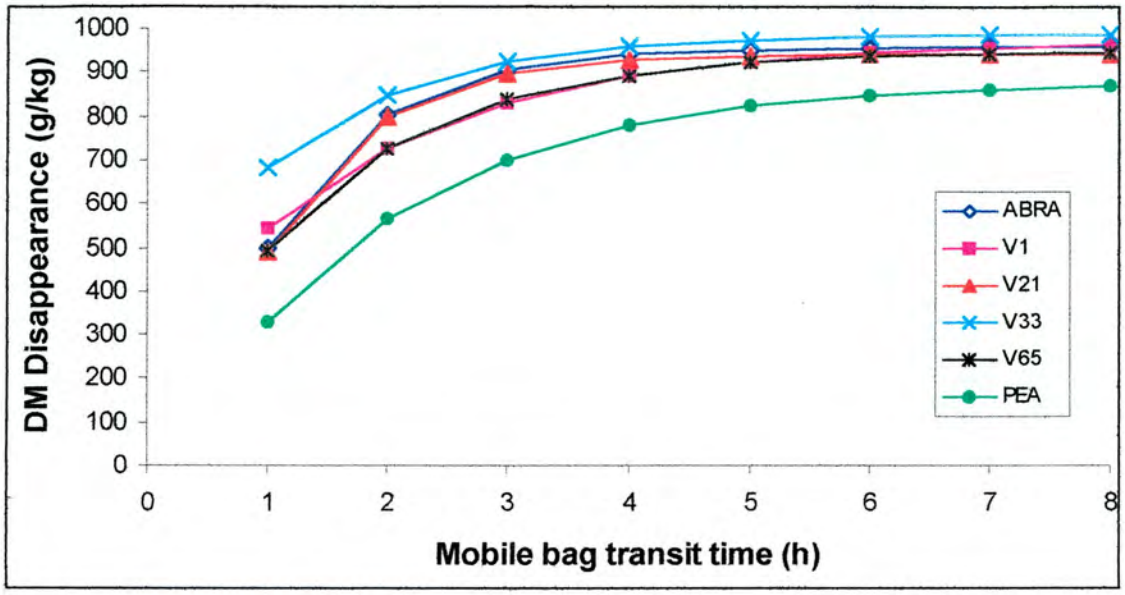


Table 5.1.3.4 Calculated effective degradabilities (ED) at five different mean retention times (MRT) for six different starch types, incubated in the precaecal segment of the gastrointestinal tract of ponies, using time independent$^{\Psi}$ and time dependent models (G2, G3, G4)$^{\infty}$.

	ABRA	V1	V21	V33	V65	PEA	sed	sig
Time independent model								
ED[1] k=0.50	778[a]	801[a]	758[a]	800[a]	597[b]	556[b]	49.0	*
ED[2] k=0.33	823[a]	845[a]	801[a]	840[a]	676[b]	614[b]	35.4	**
ED[3] k=0.25	851[a]	872[a]	827[a]	866[a]	726[b]	654[c]	28.2	*
ED[4] k=0.20	867[a]	892[a]	845[a]	884[a]	761[b]	683[c]	25.4	*
ED[5] k=0.14	891[a]	918[a]	869[a]	909[a]	805[b]	723[c]	26.5	*
Time dependent model - G2								
ED[1] λ=1.00	755[a]	782[a]	738[a]	782[a]	561[b]	531[b]	55.4	*
ED[2] λ=0.67	804[a]	826[a]	783[a]	823[a]	643[b]	589[b]	41.0	**
ED[3] λ=0.50	834[a]	856[a]	811[a]	850[a]	696[b]	630[b]	32.2	**
ED[4] λ=0.40	855[a]	877[a]	831[a]	870[a]	734[b]	660[c]	27.4	*
ED[5] λ=0.29	880[a]	905[a]	857[a]	897[a]	783[b]	703	25.2	*
Time dependent model - G3								
ED[1] λ=1.50	732[a]	763[a]	718[a]	765[a]	534[b]	506[b]	61.8	*
ED[2] λ=1.00	784[a]	807[a]	764[a]	805[a]	608[b]	564[b]	47.1	*
ED[3] λ=0.75	817[a]	838[a]	794[a]	834[a]	664[b]	605[b]	37.4	**
ED[4] λ=0.60	839[a]	860[a]	812[a]	854[a]	704[b]	633[b]	31.1	**
ED[5] λ=0.43	867[a]	891[a]	844[a]	883[a]	758[b]	681[c]	25.5	*
Time dependent model - G4								
ED[1] λ=2.00	713[a]	748[a]	702[a]	751[a]	49.5[b]	48.7[b]	66.7	*
ED[2] λ=1.33	76.7[a]	79.2[a]	74.9[a]	79.2[a]	58.0[b]	54.4[b]	5.21	*
ED[3] λ=1.00	801[a]	823[a]	780[a]	820[a]	638[b]	585[b]	41.9	**
ED[4] λ=0.80	825[a]	846[a]	803[a]	842[a]	680[b]	617[b]	34.8	**
ED[5] λ=0.57	843[a]	864[a]	820[a]	858[a]	712[b]	642[c]	30.0	*

Values on the same row not sharing common superscripts differ significantly (P<0.05)
$^{\Psi}$Effective degradabilities calculated according to the models of Ørskov and McDonald (1979)
$^{\infty}$Effective degradabilities calculated according to the model of Ellis *et al.* (1994)
[1]MRT = 2 h, [2]MRT = 3 h, [3]MRT = 4 h, [4]MRT = 5 h, [5]MRT = 6 h

The effect of model on calculated ED are given below in Table 5.1.3.5. For all MRT, with the exception of MRT = 2 h, calculated ED were significantly lower for the G4 model (P<0.01) compared to the TI and G2 models. Incorporating a G3 function also resulted in significantly lower (P<0.05) ED compared to the TI model but was not significantly different to the G2 or G4 models for all MRT.

Table 5.1.3.5 Effect of time independent (TI) and time dependent (G2, G3 or G4) models on calculated effective degradabilities at five different mean retention times of pure starch incubated in the precaecal segment of the gastrointestinal tract of ponies

	TI	G2	G3	G4	sed	sig
MRT = 2 h	715[a]	692[ab]	668[b]	650[b]	22.0	*
MRT = 3 h	766[a]	745[ab]	722[bc]	704[c]	16.6	*
MRT = 4 h	798[a]	780[ab]	759[bc]	741[c]	13.0	**
MRT = 5 h	822[a]	804[ab]	785[bc]	769[c]	11.1	**
MRT = 7 h	853[a]	837[ab]	821[bc]	807[c]	9.7	**

[a, b, c] Values in the same row not sharing common subscripts are significantly different (P<0.05)

5.1.4 Discussion

5.1.4.1 Development of the mobile bag technique

The methodology of the mobile bag technique (MBT) in equines differs in some respects from that used in pigs and ruminants. The introduction of bags directly into the stomach of ponies appears to present no problems with regards to subsequent passage through the digestive tract. Of the 410 bags successfully introduced into the stomach of ponies in this experiment, 96% of them were recovered on the magnet in the caecum within eight hours, the remaining 4% were recovered in the faeces within 72 h. This is in contrast to the work of Sauer *et al.* (1983) where they introduced bags directly into the stomach of pigs *via* a gastric cannula. After four days there was no evidence of bags in the faeces. The pigs were starved for a day allowing the pyloric sphincter to relax sufficiently to allow passage of the bags and subsequent recovery in the faeces 24 - 48 h later.

In subsequent pig studies, bags underwent simulated gastric digestion (pepsin/HCl) before insertion *via* duodenal cannula into the digestive tract (Graham *et al.*, 1985;

Cherian *et al.*, 1989; de Lange *et al.*, 1991; Leibholz, 1991). In ruminant studies bags were either introduced directly into the duodenum (Varvikko and Vanhatalo, 1989) or feed samples underwent one of three pre-treatments: incubation of feeds in the rumen (de Boer *et al.*, 1987; Vanhatalo and Keetoja, 1995; O'Mara *et al.*, 1997); an *in vitro* pepsin/HCl incubation only (Jarosz *et al.*, 1994; Mgheni *et al.*, 1994)or an incubation in the rumen followed by *in vitro* pepsin/HCl incubation. The introduction of bags directly into the stomach of equids overcomes the need for simulated gastric digestion of bags prior to insertion into the digestive tract. Thus the MBT may be a more suitable technique for use in equine studies than for those in ruminants or pigs.

In the current study, the use of a flexible naso-gastric tube pre-loaded with bags allows for a substantial number of bags (24 in this case) to be simultaneously introduced directly into the equine stomach. Macheboeuf *et al.* (1995) also used a naso-gastric tube to introduce thirty bags at one time into the equine stomach. In this experiment the bags were introduced prior to the 09:30 h meal, however, Macheboeuf *et al.* (1995) introduced bags during the morning feed (after the concentrate meal, prior to the forage meal). No studies have yet investigated the effect of timing the introduction of bags into the equine stomach. However, introducing bags prior or during meals appears to be a standard practice in all species. In studies in pigs smaller numbers of bags (2 - 6) were introduced during meals and the whole procedure could take up to an hour (Graham *et al.*, 1985; Cherian *et al.*, 1989; de Lange *et al.*, 1991). Procedures for introducing bags into the duodenum of ruminants also take time and only small numbers of bags are inserted. Accordingly, De Boer *et al.* (1987) inserted one bag every 45 minutes, Becker *et al.* (1996) inserted one bag every 15 minutes during a meal (total of six bags per meal) whereas Vanhatalo and Keetoja (1995) inserted four or five bags at any one time, aiming to insert twenty bags per animal per day. In contrast to the procedures in pig and ruminant studies, use of a naso-gastric tube allows the rapid delivery of a substantial number of bags directly into the digestive tract of equids, thereby reducing the frequency of the procedure and

enabling the examination of a greater number of samples than has been the case in pig or ruminant studies.

The literature indicates that, to date, residues from mobile bags have been pooled and chemical analyses carried out on the pooled residue to give a single measure of digestibility. However, few studies have examined the effect of mobile bag transit time through the digestive tract on digestibility. In studies with ruminants it was noted that bags recovered in the faeces had a retention time almost double that of bags recovered from the ileum (25 and 12 h respectively) (Vanhatalo and Keetoja, 1995). These authors also noted that retention time was related to the level of feeding and that the shortest retention times were recorded when diets were fed at a high level. Beckers *et al.* (1996) concluded from their studies in ruminants that there was no relationship between transit time through the intestines and digestibility in the intestines for concentrates. However, Vanhatalo and Keetoja (1995) did note that site of recovery, and consequently transit time was important for degradation of forage feeds. In studies in pigs Graham *et al.* (1985) found that retention time did not influence the extent of barley degradation but did for whole crop peas. In the present study there were no significant differences between feeds in terms of transit time through the small intestine, however, PEA starch had a significantly ($P<0.05$) lower DMD than ABRA, V1, V21 and V33 starch but was similar in DMD to V65 starch. This difference structure can alter the granules susceptibility to hydrolysis. The modified starches (V21, V1, V33 and V65) were produced by treating the purified ABRA starch with sodium trimetaphosphate which replaced some of the native hydrostatic bonds with phosphate bonds (known as cross-linking). These bonds are stronger than the native hydrostatic bonds and are less susceptible to adverse environmental conditions such as pH. The pH of digesta in the pre-caecal segment of the equine digestive tract has been quoted as 2 –3 in the pyloric region of the stomach rising to approximately 7 by the end of the ileum. Such initial acidic conditions may well have resulted in a weakening of native hydrostatic bonds resulting in the starch

being more susceptible to hydrolysis. V65 was considered to be highly crosslinked and this may well have made it less susceptible to degradation in the pre-caecal segment of the equine digestive tract. The lower DMD of PEA starch was assumed to be due to differences in starch granule shape, leguminous starch granules tend to be lenticular in shape whereas graminaceous starches tend to spherical (see 5.1.4.2).

The objective of this experiment was to determine if degradation profiles could be constructed for highly digestible feedstuffs using the mobile bag technique. Hyslop *et al.* (1998), in a study using ponies, constructed degradation profiles for DM and NDF content of four different forages using the models of Ørskov and McDonald (1979) and Dhanoa (1988). Due to the tubular nature of the equine small intestine, bags cannot be instantly recovered at the end of the small intestine and therefore a lag occurs between introduction of bags into the stomach and the site of recovery. The model of Dhanoa (1988) includes a lag time and was used in the present study to construct degradation profiles. The mean calculated lag time across feeds was 0.74 h and the earliest recovery of bags was 1 h. In this study the correlation coefficient (R^2) between observed DMD and fitted DMD was 0·615. Hyslop *et al.* (1998) presented R^2 of 0·954 and 0·824 for DM and NDF degradation profiles respectively. However, in the study of Hyslop *et al.* (1998), a larger number of ponies were used allowing a greater number of bags to be incubated. They also pooled residues from six or seven bags to provide subsample on which they carried out their analysis and this would have reduced the variability in their data.

The model of Ørskov and McDonald (1979) assumes that passage through the incubating segment can be described by an exponential outflow pattern, i.e. the segment behaves as a continuous flow stirred tank (see section 2.2.2) and every particle has the same equal opportunity to pass out of the segment regardless of time spent in that segment. The small intestine however is a long tubular segment and particles must travel its entire length before passing into the next section, thus passage

through the small intestine is time dependent. The model of Dhanoa (1988) incorporates a lag phase that can be applied to constituent disappearances of feedstuffs incubated in the pre-caecal segment of the gastrointestinal tract of ponies. Pond *et al.* (1988) incorporated the concept of time dependency into their models for passage rates, replacing k with λ. By incorporating λ into equations for calculating effective degradabilities (Ellis *et al.*, 1994) it is possible to calculate ED for feedstuffs incubated in time-dependent segments of the gastrointestinal tract. In this experiment ED were calculated for time-independent and time-dependent models to investigate the effect of model on calculated ED. From the results of section 3.2.3.1 it is apparent that a G4 time-dependent model best represents the passage of digesta through the digestive tract of ponies. Incorporation of a time-dependent G4 function resulted in lower calculated ED compared to time-independent models in this experiment. These results suggest that an inappropriate model can lead to significant over-estimation of effective degradabilities even for rapidly degradable feedstuffs.

5.1.4.2 The effect of chemical modification of starch on starch degradation in the pre-caecal segment of the equine digestive tract

The purified starches used in this experiment included a graminaceous (ABRA) and a leguminous (PEA) starch as well as four chemically modified starches. DM disappearance of PEA (609 g/kg) from bags passing through the small intestine was significantly lower ($P<0.05$) than that of ABRA (719 g/kg). The calculated effective degradability values for PEA, regardless of model used, were significantly lower ($P<0.05$) than ABRA reflecting the difference in DM loss in the small intestine. Legume starch granules tend to be lenticular in shape whereas graminaceous starch granules tend to be spherical. This difference in granule structure may well account for the difference in DM disappearance. Gallant *et al.* (1973) and Kienzle *et al.* (1998) have investigated the amylolytic digestion of different botanical starch sources in monogastrics and concluded that different sources were digested in different ways

and to a greater or lesser extent. Gallant *et al.* (1973) incubated different botanical starch sources (potato, manioc, wheat, maize, waxy maize and amylomaize) *in vitro* with piglet pancreatic juice. Their results showed that the extent of damage to the starch granule was different for each botanical starch source and that the damage was either by exocorrosion alone or a combination of exocorrosion and endocorrosion. Kienzle *et al.* (1998) examined starch granules in the jejunal chyme of horses under a scanning electron microscope and also found that different botanical sources were hydrolysed in different ways. For maize and barley, starch granules underwent endocorrosion whereas oat starch granules underwent extensive exocorrosion. The apparent starch digestibilities were measured as being 0.88, 0.47 and 0.22 for oats, maize and barley respectively when measured in the small intestine of horses (Kienzle *et al.*, 1998), which may suggest that exocorrosion may result in either an enhanced rate or extent of starch breakdown.

Cross-linking of starch is carried out to stabilise the overall structure and to make it more resistant to damage occurring at low pH, high shear forces and high temperatures. It was therefore expected, as the level of cross-linking increased in the purified starch sources used in the present study, that DM losses would progressively decrease with increased cross-linkage. Although the highest level of cross-linking (V65) had lower DM losses (711 g/kg) in the small intestine than ABRA (791 g/kg) they were not significantly different ($P>0.05$). However, calculated effective degradability values were significantly lower ($P<0.05$) for V65 compared to ABRA, V1, V21 and V33 but were not significantly different to PEA ($P>0.05$) regardless of the model and MRT employed. The effect of cross-linking on wheat starch digestibility in horses would, therefore, appear to be limited to reducing the ED of those starches which were very highly cross-linked.

5.1.5 Conclusions

The experiment reported here demonstrates that the mobile bag technique can be used successfully in equines to calculate effective degradability of feedstuffs and with few of the problems encountered with other species. Using a naso-gastric tube pre-loaded with bags allows rapid introduction of bags (up to 24 per pony per introduction) directly into the stomach without the need for prior pepsin/HCl digestion. The results of this experiment show that it is possible to construct degradation curves and calculate effective degradabilities from mobile bag data. The calculation of effective degradabilities should include the use of an appropriate model and MRT.

To summarise

1. Use of a naso-gastric tube to introduce MB into ponies offered advantages in terms of increased sample delivery, compared to other methods of bag administration used with other species.

2. The MBT is a suitable technique for use in ponies with few of the problems encountered in other species.

3. Mobility of bags did not appear to be impeded in any section of the GI tract and delivery of bags directly into the stomach negated the need for pre-treatment of samples

4. The results clearly demonstrated that models developed for ruminant studies can be used to derive degradation kinetics of feedstuffs in the pre-caecal segment of the equine digestive tract.

Chapter 6. Effect of physical processing on the degradation of starch-based feedstuffs in both the total and pre-caecal segment of the digestive tract of ponies as measured by the mobile bag technique

6.1 Introduction

Several workers have used the mobile bag technique to determine the degradation of a variety of feeds over the entire equine digestive tract in both ponies and horses (Ellis and Beever, 1984; Macheboeuf *et al.*, 1995; Hyslop and Cuddeford, 1996; Longland *et al.*, 1997; Moore-Colyer *et al.*, 1997a; Moore-Colyer *et al.*, 1997b). Although the majority of theses studies investigated the degradation of different sources of fibre, Araujo *et al.*, (1996a) examined the degradation of concentrates and reported DM disappearances of 92, 96, 78, 75 and 99% for maize, soyabean meal, wheat meal, cottonseed meal and corn starch respectively. Although Araujo *et al.*, (1996a) described disappearances of feed constituents from bags as apparent digestibility coefficients they do not report any correlations between these coefficients and *in vivo* digestibilities. However, Macheboeuf *et al.*, (1995) reported a correlation of 0.99 between *in vivo* digestibility of DM and DM disappearance from mobile bags which had been allowed to travel through the whole of the equine digestive tract.

Following on from the development of the mobile bag technique in the previous chapter and from the work of Hyslop and co-workers (Hyslop and Cuddeford, 1996; Hyslop *et al.*, 1998) two experiments were carried out to measure the degradation of starch-based feedstuffs in both the pre-caecal segment and the total digestive tract of ponies using the mobile bag technique. The effect of physical processing on the degradation kinetics of barley and the degradation of maize and peas were investigated in Experiments 5 and 6 respectively.

6.2 Materials and Methods

6.2.1 Experiment 5: Effect of physical processing on barley degradation in the small intestine and total tract of ponies

6.2.1.1 Animal Management

The two caecally-fistulated Welsh-cross pony geldings described previously (see section 5.2.1.1) were used. Housing and management of ponies was as previously described (section 5.2.1.1). Ponies received a daily basal diet of 3kg grass nuts (GN), 1kg micronised flaked barley (MFB) and 60mg min-vit supplement (see appendix 1) in two equal meals at 09:30 h and 17:00 h. Hay was available ad libitum between 17:30 h and 09:00 h

6.2.1.2 Preparation of Feed Samples

The three processed barleys (UB, MB and EB) that were used in Experiments 1 and 2 were employed in this trial. All feedstuffs were milled through a 0.5mm steel mesh screen and then sieved across a 45μm screen. Particles of < 45μm were not included in the samples as these particles could be lost immediately through the bag pores.

6.2.1.3 Preparation of Bags

Mobile bags were made of a monofilamentous polyester fibre (Sericol Ltd., Westwood Road, Broadstairs, Kent, CT10 2PA) with a pore size of 41μm. Bags were either 6cm (large) or 4cm (small) long with a diameter of 1cm. Bags were manufactured in the laboratory as described previously in section 5.2.1.3. Approximately two thirds of both the large and small bags contained two small metal washers sealed into a small compartment at one end, whereas the remaining bags did not. All bags were numbered for ease of identification. Samples of feedstuffs were weighed accurately into individual bags: 400 mg into large bags no washers; 300 mg

into large bags with washers; 265 mg into small bags with no washers and 165 mg into small bags with washers. Bags were then closed by heat-sealing the open end.

6.2.1.4 Bag administration and retrieval

On day 1 (Monday) of each working week, prior to the 09:30 meal, twelve large and twelve small bags (four bags per feedstuff per bag size) with washers were pre-loaded into a naso-gastric tube (one per pony). Bags were then administered directly into the stomach of each pony *via* a naso-gastric tube (as described in section 5.2.1.4), whereupon the magnetic capture device (described in section 5.2.1.4) was placed in the caecal fistula. The animals then received their 09:30 meal.

The LED of the magnetic capture device was activated, as before, when bags attached to the magnet and the display was checked every 10 minutes. When the LED was activated the capture device was removed from the caecum and the adherent bags removed. The LED on the capture device was then deactivated and the capture device returned to the caecum. The bag number and the time of its recovery were recorded. After eight hours the magnetic capture device was removed from the caecum and a further set of 12 large and 12 small bags, which did not contain magnetic washers, were then administered into the stomach of each pony. As these bags would not attach to the caecal capture device they travelled through the entire gastro-intestinal tract and were recovered in the faeces.

On day 3 (Wednesday) the procedure was repeated with the exception that the second set of bags was not administered. Any bags not recovered at the magnet were recovered in the faeces, which were checked regularly. When bags were recovered from the faeces their number and time were recorded. Faeces were checked regularly from 17:00 h on day 1 through to 17:00 on day 7 (Sunday). This procedure was carried out for ten weeks.

6.2.1.5 Post-incubation treatment of bags

When bags were removed from the caecum or faeces they were rinsed under running tap water to remove adherent digesta/faecal material from the exterior of the bag. The recovered bags were then placed in a fridge (4°C) until they were washed in an automatic washing machine (Indesit 824) on a cold-water programme (see section 4.1.2.5).

DM losses from feedstuffs in 'zero time' bags were measured by subjecting at least four bags of each design per feedstuff to the cold water washing procedure in a washing machine as described above. These bags were not administered into the stomach. The bags were then dried in a forced draught oven at 60°C for at least 48 hours. Zero time washing DM losses were determined once per feedstuff.

6.2.1.6 Analysis of residues

The DM disappearance (DMD) of feed particles from each bag was calculated. For each two week period, residues (one replicate) from bags recovered on the magnet from both ponies were pooled to give seven sub-samples (i.e. early bags grouped together and later bags grouped together) per feedstuff. A mean weighted transit time was calculated for each sub-sample according to the transit times of individual bags within each subsample. The pooled residues were then analysed for starch content. Due to the small quantities of residue remaining after starch analysis, these were then bulked for crude protein (CP) analysis and this was determined once for each two week block. A mean weighted transit time was calculated for the bulked residues.

For bags that were recovered in the faeces, the DM disappearance of feed from each bag was calculated and residues from bags were then bulked across replicates for both

ponies for subsequent STC and CP analysis for each week and the mean weighted transit time of the bags was calculated.

DM, STC and CP disappearances (DMD, STCD and CPD respectively) from feeds were calculated for bags, which had undergone the cold water washing procedure. Single, mean weighted transit times were also calculated for each two week replicate for DMD, STCD and CPD for bags recovered at the magnet (pre-caecal bags) and for bags recovered in the faeces (total tract bags). This gave five replicates in total for each feed which had been subjected to passage through the fore gut (pre-caecal bags: PC), the whole gut (faecal bags: TT) or a washing machine cycle (washing machine bags: WM).

6.2.1.7 Modelling Degradation Profiles and Statistical Analysis

The model of Dhanoa (1988) was fitted to the seven subsamples for each two week replicate and degradation parameters derived for both DM and STC. The same model was used to fit degradation profiles to DMD from faecal bags. MLP (Maximum Likelihood Programme, Lawes Agricultural Trust, 1993) was used to fit degradation profiles to the data. Effective degradabilities were calculated according to a Gamma 4 time dependent equation as in described in section 5.1.2.6 (Equation 5.1).

Genstat 5 (Lawes Agricultural Trust, 1990) was used to carry out analysis of variance on degradation parameters and effective degradabilities for DM and STC where variance due to feed and replicate (two week blocks) were identified.
DMD, STCD, CPD and MTT from zero time bags, pre-caecal bags and faecal bags were also subjected to analysis of variance where variance due to feed and replicate were identified.

6.2.2 Experiment 6. Effect of physical processing on degradation of maize and peas in the pre-caecal segment and total tract of ponies as measured by the mobile bag technique.

6.2.2.1 Animal management

The two caecally fistulated ponies used in Experiment 5 were used in Experiment 6. The same housing, diet and management regime described in section 6.2.1.1 was used throughout this experiment.

6.2.2.2 Feed and bag preparation

Unprocessed maize (UM), micronised maize (MM), extruded maize (EM), unprocessed peas (UP), micronised peas (MP) and extruded peas (EP) were used. These feeds were from the same batches as those in Experiment 4 (section 4.2.2). All feeds were ground to pass through a 0.5mm screen and were sieved across a 45μm screen to remove particles smaller than 45μm. Bags were prepared in the laboratory as described in section 6.2.1.3

6.2.2.3 Bag administration and retrieval

Twenty-four bags with washers were pre-loaded in a naso-gastric tube for each pony. Each naso-gastric tube contained two large bags and two small bags per feedstuffs. Bags were administered directly into the stomach of each pony on day 1 (Monday) prior to the 09:30 h meal. The magnetic capture device was then placed directly in the caecum and the fistula closed using the specially designed cap. As in Experiment 5 the LED was checked every 10 min for a flashing light. When the LED was activated the same procedure was followed as described in section 6.2.1.4. At 17:00 h the device was removed from the caecum. A further set of 24 bags (without washers, two large and two small per feedstuff) were then administered into the stomach of each pony, after which the ponies received the 17:00 h meal.

On day 3 (Wednesday) a further set of twenty-four bags (with washers) were administered directly into the stomach of each pony and the procedure repeated. As in Experiment 5, bags with washers were administered once on day 3 with no further administration of bags on that day. This procedure was carried out for nine weeks.

6.2.2.4 Post-incubation treatment

When bags were removed from the caecum or faeces the post incubation procedure was as described in section 6.2.1.5.

6.2.2.5 Analysis of residues

DMD of feeds from bags was calculated for each bag. For each three week period residues from bags recovered on the magnet for both ponies were bulked together for subsequent starch (STC) and CP analysis and a mean weighted transit time calculated for each three week replicate.

Bags recovered in the faeces were treated in the same way as those described in section 6.2.1.5 to give DM disappearance for each bag and STC and CP disappearance for each week with a mean weighted transit time.

DMD, STCD and CPD were calculated for bags that had undergone the washing machine cycle described previously. This gave three replicates in total for feeds that had been subjected to passage through the foregut (PC bags), the whole digestive tract (TT bags) or a washing cycle (WM bags).

6.2.2.6 Statistical analysis

Statistical analysis of washing machine bags, pre-caecal bags and total tract bags was carried out as in section 6.2.1.7. Variance due to feed and replicate (three week period) was identified.

6.3 Results

6.3.1 Experiment 5 – Processed Barley Mobile Bags

6.3.1.1 Constituent losses

The composition of the basal diet and the barleys are given in Table 6.3.1.1. The values observed are in the range quoted for micronised flaked barley (MFB) and dried grass meal by MAFF (1990). The hay was of poor quality and this is reflected in its high NDF value and low CP value. In other mobile bag studies to date, data from mobile bag experiments have been analysed as single time point disappearances weighted for a mean transit time. In this experiment, data have been analysed in this manner but degradation profiles have also been fitted and effective degradabilities calculated.

Table 6.3.1.1 Chemical composition of the experimental feedstuffs: unprocessed (UB); micronised (MB) and extruded barley (EB) and of the basal diet of grass nuts (GN), micronised flaked barley (MFB) and hay fed to the host ponies.

	UB	MB	EB	GN	MFB	HAY
DM (g/kg)	890	900	900	903	876	817
OM	978	978	978	917	975	912
Starch	585	601	584	37	630	23
CP	118	131	115	141	123	51
ADF	63	44	49	368	42	459
NDF	205	159	173	569	172	831

DMD, STCD and CPD from bags placed in the washing machine (WM) are given in Table 6.3.1.2. UB had significantly higher ($P<0.001$) DMD than MB (489 and 304 g/kg respectively), which in turn had significantly higher ($P<0.001$) WM losses than EB (269 g/kg). STCD from WM bags was 717 g/kg for UB, which was significantly greater ($P<0.01$) than either MB (427 g/kg) or EB (459 g/kg). A similar pattern was seen for CPD from WM bags where MB and EB had significantly lower ($P<0.001$) CPD (203 and 208 g/kg respectively) compared to UB (449 g/kg).

Table 6.3.1.2 Dry matter (DM), starch (STC) and crude protein (CP) disappearances (g/kg) of unprocessed (UB), micronised (MB) and extruded barley (EB) from nylon bags that had been cold water washed in a washing machine

	UB	MB	EB	sed	sig
DM	489[a]	304[b]	269[c]	2.18	***
STC	717[a]	427[b]	459[b]	64.30	**
CP	449[a]	203[b]	208[b]	11.87	***

[a, b, c] Values on the same row not sharing common superscripts differ significantly ($P<0.05$)

Single time point disappearances for DM, STC and CP from the feeds along with the weighted mean transit times (MTT) for bags recovered at the end of the precaecal

segment of the digestive tract (PC) and in the faeces (TT) bags are given in Table 6.3.1.3. Although not shown here there were significant differences between site of recovery (pre-caecal bags *vs.* total tract bags) for MTT ($P<0.001$), DMD ($P<0.01$), STCD ($P<0.05$) and CPD ($P<0.001$). The MTT of TT bags was significantly greater than that of PC bags. TT bags also had greater DMD, STCD and CPD than PC bags. MTT of bags through the pre-caecal segment of the tract was 3.64 h, across all three forms of barley, with transit time ranging from 1 - 8 h. Transit time of bags through the whole tract ranged from 9 - 208 h with a MTT of 40.3 h. There were no significant differences between feedstuffs in terms of MTT for either PC or TT bags.

Table 6.3.1.3 Dry matter (DM), starch (STC) and crude protein (CP) disappearances (g/kg) from unprocessed (UB), micronised (MB) and extruded barley (EB) incubated in nylon bags recovered at the end of the pre-caecal segment of the digestive tract of ponies (PC) or in the faeces of ponies, calculated according to a mean transit time (MTT) of bags

		UB	MB	EB	sed	sig
MTT (h)	PC	3.59	3.73	3.59	0.095	NS
	TT	39.65	41.72	39.57	2.690	NS
DM	PC	817[a]	844[b]	826[a]	6.73	*
	TT	900[a]	906[a]	898[b]	3.09	*
STC	PC	977[a]	990[b]	983[ab]	3.92	*
	TT	1000	1000	1000	0.18	NS
CP	PC	899[a]	900[a]	888[b]	3.11	*
	TT	954	951	946	3.84	NS

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

MB had a small but significantly higher ($P<0.01$) DMD than either UB or EB for PC bags (844, 817 and 826 g/kg for MB, UB and EB respectively). DMD from faecal bags was significantly lower ($P<0.05$) for EB (898 g/kg) compared to either UB (900 g/kg) or MB (906 g/kg). STCD in PC bags for UB was 977 g/kg which was significantly lower ($P<0.01$) than that of MB (990 g/kg) but was similar to that of EB (983 g/kg). STCD from TT bags was complete (1000 g/kg) across all feeds. EB had a significantly lower CPD from PC bags than either UB or MB. CPD ranged from 946 - 954 g/kg across feeds for TT bags.

6.3.1.2 Degradation profiles

Degradation profiles were derived for the three barleys using the model of Dhanoa (1988) and fitted profiles are depicted in Figures 6.3.1.1 - 6.3.1.3. The derived degradation parameters along with calculated effective degradabilities (ED) are given in Table 6.3.1.4. ED were calculated according to a Gamma 4 time-dependent model (G4) (Pond *et al.* 1988). From Figure 6.3.1.1 it can be seen that the dry matter degradation profiles are similar for UB and MB but that MB had slightly higher DM disappearance. EB had a different profile due to a longer lag time. These differences are reflected in the degradation parameters, which are given in Table 6.3.1.4 although there were no significant differences between feedstuffs for parameter *a*, *b*, *c* or *lag*. However, MB did have a significantly higher ($P<0.01$) value for potential degradation parameter $a+b$ (872 g/kg) compared to UB or EB (840 and 847 g/kg respectively). This difference was also reflected in the calculated ED at MRT = 7h (842, 811 and 803 g/kg for MB, EB and UB respectively. At MRT = 5h MB (833 g/kg) was not significantly different to EB (800 g/kg) which in turn was not significantly different to UB (793 g/kg). For shorter MRT there were no significant differences between feedstuffs, however MB always had higher DMD than either UB or EB.

Figure 6.3.1.1 Derived dry matter (DM) degradation profiles of unprocessed (UB), micronised (MB) and extruded barley (EB) for the pre-caecal segment of the equine digestive tract.

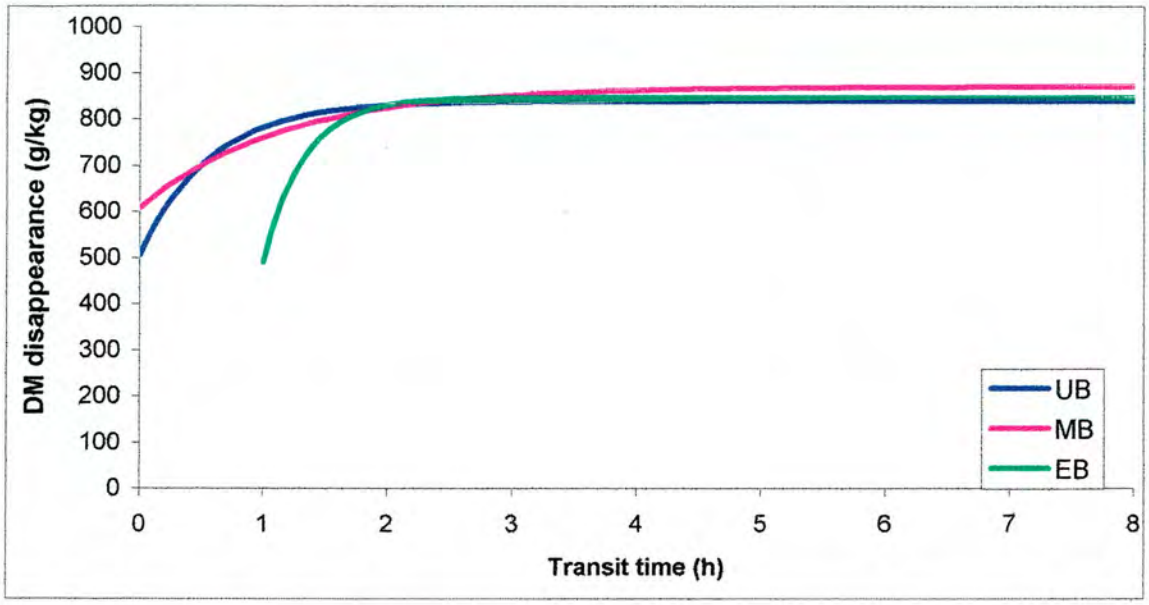


Table 6.3.1.4 Derived dry matter (DM) degradation parameters[¥] and calculated effective degradabilities[Ψ] at three different mean retention times (MRT) for unprocessed barley (UB), micronised barley (MB) and extruded barley (EB)

	UB	MB	EB	sed	sig
Degradation parameters					
a	671	732	641	48.0	NS
b	169	140	206	48.5	NS
c	1.75	0.86	3.06	1.792	NS
lag (h)	0.39	0.74	1.18	0.580	NS
a+b	840[a]	872[b]	847[a]	7.3	**
Effective degradabilities					
MRT = 2h	762	805	763	27.4	NS
MRT = 3h	776	818	781	21.7	NS
MRT = 4h	786	827	792	17.9	NS
MRT = 5h	793[a]	833[b]	800[ab]	15.2	*
MRT = 7h	803[a]	842[b]	811[a]	11.7	*

[¥] Degradation parameters calculated according to the model of Dhanoa (1988)

[Ψ] Effective degradabilities calculated incorporating a G4 time dependency (Ellis *et al.*, 1994)

[a, b] Values on the same row not bearing common superscripts are significantly different

The degradation profile for starch disappearance in the precaecal segment of the equine digestive tract is shown in Figure 6.3.1.2, where it can be seen that MB and EB have similar profiles but UB differs slightly. This difference is not reflected in the degradation parameters given in Table 6.3.1.5. The degradation rate parameter *c* is smaller for UB compared to either MB or EB which would account for the difference in degradation profiles. It is also interesting to note that EB had a smaller value for degradation parameter *a* (rapidly soluble fraction) compared to either UB or MB. Calculated ED for each feedstuff were not significantly different (P>0.05) for each MRT. However, calculated ED were always higher for MB at each MRT compared to UB which in turn always had higher ED than EB. The higher values for UB compared to EB are probably a result of the higher value of parameter *a* for UB compared to EB despite UB having a slower degradation rate.

Figure 6.3.1.2 Starch degradation profiles of unprocessed barley (UB), micronised barley (MB) and extruded barley (EB) which had passed through the pre-caecal segment of the equine digestive tract in mobile bags.

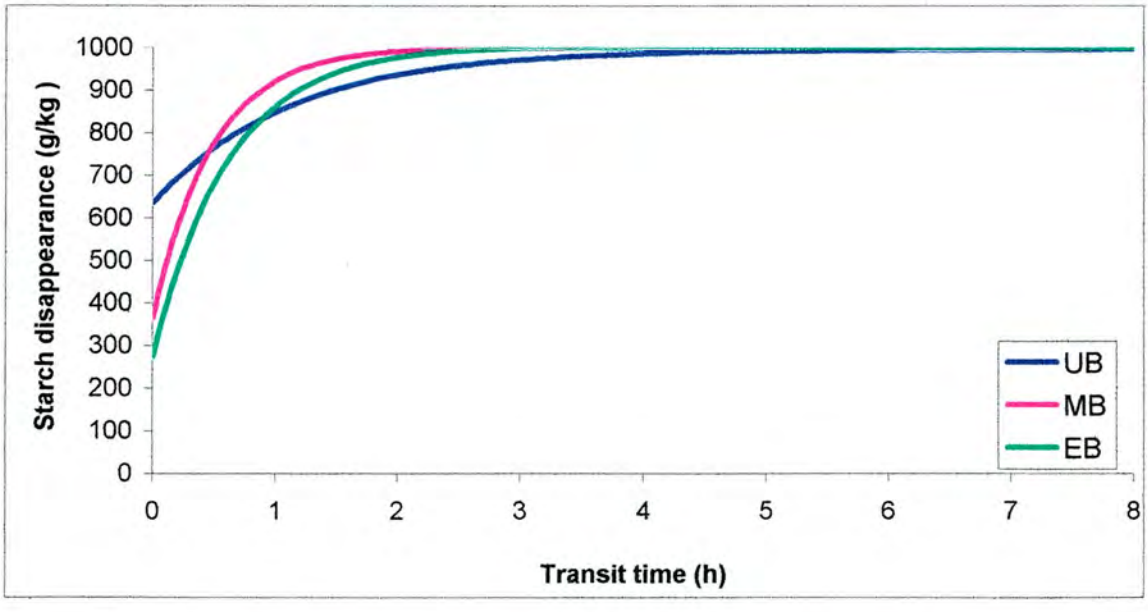


Table 6.3.1.5 Derived starch degradation parameters[¥] and calculated effective degradabilities at different mean retention times (MRT) for unprocessed (UB),

Table 6.3.1.5 Derived starch degradation parameters[¥] and calculated effective degradabilities at different mean retention times (MRT) for unprocessed (UB), micronised (MB) and extruded barley (EB) when incubated in bags which have passed through the pre-caecal segment of the equine digestive tract.

	UB	MB	EB	sed	sig
Degradation parameters					
a	718	847	591	180.5	NS
b	278	153	413	178.8	NS
c	0.89	2.09	1.63	0.635	NS
Lag	0.30	0.68	0.35	0.234	NS
a+b	996	1000	1000	3.1	NS
Effective degradabilities					
MRT = 2	890	937	854	62.2	NS
MRT = 3	914	951	888	46.7	NS
MRT = 4	929	960	909	37.4	NS
MRT = 5	939	969	924	31.3	NS
MRT = 7	952	974	943	23.6	NS

The degradation profile for DM disappearance through the whole of the equine digestive tract is shown in Figure 6.3.1.3. The profiles are similar for all three barleys. Degradation parameters are given in Table 6.3.1.6. There were no significant differences between barleys for *a*, *b*, *c* and *lag* parameters but MB had a small but significantly ($P<0.05$) higher *a+b* value (911 g/kg) compared to UB or EB (904 and 902 g/kg respectively). This higher value was not however reflected in the calculated ED values given in Table 6.3.1.6. ED values were not significantly different between feeds for each of the proposed MRT, however calculated ED values were lower for EB compared to either UB or MB.

Figure 6.3.1.3 Derived dry matter (DM) degradation profiles of unprocessed (UB), micronised (MB) and extruded barley (EB) which has passed through the entire equine digestive tract in mobile bags

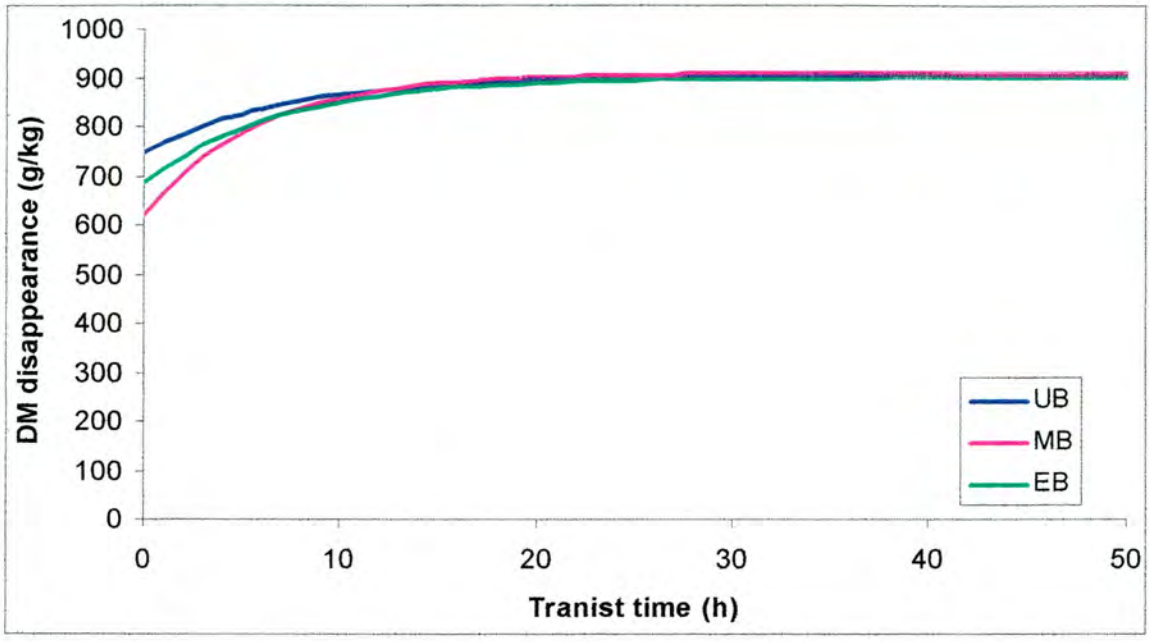


Table 6.3.1.6 Derived dry matter (DM) degradation parameters[¥] and calculated effective degradabilities at three different mean retention times (MRT) for unprocessed (UB), micronised (MB) and extruded barley (EB) when incubated in bags which had passed through the entire equine digestive tract.

	UB	MB	EB	sed	sig
Degradation parameters					
a	748	629	688	81.4	NS
b	156	282	213	82.9	NS
c	0.140	0.169	0.144	0.0323	NS
lag	0.04	0.14	0.04	0.078	NS
a+*b*	904[a]	911[b]	902[a]	2.7	*
Effective degradabilities					
MRT = 20	872	858	859	12.5	NS
MRT = 40	886	882	878	6.3	NS
MRT = 60	891	890	885	4.1	NS

[a, b] Values on the same row not sharing common superscripts are significantly different (P<0.05)

6.3.2 Experiment 6

The chemical composition of the basal diet and that of the six feeds used in the bags is given in Table 6.3.2.1. The values observed for the basal diet were similar to those observed in experiment 5. The DM content of maize and peas was higher than published values (MAFF 1990) but similar to the same feeds used in Experiment 4 (see Table 4.3.2.1)

Table 6.3.2.1 Chemical composition of the basal diet of grass nuts (GN), micronised flaked barley (MFB) and hay fed to ponies and the chemical composition of unprocessed (UM), micronised (MM) and extruded (EM) maize and unprocessed (UP), micronised (MP) and extruded peas (EP) (g/kg DM) incubated in nylon bags in the GI tract of ponies

	UM	MM	EM	UP	MP	EP	GN	MFB	HAY
DM (g/kg)	924	923	926	927	934	936	894	857	861
OM	986	990	988	971	973	973	908	977	932
STC	739	747	809	465	462	455	60	612	45
CP	88	85	89	237	245	247	140	135	54
NDF	82	74	58	118	62	87	533	228	869
ADF	30	32	29	63	58	67	339	52	435

DMD and STCD from bags that were washed only (WM) are given in Table 6.3.2.2 and constituent losses along with weighted mean transit time (MTT) for PC and TT bags are given in Table 6.3.2.3

Table 6.3.2.2 Dry matter (DM), starch (STC) and crude protein (CP) disappearances from unprocessed (UM), micronised (MM) or extruded maize (EM) and unprocessed (UP), micronised (MP) or extruded peas (EP) from nylon bags that had been cold water washed in a washing machine.

	UM	MM	EM	UP	MP	EP	sed	sig
DM (g/kg)	212[a]	225[a]	106[b]	713[c]	561[d]	210[a]	9.9	***
STC (g/kg)	239[a]	225[ab]	184[b]	852[c]	551[d]	95[e]	23.6	*
CP (g/kg)	65[a]	45[a]	177[ab]	880[c]	773[c]	438[b]	134.5	*

[a, b, c, d] Values on the same row not sharing common superscripts differ significantly (P<0.05)

There were significant differences (P<0.05) between feeds for constituent losses when bags underwent the cold water washing procedure. DMD from UP and EM were significantly(P<0.001) higher and lower respectively than from the other feeds. DMD from MP (561 g/kg) was significantly higher (P<0.001) than those from UM, MM and EP (212, 225 and 210 g/kg respectively). There were no differences between UM, MM and EP. UP had significantly higher (P<0.05) STCD (852 g/kg) and EP had significantly lower (P<0.05) STCD (95 g/kg) compared to the four other feeds. EM had significantly lower (P<0.05) STCD (184 g/kg) than MP (561 g/kg) and UM (239 g/kg) but was similar to MM (225 g/kg). UM had significantly lower STCD than MP but similar to MM.

UP also had significantly higher (P<0.05) CPD (880 g/kg) compared to the other feeds with MM (45 g/kg) having the lowest (P<0.05). UM (65 g/kg) and EM (177 g/kg) had similar CPD to MM. MP (773 g/kg) had similar CPD to UP but had significantly higher losses compared to EP (438 g/kg). EP was not significantly different to EM.

Table 6.3.2.3 Calculated mean bag transit time (MTT), Dry matter (DM), starch (STC) and crude protein (CP) disappearances of unprocessed (UM), micronised (MM) or extruded maize (EM) and unprocessed (UP), micronised (MP or extruded peas (EP) from bags that were recovered at the end of the pre-caecal segment of the digestive tract of ponies (PC) or in the faeces of ponies (TT),

		UM	MM	EM	UP	MP	EP	sed	sig
MTT	PC	5.1^{ab}	5.3^{b}	4.9^{a}	5.1^{a}	5.1^{a}	5.1^{a}	0.13	*
(h)	TT	43.6	43.6	44.7	42.3	40.1	49.9	6.17	NS
DM	PC	833^{a}	907^{b}	811^{a}	817^{a}	874^{c}	835^{a}	12.1	*
(g/kg)	TT	905^{a}	922^{b}	921^{b}	947^{c}	955^{c}	932^{d}	4.1	*
STC	PC	941^{a}	992^{cd}	962^{ab}	967^{b}	998^{d}	977^{bc}	9.4	*
(g/kg)	TT	990^{a}	1000^{b}	996^{ab}	997^{ab}	998^{b}	997^{db}	3.0	*
CP	PC	858^{a}	816^{a}	603^{b}	976^{c}	969^{c}	978^{c}	25.6	*
(g/kg)	TT	793^{a}	845^{b}	834^{b}	967^{c}	974^{c}	957^{c}	11.2	*

^{a,b,c,d} Values on the same row not sharing common superscripts are significantly different (P<0.05)

Transit time through the pre-caecal segment of the equine digestive tract ranged from 1 - 8 h with an average transit time of 5.1 h. There was no difference in transit time due to feed for PC bags. A significant difference (P<0.05) in transit time did occur between bags containing MP (40.1 h) and those containing EP (49.9 h) for TT bags. There were no differences in transit time between the bags containing the four other feeds and either MP or EP. Average mean transit time for bags through the whole of the equine digestive tract was 44 h and ranged from 10 - 250 h.

In the main, constituent losses tended to be higher in TT bags than PC bags however, CPD from UM was significantly lower (P<0.05) in TT bags (793 g/kg) compared to PC bags (858 g/kg). UP and EP also had lower CPD from TT bags (967 and 957 g/kg respectively) but this was not significantly different to PC bags (976 and 978 g/kg respectively). MM had significantly higher DMD for PC bag (907 g/kg) compared to MP (874 g/kg), which in turn had significantly higher (P<0.05) DMD than the four other feeds. DMD for UM and EP were similar (833 and 835 g/kg respectively) and

DMD for EM and UP were similar (811 and 817 g/kg), however EM had significantly lower ($P<0.05$) DMD than UM or EP for PC bags. DMD in TT bags was significantly higher ($P<0.05$) for UP and MP (947 and 955 g/kg) compared to EP and the maize feeds. EP (932 g/kg) did not differ significantly in terms of DMD compared to MM and EM (922 and 921 g/kg respectively) but had significantly higher losses compared to UM (905 g/kg).

Disappearance of starch was almost complete in PC bags for all feeds with STCD ranging from 941 - 998 g/kg. MP and MM had similar STCD (998 and 992 g/kg). MM had similar STCD to EP (977 g/kg) which in turn had similar losses to EM and UP (962 and 967 g/kg respectively). UM had significantly lower ($P<0.05$) STCD (941 g/kg) compared to all other feeds for PC bags. STCD from TT bags were significantly higher ($P<0.05$) than PC bags for UM, EM, UP and EP. UM had slightly, but significantly lower STCD for TT bags compared to all other feeds. STCD in TT bags ranged from 990 - 1000 g/kg.

All three pea feedstuffs had significantly higher ($P<0.05$) CPD compared to the three maize feeds for both PC and TT bags. CPD losses for UP, MP and EP were similar for both PC and TT bags with losses ranging from 957 - 978 g/kg. CPD in PC bags were significantly lower ($P<0.05$) for EM (603 g/kg) compared to either UM (858 g/kg) or MM (816 g/kg). However in TT bags CPD were significantly lower ($P<0.05$) for UM (793 g/kg) compared to MM (845 g/kg) but were similar to EM (834 g/kg).

Due to the high DMD and STCD from the bags it was not possible to construct degradation curves or derive degradation parameters in this experiment.

6.4 Discussion

In Experiment 1a the results showed no differences in total tract *in vivo* apparent digestibilities of the three forms of barley but did reveal differences in intra-caecal

fermentation parameters. The results suggest that there may be differences in pre-caecal digestibility of starch when cereals are physically processed. An objective of Experiment 5 was to test this hypothesis using the mobile bag technique to study pre-caecal feed degradation.

The three barleys used in Experiment 5 were from the same batches of feed used in Experiment 1, which allows direct comparisons to be made between *in vivo* apparent DM, STC and CP digestibilities and their corresponding disappearance from mobile bags (see Table 6.4.1). DMD from PC bags was similar to DMIV but DMD from TT bags was higher than DMIV. STCD from both PC and TT bags was higher than STCIV values. This was also the case for CP.

Table 6.4.1 Apparent *in vivo* digestibilities (IVD) and mobile bag disappearances from the precaecal segment (PC) and the total digestive tract (TT) of unprocessed (UB/RB) micronised (MB) and extruded barley (EB) as measure in ponies

	UB/RB	MB	EB
Dry matter (g/kg)			
IVD	825	834	823
PC	817	844	826
TT	900	906	898
Starch (g/kg)			
IVD	967	969	966
PC	977	990	983
TT	1000	1000	1000
Crude protein (g/kg)			
IVD	709	717	685
PC	899	900	888
TT	954	951	946

Differences between *in vivo* apparent digestibilities and disappearances from mobile bags may occur for three reasons: 1.) microbial contamination of faeces and bags; 2.) particle size of feeds and 3.) disappearance of feed from a bag is not directly analogous to its digestion. Each is discussed in turn below.

Faecal material contains microbial debris, endogenous material resulting from the sloughing of cells from the digestive tract and material resulting from enzymes and other substances secreted into the digestive tract as well as undigested feed residues (McDonald *et al.*, 1996). This leads to an underestimation of the proportion of feed actually degraded and absorbed. It has generally been assumed that washing of *in sacco* bags after incubation in the digestive tract removes contaminating bacterial and endogenous residues (Moore *et al.*, 1994; Huntington and Givens, 1995) and it has therefore been suggested that disappearances of material from mobile bags are an estimate of true rather than apparent digestibility (de Boer *et al.*, 1987; Stern *et al.*, 1997). The higher feedstuff losses from MB compared with those *in vivo* may well be an indication of the true digestibility of these feeds. However, procedures for post-incubation washing of bags differ between studies, ranging from gentle rinsing by hand to severe machine washing for several minutes (Cherney *et al.*, 1990) resulting in differences in feedstuff values and degradation profiles (Philippeau and Michalet-Doreau, 1997). These differences may well represent, on the one hand, incomplete removal of non-dietary biomass and on the other, its complete removal together with varying amounts of feed residues which had escaped degradation during incubation.. Clearly, post-incubation washing of bags should reflect a balance between complete removal of contaminating biomass and maintenance of feed residue integrity. However, the effects of different post-incubation procedures on feedstuff DMD were not examined in the current study, yet the method employed was relatively severe. Thus, the higher MB feedstuff losses compared with those *in vivo* may reflect losses which were due to removal of both contaminating biomass and feed residue components in the washing process.

Particle size of feedstuffs has been shown to have a large influence on value arising from *in sacco* studies. Michalet-Doreau and Cerneau (1991) ground a variety of feedstuffs through three different screen sizes (0.8, 3.0 and 6.0 mm) prior to

incubation of feedstuffs in the rumen *in situ*. They found that feed nitrogen degradability in the rumen decreased when the grinding screen changed from 0.8 to 6.0mm and this effect was greatest for peas and maize. They also noted that mean particle size was different for feeds that had been ground through the same screen size. Although not reported here, mean particle sizes for the feeds used in this thesis were significantly different ($P<0.05$) between the same feeds ground through a 1.0mm screen (*in situ* experiments) and those ground through a 0.5mm screen (mobile bag experiments). Mean particle size of MB, EB, EM, UP, MP and EP was significantly larger when these feeds were ground through a 1.0mm compared to a 0.5mm screen (McLean *et al.*, 1999). The smaller mean particle size of feeds in Experiments 5 and 6 may have resulted in greater losses of particles through the bag pores and consequently may have led to an overestimation of digestibility.

The aim of grinding feedstuffs prior to incubation *in sacco* is to provide a sample that is homogeneous and simulates the particle distribution resulting from mastication (Huntington and Givens, 1995). Many workers have investigated the effect of particle size on *in sacco* losses (Galyean *et al.*, 1981; Ehle *et al.*, 1982; Emanuele and Staples, 1988; Nocek and Kohn, 1988b; Michalet-Doreau and Cerneau, 1991). Particle size reduction in the rumen has also been well studied (Weston and Kennedy, 1984; Mertens *et al.*, 1984; Knipfel, 1984; Deswysen *et al.*, 1984; Wadsworth, 1984; Pond, 1984) but in equines this information is sparse. It has been suggested that in equines, feed particles must be less than 1.6 mm before they can be swallowed (Frape, 1998). No consistent recommendations have been made for the optimum mean particle size of feedstuffs for *in sacco* studies. The AFRC 1992 recommend that concentrates are ground through a 2.0mm screen whereas Nocek (1988a) recommends the use of a 5.0mm screen for concentrates. Michalet-Doreau and Cerneau (1991) recommend that the mean particle size of feedstuff is given rather than milling screen size as not all feeds will have the same mean particle size for a given milling screen.

Feed particles that disappear from bags can either be: 1.) digested and subsequently absorbed into the blood stream; 2.) be degraded by the hindgut microflora or 3.) pass through into the next segment of the digestive tract. Therefore, disappearance of feed particles from a bag is not directly comparable to the digestibility of that feed. Macheboeuf *et al.* (1995) correlated *in vivo* digestibilities with disappearances from bags which had travelled the whole of the equine digestive tract and calculated correlation coefficients of, 0.99, 0.77 and 0.75 for DM, nitrogen and estimated true nitrogen digestibility respectively. In the experiments reported here the three barley feeds had known *in vivo* apparent digestibility values however, this number of feeds is too small to carry out any meaningful statistical correlations. Disappearance of feedstuff from PC and TT bags was higher than *in vivo* values especially for CP.

The average transit times of bags through both the pre-caecal segment and the total digestive tract of ponies were slightly different between the two experiments. However, the transit times of TT bags in Experiment 5 were not dissimilar to the MRT of each diet recorded in Experiment 1b (see section 3.2.3). Araujo *et al.*, (1996b) compared transit time, through the entire equine digestive tract, of three different sizes of mobile bags with digesta passage measured using polythene particles. Digesta passage was measured as being between 48 and 66 h and transit time of bags through the total tract were 42, 54 and 48 h for small, medium and large bags respectively. Araujo *et al.*, (1984) recorded an average transit time of 46.3 h for bags containing different feedstuffs passing through the whole digestive tract of equids. This is similar to an average of 44 h measured in Experiment 6 but slightly higher than the average of 40.3 h recorded in Experiment 5. Transit time through the pre-caecal segment was also faster for bags in Experiment 5 compared to Experiment 6 (3.7 h *vs.* 5.1 h respectively). The results here concur with those of Macheboeuf *et al.*, (1995) who recorded transit times of 4.2 h and 38.8 h for bags travelling through the pre-caecal segment and the entire equine digestive tract respectively. In studies carried out in pigs and ruminants, transit times of bags vary widely within and

between experiments, however differences in transit time do not appear to have an effect on estimates of digestion in the small intestine of these animals (Hvelplund, 1985; Graham *et al.*, 1985). This is in contrast to the work described here where site of recovery and consequently transit time of bags had a significant effect on DMD, STCD and CPD. The hindgut of the horse plays an important role in the degradation of feed and may well contribute more to the digestive process than the hindgut of pigs or ruminants.

In Experiment 5, degradation profiles were constructed and effective degradabilities were calculated for the different feeds using models originally developed for use in ruminant studies. Hyslop *et al.* (1998) were also able to use ruminant models to construct degradation profiles for fibrous feeds which had been incubated in mobile bags. In the current study, only three feeds (UB, MB and EB) had degradation profiles constructed from both *in situ* and mobile bag data so meaningful statistical comparisons are not possible. However, it is encouraging to note that the calculated effective degradabilities for these feeds were similar for both methods and further work in equines is needed, (where suitable conditions for post-incubation washing of bags have been determined), using a wide range of feedstuffs to establish if such relationships are the norm. If so, then the potential exists for degradation profiles to be constructed for different feeds without the need for fistulated animals.

Tables 6.4.2 summarise the effect of processing on degradation of barley, maize and peas in the digestive tract of ponies. In the main, micronising increased DM and starch degradation in the pre-caecal segment of the digestive tract but decreased CPD of both maize and peas compared to their respective unprocessed counterparts. Extrusion also increased STCD for all three feeds but generally decreased CPD with the exception of peas.

Table 6.4.2 Effect of processing on the degradation of barley, maize and peas in the precaecal segment (PC) and total digestive tract (TT) of ponies

	Unprocessed		Micronised		Extrusion	
	PC[1]	TT[2]	PC	TT	PC	TT
Dry matter (g/kg)						
Barley	817	900	844	906	826	898
Maize	833	905	907	922	811	921
Peas	817	947	874	955	835	932
Starch (g/kg)						
Barley	977	1000	990	1000	983	1000
Maize	941	990	992	1000	962	976
Peas	967	997	998	998	977	997
Crude Protein (g/kg)						
Barley	899	954	900	951	888	946
Maize	858	793	816	845	603	834
Peas	976	967	969	974	978	957

[1]PC = Precaecal segment of the digestive tract
[2]TT = whole of the digestive tract

The effects of processing noted in these studies were consistent with those in the previous *in situ* studies with micronising having a greater effect on nutrient degradation than extrusion. As discussed in section 4.4.3, the process of micronisation differs from that of extrusion. When grains are micronised, the starch granules are gelatinised by a hydrothermal process whereas extrusion employs both mechanical forces and gelatinisation to disrupt the starch granule. This study would suggest that the different processing methods lead to differences in degradation parameters.

The crude protein component of both barley and maize was adversely affected with a reduction in pre-caecal degradation compared to their unprocessed counterparts. Heat treatment of cereal grains can result in several types of reactions which can reduce the digestibility of protein. These include Maillard reactions as well as the formation of iso-peptide bonds (Voragen *et al.*, 1995). Maillard reactions take place between reducing sugars and free amino acids resulting in a reduction of digestibility of

proteins through cross-linking reactions. It may well be that the more intensive process of extrusion results in more Maillard reactions taking place and consequently crude protein degradability is reduced pre-caecally. However, it would appear that the products of such reactions are fermented in the hindgut of ponies as total tract degradability was increased for extruded feeds compared to their unprocessed counterparts.

6.5 Conclusion

The mobile bag technique has considerable potential in the field of equine nutrition, and if developed further, may: a) enable the prediction of *in vivo* apparent digestibilities of feeds in different segments of the digestive tract without the need for fistulated animals and b) allow for the rapid, simultaneous comparison of a large number of samples.

Areas that need to be further developed are; the appropriate particle size of feeds to be incubated in mobile bags; correlation between disappearances from bags and *in vivo* apparent digestibilities; the effect of seasonality on transit time of bags; appropriate models to calculate effective degradabilities in different segments of the equine digestive tract.

To summarise

1. The mobile bag technique can be used to determine degradability of different feedstuffs in different segments of the equine digestive tract.
2. Degradabilities can be determined for more than one feed at one time offering the opportunity for rapid, simultaneous comparisons of a large number of feeds
3. Certain procedural aspects require further investigation before the mobile bag technique can become a standard technique in equine nutrition research

Chapter 7 General Discussion

The modern domesticated horse consumes a variety of feeds which typically include fresh or conserved forage and varying amounts of cereal grains which contain between 470 and 730g starch/kg DM. It is known that feeding too much starch can cause hindgut dysfunction resulting in a number of disorders such as acidosis, gastric ulceration, colic and laminitis (Garner *et al.*, 1977; Carroll *et al.*, 1987; Clarke *et al.*, 1990; Rowe *et al.*, 1994). For horses which receive a large proportion of their daily feed as cereals the need to prevent such hindgut dysfunction is paramount for the horse's welfare. It has been suggested that processing cereal grains disrupts the starch within the grain resulting in an increase in starch susceptibility to both enzymatic hydrolysis and microbial fermentation. Several studies have shown that processing cereal grains for equines has little effect on total tract apparent digestibility in equines (Householder *et al.*, 1977; Arnold *et al.*, 1981; Hinkle *et al.*, 1983) however few have investigated the effect of processing on either the site of starch digestion or the hindgut environment. The work of this thesis was therefore to investigate the effects of physical processing of starch-based feedstuffs on their site of degradation and on the hindgut environment of ponies. The research was divided into two broad areas, the effects of physical processing of grains on starch digestion in the equine digestive tract and the development of *in sacco* methodologies to enable degradation parameters of processed grains to be determined in different segments of the equine digestive tract.

7.1 Physical processing of starch-based feedstuffs

7.1.1 Hindgut environment

Several studies have investigated the effect of processing on the digestibility of starch in the small intestine of equines and have noted substantial increases (Householder *et al.*, 1977; Meyer *et al.*, 1993; Meyer *et al.*, 1995). Although the effect of processing

barley on starch digestibility has not been investigated, Meyer *et al.* (1995, 1993) noted that starch digestibility of unprocessed barley in the equine small intestine was approximately 22%. This is in contrast to the work of Arnold *et al.* (1981) who noted a much higher digestibility of starch in the equine small intestine but attributed this to being an artefact due to one pony. If the digestibility of barley starch in the small intestine of equines is as low as recorded by Meyer *et al.* (1993; 1995) then substantial quantities of starch will reach the hindgut and be fermented by the microbial population, resulting in low pH and high lactate levels.

Inclusion of rolled barley (RB) in the diet significantly reduced intra-caecal pH and acetate molar proportions whilst lactate concentration and propionate molar proportions were increased compared to a hay cubes (HC) diet when measured 5 h post feeding. In Experiment 1a intra-caecal lactate levels remained low in ponies fed the HC diet. When RB, but not EB or MB, was included in the diet, lactate levels were thirty-three fold greater when averaged over the 4-8h following the morning meal. However, lactate production was different between ponies. At 5 hrs post feeding lactate production reached 0.5mmol/l in pony 1 and by 6 hrs post feeding it had dropped to 016 mmol/l. Yet in pony 3 lactate production had reached 1.96 mmol/l by 4 h post-feeding, continued to rise and had reached 8.76 mmol/l by 8 h post-feeding. Pony 3 was almost certainly suffering from sub-clinical acidosis when on the RB diet, yet the remaining two ponies displayed no signs of acidosis when on the same diet. In a study carried out in America where laminitis was induced by carbohydrate overload, 16 % of the horses died, 68% developed acute laminitis whilst the remaining 16% demonstrated no clinical signs (Garner *et al.*, 1977). Both sets of results demonstrate that individual animals vary greatly in their response to a particular diet.

Both Potter *et al.* (1992) and Meyer *et al.* (1995) suggest that if the capacity of the small intestine for starch digestion is exceeded, then large changes in intra-caecal

fermentation parameters will occur. The main objective of processing cereal grains is to disrupt the granule structure resulting in an increase in enzymatic digestion of starch. If this is the case and starch becomes more readily degraded by mammalian enzymes, then in the horse, greater amounts of starch would be degraded in the small intestine and less would reach the hindgut. If less starch reaches the hindgut then fewer changes will occur and a more stable environment can be maintained. When micronised or extruded barley was included in the diets, changes in intra-caecal fermentation parameters were not as great as when rolled barley was included. This suggests that micronising or extrusion may increase the digestibility of starch in the equine small intestine.

Meyer *et al.* (1995) recommend that, to prevent caecal acidosis and hindgut dysfunction, starch intake per meal should not exceed 2 g/kg LW. In Experiment 1 starch intake was similar to this recommendation (2.1 g/kg LW per meal) yet significant, unfavourable, changes occurred in intra-caecal fermentation parameters when barley that had undergone minimal processing was included in the diet. Potter *et al.* (1992) suggested that starch digestibility in the small intestine of equids would be exceeded if more than 4.0 g/kg LW of starch was fed per meal. In light of the results of Experiment 1a, it would appear that the recommendations of Meyer *et al.* (1995) may well be too high to prevent hindgut dysfunction in equines when unprocessed starch sources or those that have undergone only minimal processing (such as rolling) are included in diets for equines and the recommendations of Potter *et al.* (1992) are certainly too high. However, the recommendations of Potter *et al.* (1992) may be appropriate for processed cereals. Blanket recommendations regarding starch intake are difficult to make as factors such as botanical source, extent of processing, processing method and an individual animal's susceptibility to cereals must be taken into account. However, as a rule of thumb, animals which are susceptible to digestive upsets as a result of high intakes of cereals should not be offered cereals which have only undergone minimal processing. Where cereals must

be included in their diets a processed form such as micronised should be used. For other animals starch intake should probably not exceed 2g/kg liveweight per meal when relatively unprocessed cereals are included in diets.

7.1.2 Site of digestion

To test the theory that digestibility of rolled barley in the equine small intestine was less than that of MB or EB a mobile bag study was conducted. In Experiment 5, the disappearance of DM, STC and CP in the pre-caecal (PC) segment of the equine digestive tract was investigated. MB had significantly ($P<0.05$) higher STCD than either UB or EB. This difference in STCD, although small (23 g/kg), may well account for the differences in intra-caecal fermentation parameters.

The effects of micronisation and extrusion on the degradation of maize and peas were also investigated using the mobile bag technique. Micronisation increased DMD of maize when measured in the pre-caecal segment of the equine digestive tract. STCD in the pre-caecal segment was also increased by both micronisation and extrusion for maize and peas. When DMD and STD were measured using mobile bags that had passed through the whole of the equine digestive tract there were no significant differences between unprocessed and processed feedstuffs for barley, maize or peas. This is in line with other published results (Householder *et al.*, 1977; Potter *et al.*, 1992) and with the total tract *in vivo* apparent digestibilities determined in Experiment 1a.

7.1.3 Processing and starch source

Although, in the main the effects of physical processing on DM, STC and CP degradation were similar for barley, peas and maize, the greatest effects were seen with maize and to a lesser extent barley. However, in light of the results from Experiment 1, it would be unwise to include maize or barley in diets for equines unless they had undergone some form of processing.

The extruded feedstuffs used in these studies were specifically created by a process using only water in the cereal mix. It is more common in extrusion for the cereals to be mixed with an oil (e.g. linseed) to lubricate the process. One effect of this is the formation of starch lipid complexes, which are relatively resistant to enzymatic hydrolysis, thus reducing digestibility of starch in the small intestine. As this study has already shown water-mix extrusion to have debatable benefits in terms of increasing nutrient digestibility in the small intestine of equines, the effects of oil-mix extrusion would be expected to be more pronounced, leading to a potentially less digestible product. The effects of extrusion on the digestibility of equine diets therefore require further study before the process can be unequivocally recommended.

Micronisation however is a relatively simple process that appears to maximise starch digestion in the small intestine of the equine. The process uses only cereal grains and water and so no starch-lipid complexes are formed. This study has shown, micronisation increases starch degradation in the small intestine of the equine. As such, where a processed cereal is to be included in an equine diet, it would be recommended that the process be micronisation.

Whereas barley and maize are significantly improved by processing, peas appear to be readily degradable in the small intestine of equines regardless of whether they have been processed or not. This suggests that peas offer great potential as a dietary ingredient for equines. Whether it would be best to feed them in a whole crop, fresh, dried or conserved form has yet to be determined.

7.2 In sacco Methodologies

Two *in sacco* methodologies were used in this thesis to determine degradation rates of starch based feeds in different segments of the equine digestive tract.

In Experiments 2 and 3 the effect of incubation sequence on DM degradation of feedstuffs was investigated. It has been shown in ruminant studies that incubation sequence has no effect (Huntington and Givens, 1997a) whereas in equine studies incubation sequence has been shown to have an effect on the degradation parameters of fibre based feedstuffs (Hyslop *et al.*, 1996). The effect of incubation sequence on the starch-based feedstuffs was minimal and calculated ED were similar for both sequences, however for DHG there were significant differences ($P<0.05$) between the calculated ED for the two sequences. Therefore when conducting in situ experiments, the effect of incubation sequence must be taken into account for forage feeds. It is however less important for cereals.

The mobile bag technique has been used in equines to investigate the degradability of fibre based feeds in the pre-caecal segment of the equine digestive tract (Macheboeuf *et al.*, 1995; Moore-Colyer *et al.*, 1997a; Moore-Colyer *et al.*, 1997b; Hyslop *et al.*, 1998) and through the whole digestive tract (Araujo *et al.*, 1996a; Araujo *et al.*, 1996b). Degradation profiles of DM and NDF have been constructed by Hyslop *et al.* (1998) from DM and NDF losses from mobile bags that have passed through the whole of the equine digestive tract. In Experiment 4 degradation profiles were constructed for purified starch sources that had been incubated in the pre-caecal segment of the equine digestive tract using mobile bags. Following on from this, Experiment 5 set out to construct degradation profiles for UB, MB and EB in the pre-caecal segment and through the whole of the digestive tract. Due to the small amounts of residues left in bags it was only possible to construct pre-caecal degradation profiles for DM and STC and total tract profiles for DM. However, differences in DM ED were detected, UB had significantly lower ($P<0.05$) ED than MB.

Effective degradabilities of UB, MB and EB were lower when calculated by incubation of *in situ* bags in the caecum of ponies than when calculated by mobile bag

residues. Feedstuffs had been ground to pass through a 1.0mm screen in the *in situ* experiments but ground to pass through a 0.5mm screen for the mobile bag experiments. Particle size has been shown to have a large influence on *in sacco* techniques (Michalet-Doreau and Cerneau, 1991). In experiment 6 it was not possible to construct degradation profiles for any of the six feedstuffs, as particle losses were high. Such large losses may have had more to do with particle size than with degradability within the digestive tract as DM disappearance of UM, MM and EM were much lower for *in situ* bags than for PC mobile bags. There is still some debate as to what is the optimum particle size for *in sacco* studies. Nocek (1988a) recommends the use of a 5.0mm screen for concentrates whereas the AFRC (1992) recommended that concentrates are ground through a 2.0mm screen. In equines it has been suggested that feed particles must be less than 1.6 mm before they can be swallowed (Frape, 1998). However, anecdotal evidence would suggest that particles larger than this can pass through the digestive tract as whole grains are frequently found in the faeces of equines. Particle size analysis of equine faecal material would give some idea as to digesta particle size. If *in sacco* techniques are to be used routinely in equine studies then further research is needed to standardise the methodology.

Transit time of bags through the digestive tract differed between experiments. The transit time of bags through the small intestine averaged 2.3 h, 3.6 h and 5.1 h for Experiments 4, 5 and 6 respectively. Time for bags to travel from the stomach to the faeces was on average 40 h and 44 h for Experiments 5 and 6 respectively. The average transit time of faecal bags in Experiment 6 was similar to the average MRT of 44.5 h for diets measured in Experiment 1b. The MRT of the diets in Experiment 1b were 46.1, 42.3, 46.9 and 43.0 h for HC, RB, MB and EB respectively. MRT of HC had been determined in an earlier experiment and recorded as 30 h (Moore-Colyer, pers. comm.). In both MRT experiments the same ponies, same management regime and same batch of HC were used. The only difference between experimental set up

was time of year. In Experiment 1b the trial was carried out between October and December whereas the earlier trial of Moore-Colyer took place between April and June. Mobile bag studies took place from April through to December, in particular Experiment 6 took placed during October through to December. Such a large difference in MRT between the two experiments (16 h approximately) suggest that time of year may well have an effect on digesta passage rate. If this is indeed the case, this has implications for both digestibility studies and mobile bag studies. Digestibility is positively correlated with digesta passage whereas intake is negatively correlated with digestibility. As MRT increases, digestibility of feedstuffs tends to increase. With an increase in digestibility, feed intake tends to decrease in equines. Thus, if passage rate increases during the winter months, intakes of feedstuffs may be less than during the summer months. This would make biological senses as, in the wild, more food is available during the summer months and equines will increase their intake in order to lay down fat reserves for the winter. Different studies carried out at different times of the year may not, therefore be strictly comparable. There is however, no information on seasonality and its effects on digestibility or digesta rates of passage in equines, and it is therefore an important area for future study if the MBT is to be used as a routine method for determining the nutritive value of feedstuffs for horses.

In Experiment 1b different compartmental models were applied to faecal excretion curves. Despite the small number of ponies used and the large variation between them, it was possible to determine, mathematically, two compartments within the equine digestive tract. A two compartment time dependent model, incorporating a Gamma 4 function (G4G1 model) gave the best statistical fit for faecal excretion curves for markers which had passed through the whole digestive tract whereas a two compartment time independent model, incorporating a Gamma 1 function (G1G1 model) gave the best fit to marker data representing the post-ileal segment of the equine digestive tract. As well as fitting the data mathematically, both models are

relevant biologically. The G4G1 model assumes an element of time dependency in the digestive tract and is normally attributed to the tubular segments of the digestive tract. Where the tubular segment of the digestive tract precedes the fermentation chamber, the non-mixing nature of the tubular segment will result in a time delay of digesta moving into the next section of the digestive tract (Pond *et al.*, 1994). A G1G1 model assumes that passage through the tract is time independent and that first order kinetics apply. Such models are assumed to apply when the fermentation chamber precedes the tubular segment. In the caecum of ponies, outflow rate was determined as being represented by first order kinetics. More animals and diets would be required in future work to add to and confirm these findings more precisely.

7.3 Conclusions

- Where horses receive a considerable portion of their diet as starch, then changes in hindgut fermentation parameters resulting in lower pH and increased lactate concentrations may be a particular problem.

- To minimise these problems cereal grains which have been processed should be included in the diet rather than those which have just been rolled.

- Peas may be a suitable substitute where processed cereal grains are not available.

- Starch intake per meal should be kept well below 2 g/kg LW.

- Incubation sequence has an effect on *in situ* degradation parameters when feedstuffs are incubated in bags in the caeca of ponies, however this effect is greater with slowly degradable feeds (fibre based feeds) than rapidly degraded feeds (starch based feeds)

- Correlations between mobile bag degradation of feedstuffs and *in vivo* apparent digestibilities need to be establish before the mobile bag technique can be adopted as a routine methodology for determining differences in degradation rates between feedstuffs.

- For effective degradabilities calculated in different segments of the digestive tract to be valid, appropriate models and estimates of digesta passage for each segment of the digestive tract must be established.

- Development of *in sacco* techniques should allow the digestion of feedstuff in the equine digestive tract to be partitioned, thereby enabling the identification of processing methods which minimise alterations in intra-caecal fermentation parameters in equines.

7.4 Future Work

A major question that this work has thrown up is why are some animals susceptible to high levels of cereals in their diets and others not? One reason may be that susceptible animals do not produce enough amylolytic enzymes in the small intestine to hydrolyse cereal starch. Analysis of the jejunal chyme of susceptible horses for amylolytic enzymes could answer this question. If a lack of amylolytic enzymes is the answer this then throws up the next question: is it genetic? Are some breeds of equines or some families more susceptible? An inability to digest soluble carbohydrates has been implicated in the aetiology of laminitis. An epidemiological study of laminitis cases may help to identify if some animals have a genetic pre-disposition to develop the condition.

Processing does alter the degradation of cereals in the equine digestive tract, however different processing methods do this to a greater of lesser extent. Intensity of processing requires further investigation and further work with extruded products may

help to answer these questions. Extrusion of cereals can result in a lowering of nutrient digestibility . It can also increase digestibility of nutrients. Other workers have highlighted temperature, moisture content and screw speed as being the most important variables in the extrusion process. Changing any one of these variables may result in a product that is more or less digestible. These variables need to be investigated further to determine what the optimum extrusion conditions are to improve the digestibility of cereals in the small intestine of equids.

Whilst recommendations as to the maximum starch intake per meal for equines have been suggested, they are not universal. Further studies with processed cereals, in particular micronised cereals, are required to determine how much can be included in diets for equines before detrimental changes occur in the hindgut environment.

The potential of peas as a dietary ingredient requires further study to determine their optimum feeding strategy – i.e. whole crop, fresh, dried and conserved peas. The different presentations may have different digestibility parameters.

In sacco methods have great potential in the field of equine nutrition. However, many of the models used are taken straight from ruminant studies. Yet equines and ruminants differ in their basic digestive physiology. Models must be determined which reflect the equid's physiology rather than the ruminant's. If *in situ* studies are to be carried out in the caecum of equids to determine digestibility then one area that requires investigation is that of simulated gastric digestion of feedstuff before incubation in the caecum.

Further studies are also required to standardise the methodologies for equine studies. Areas requiring further research include: optimum particle size of feedstuff; the effect of post incubation treatment (i.e. hand washing *vs.* machine washing of bags) and the effect of transit time on nutrient losses from bags.

To determine effective degradabilities using *in sacco* techniques passage rates through the digestive tract must be determined. Therefore further studies are required to determine effects of season on passage rate; to determine passage rates for different feedstuffs and to determine the applicability of compartmental models to faecal excretion curves.

A major drawback to both *in situ* and mobile bag methodologies is the need to combine residues from bags to determine nutrient degradability other than dry matter. Analytical methods need to be investigated that remove the need for wet chemistry techniques, such as NIR. Analysis of residues from individual bags would allow greater prediction of degradation parameters using degradation models. It would also reduce the number of bags required per feedstuff, allowing a more rapid determination of degradation.

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Appendix 1

Chemical composition of mineral and vitamin supplement added to diets of the ponies (60g per pony per day)

Nutrient	Quantity
Calcium	160 g/kg
Phosphorus	117 g/kg
Magnesium	67 g/kg
Sodium	67 g/kg
Copper	683 mg/kg
Zinc	2730 mg/kg
Iron	2730 mg/kg
Manganese	2730 mg/kg
Iodine	6.7 mg/kg
Cobalt	6.7 mg/kg
Vitamin A	136670 IU
Vitamin D_3	20500 IU
Vitamin E	3417 IU

Appendix 2

Repeated measures analysis of variance with ante-dependence order of 1 (using previous value as a covariate)

Table A2.1 Intra-caecal changes in pH following the 09:00 hour meal in ponies offered hay cubes (HC), rolled barley: hay cubes (RB:HC), micronised barley: hay cubes (MB:HC) or extruded barley: hay cubes (EB:HC)

h post feeding	HC	RB:HC	MB:HC	EB:HC	sed	sig
1	6.86	6.79	6.81	6.83	0.1114	NS
2	6.61	6.66	6.58	6.67	0.1421	NS
3	6.56	6.36	6.43	6.46	0.0575	NS
4	6.48	6.26	6.34	6.35	0.1693	NS
5	6.38	6.34	6.38	6.36	0.1126	NS
6	6.46	6.26	6.37	6.41	0.1044	NS
7	6.41	6.46	6.45	6.27	0.2095	NS
8	6.52	6.38	6.35	6.37	0.1203	NS
Mean	6.52	6.43	6.46	6.51		

Table A2.2 Intra-caecal changes in lactate (mmol/l) following the 09:00 hour meal in ponies offered hay cubes (HC), rolled barley: hay cubes (RB:HC), micronised barley: hay cubes (MB:HC) or extruded barley: hay cubes (EB:HC)

h post feeding	HC	RB	MB	EB	sed	sig
1	0.104	0.660	0.130	0.095	0.0442	NS
2	0.183	0.065	0.118	0.105	0.0323	NS
3	*	0.603	0.249	0.389	0.4155	NS
4	0.036	1.206	0.377	0.202	0.3426	NS
5	0.505	0.614	0.532	0.428	0.2336	NS
6	0.424	*	0.434	0.494	0.2525	NS
7	0.641	1.216	1.067	0.760	0.4680	NS
Mean	0.488	0.537	0.453	0.498		

Table A2.3 Intra-caecal changes in acetate (mmol/mol) following the 09:00 hour meal in ponies offered hay cubes (HC), rolled barley: hay cubes (RB:HC), micronised barley: hay cubes (MB:HC) or extruded barley: hay cubes (EB:HC)

h post feeding	HC	RB	MB	EB	sed	sig
1	19.94	14.86	31.52	18.08	3.721	NS
2	20.73	29.75	27.52	29.85	5.370	NS
3	31.28	30.54	37.72	28.92	9.480	NS
4	34.05	34.90	35.93	34.14	4.960	NS
5	41.02	24.49	32.45	30.62	5.700	NS
6	30.80	35.96	35.64	26.58	7.550	NS
7	26.75	22.79	30.90	40.92	11.510	NS
8	39.54	35.24	25.36	24.82	5.720	NS
Mean	32.39	30.16	31.57	27.06		

Table A2.4 Intra-caecal changes in propionate (mmol/mol) following the 09:00 hour meal in ponies offered hay cubes (HC), rolled barley: hay cubes (RB:HC), micronised barley: hay cubes (MB:HC) or extruded barley: hay cubes (EB:HC)

H post feeding	HC	RB	MB	EB	sed	sig
1	2.56	1.73	5.97	5.10	3.282	0.05
2	4.46	7.361	3.79	4.28	2.038	NS
3	4.84	7.48	7.13	6.04	1.930	NS
4	7.80	13.05	5.10	4.39	4.381	NS
5	8.60	9.96	5.70	5.70	3.157	NS
6	6.95	18.06	2.87	2.79	4.675	NS
7	5.96	16.39	2.42	3.92	9.679	NS
8	6.53	25.93	*	*	6.7129	0.03
Mean	7.35	14.80	1.49	2.30		NS

Table A2.5 Intra-caecal changes in butyrate (mmol/mol) following the 09:00 hour meal in ponies offered hay cubes (HC), rolled barley: hay cubes (RB:HC), micronised barley: hay cubes (MB:HC) or extruded barley: hay cubes (EB:HC)

h post feeding	HC	RB	MB	EB	sed	sig
1	3.12	3.32	5.82	3.93	3.706	NS
2	3.25	3.77	5.91	6.96	0.881	0.04
3	5.11	5.34	7.77	6.27	7.582	NS
4	5.13	5.20	9.06	10.64	1.260	0.02
5	7.02	6.97	5.84	7.56	1.855	0.02
6	8.11	8.63	6.26	2.60	1.679	0.02
7	6.16	6.18	4.31	9.89	1.970	
8	7.73	6.10	5.73	7.41	0.367	
Mean	5.02	4.91	7.92	6.79		

Table A2.6 Intra-caecal changes in total volatile fatty acids (mmol/l) following the 09:00 hour meal in ponies offered hay cubes (HC), rolled barley: hay cubes (RB:HC), micronised barley: hay cubes (MB:HC) or extruded barley: hay cubes (EB:HC)

h post feeding	HC	RB	MB	EB	sed	sig
1	20.68	20.75	46.15	28.37	3.648	0.053
2	30.06	40.29	36.64	38.10	8.790	NS
3	41.64	45.69	50.38	41.34	13.620	NS
4	47.08	52.46	49.91	50.02	8.070	NS
5	54.49	46.26	44.60	45.58	7.390	NS
6	39.19	57.40	48.18	39.67	7.76	NS
7	68.40	36.62	42.68	61.05	12.80	NS
8	58.44	43.35	46.23	40.09	2.363	NS
Mean	45.26	43.76	44.34	39.06		NS

Appendix 3

Mathematical equations for compartmental models

Model 4 Grovum and Williams (1973)

$Y = A(e^{-k1(t-TD)} - e^{-k2(t-TD)})$ when $t>TD$; $Y = 0$, $t \leq TD$

Where A is the scale parameter (related to marker dose); k_1 and k_2 are rates of passage (per hour) in two separate mixing compartments and TD is time delay in the tubular portions of the gastrointestinal tract.

$TMRT = MRT_1 + MRT_2 + TD$ Where $MRT_1 = 1/k_1$ and $MRT_2 = 1/k_2$

Model 5 Dhanoa *et al.* (1985)

$Y = Ae^{-k1t}\exp[-(N-2)e^{-(k2-k1)t}]$

Where A is a scale parameter dependent on k_1, k_2 and N; k_1 and k_2 are rate constants (per hour) representing the two main compartments having the longest MRT; N represents the number of compartments in the model. The time delay (TD) is the summation of the true delays between anatomical compartments and MRT in the (N-2) minor mixing compartments.

$TD = \Sigma(1/[k_2 + (i-2)(k_2 - k_1)]$ when $k_1 > k_2$

$TMRT = MRT_1 + MRT_2 + TD$ where $MRT_1 = 1/k_1$ and $MRT^2 = 1/k_2$

Models 6 - 9 Pond *et al.* (1988)

$Y = Y_0 [\delta^n e^{-k2t-TD} - e^{-\lambda 1t} \Sigma \delta t\text{-}TD(\lambda 1t)^{n-}i/(n-i)!]$

Where Y0 is the initial marker concentration in the first mixing compartment; k_2 is a time independent passage rate constant; λ_1 is time dependent passage rate constant; TD is time delay through the tubular segments; $\delta = \lambda_1(\lambda_1 - k_2)$ and n= order of gamma distribution

$TMRT = MRT_1 + MRT_2 + TD$ where $MRT_1 = n/\lambda$ and $MRT_2 = 1/k_2$

Appendix 4

Publications

Published papers:-

McLean, B.M.L., Hyslop, J.J., Longland, A.C. Cuddeford, D., Hollands, T.. 2000 Physical processing of barley and its effect on intra-caecal fermentation parameters and *in vivo* apparent digestibility in ponies. *Animal Feed Science & Technology* **85** p79 - 87

Hyslop, J.J., Stefansdottir, G.J., McLean, B.M.L., Longland, A.C., Cuddeford, D.. 1999 *In situ* incubation sequence and its effect on degradation of feed components when measured in the caecum. *Animal Science* **69** p147 - 156

Papers presented at conferences:-

McLean, B.M.L., Hyslop, J.J., Longland, A.C., Cuddeford, D.. 1998 Physical processing of barley and its effects on intra-caecal pH and volatile fatty acid parameters in ponies offered barley based diets. *Proceedings of the 2nd Annual Meeting of the European Society of Veterinary and Comparative Nutrition, Vienna, Austria.*

Posters presented at conferences:-

McLean, B.M.L., Hyslop, J.J., Longland, A.C. Cuddeford, D., Hollands, T.. 1999 *In vivo* apparent digestibility in ponies given rolled, micronised or extruded barley. *Proceedings of the British Society of Animal Science Winter meeting.*

McLean, B.M.L., Hyslop, J.J., Longland, A.C. Cuddeford, D., Hollands, T.. 1999 Effect of screen diameter on particle size and water holding capacity of 15 starch based equine feedstuffs ground through a 1.0mm or 0.5mm screen. *Proceedings of the British Society of Animal Science Winter meeting.*

McLean, B.M.L., Hyslop, J.J., Longland, A.C. Cuddeford, D., Hollands, T.. 1999 *In situ* degradation of crude protein in physically processed barley, maize and peas in the caecum of ponies. *Proceedings of the British Society of Animal Science Winter meeting.*

McLean, B.M.L., Hyslop, J.J., Longland, A.C. Cuddeford, D., Hollands, T.. 1999 Development of the mobile bag technique to determine the degradation kinetics of purified starch sources in the pre-caecal segment of the equine digestive tract. *Proceedings of the British Society of Animal Science Winter meeting.*

McLean, B.M.L., Hyslop, J.J., Longland, A.C. Cuddeford, D., Hollands, T.. 1999 Gas production *in vitro* from purified starches using equine faeces as the source of inocula. *Proceedings of the British Society of Animal Science Winter meeting.*

McLean, B.M.L., Hyslop, J.J., Longland, A.C. Cuddeford, D., Hollands, T.. 1999 Effect of physical processing on *in situ* degradation of maize and peas in the caecum of ponies. *Proceedings of the British Society of Animal Science Winter meeting.*

McLean, B.M.L., Hyslop, J.J., Longland, A.C. Cuddeford, D., Hollands, T.. 1999 Gas production *in vitro* from either unprocessed, micronised or extruded maize, peas, wheat, naked oats and barley using equine faeces as the source of inocula. *Proceedings of the British Society of Animal Science Winter meeting.*

Hyslop, J.J.,, McLean, B.M.L., Moore-Colyer, M.J.S., Longland, A.C., Cuddeford, D., Hollands, T.. 1999 Measurement of caecal outflow rate in ponies using Chromium mordanted feeds. *Proceedings of the British Society of Animal Science Winter meeting.*

McLean, B.M.L., Hyslop, J.J., Longland, A.C., Cuddeford, D.. 1998 Effect of physical processing on *in situ* degradation of barley in the caecum of ponies. *Proceedings of the British Society of Animal Science Winter meeting.*

Workshop proceedings:-

McLean, B.M.L., Hyslop, J.J., Longland, A.C., Cuddeford, D.. 1998 Partition of starch digestion in the horse. *Proceedings of the Horserace Betting Levy Board Veterinary Advisory Committee's Equine Nutrition Workshop, London, UK*