

# PARTITIONING DIGESTION IN HORSES AND PONIES

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## Introduction

The only certain way to partition digestion between different parts of the digestive tract is to cannulate each compartment and then measure the exact rate of degradation of a feed therein. The late Frank Alexander used cannulated ponies (Alexander and Donald, 1949) to determine the digestive processes operating within the large intestine and revived interest in the digestive physiology of horses. An alternative approach to cannulation was to slaughter animals and then tie off segments of the gut (Hintz et al., 1971). However, this drastic approach has limited value in terms of understanding the dynamics of the digestive process. Ileal cannulation, together with the use of chromic oxide as a marker, was the method employed to examine digestion of starch in the small and large intestine during the late 1970s and 1980s (Potter et al., 1992). Also cannulation of the terminal jejunum of horses was undertaken to determine preileal starch digestion of whole, rolled or ground oats and maize (Kienzle et al., 1992). More recently, both cecal and colonic cannulations have been performed in the same animal (Drogoul et al., 1995) to simultaneously assess feed degradation in different compartments of the horse's large intestine. Easy access to the rumen of both sheep and cattle has allowed ruminant nutritionists to characterize the digestive process in these animals in considerable detail.

The relative ease of cannulating ruminants and the durability of the animal model have enabled research groups all over the world to develop and refine techniques that allow significant advances to be made in terms of feed evaluation and in defining animal requirements. The relative paucity of equine experimental models and the apparent reluctance to transfer ruminant methodologies to the equine are in part responsible for the lack of progress in the field of equine nutrition. Only recently have techniques that have been developed in pigs, cattle and sheep been adapted for use with equids. These fundamental techniques are the *in situ* incubation method (Mehrez and Orskov, 1977), the mobile nylon bag method (Sauer et al., 1983) and the use of markers, singly and in combination.

## In Situ Methods

These methods were developed to measure the degradation of feeds that were incubated in the rumen. Mehrez and Orskov (1977) evaluated the artificial fiber bag technique for assessing the proportions of dietary dry matter and nitrogen which "disappeared" in the rumen. They found that the technique was satisfactory as a simple and rapid guide for measuring nutrient disappearance from Dacron bags containing feeds that were suspended in the rumen. These original bags were

hand-sewn, using material from old, discarded parachutes. This method was developed by Orskov and McDonald (1979) in order to estimate protein degradability in the rumen. Importantly, they weighted the incubation measurements according to rate of passage. This was achieved by conducting a separate experiment in which the rate of passage of the protein source was measured using a marker, chromium. Potential degradability, 'p', was related to incubation time, 't', by the following equation:

$$p = a + b(1 - e^{-ct}) \quad (1)$$

where 'a' is the rapidly degradable (soluble) fraction, 'b' is the slowly degraded fraction, and 'c' is the rate at which 'b' is degraded. Orskov and McDonald (1979) showed that, by measuring the rate of passage from the rumen to the abomasum with chromium, a rate constant, 'k', could be derived. Thus, allowing for rate of passage, the percentage degradation, 'P', was shown to be:

$$P = a + [bc/(c+k)](1 - e^{-(c+k)t}) \quad (2)$$

by time, 't', after feeding. As t increases, this tends to the asymptotic value  $a + bc/(c+k)$ , providing an estimate of degradability under the prevailing feeding conditions. Equation 1 provides an empirical fit to incubation data and a, b and c are constants that can be fitted by an iterative, least-squares procedure. There are two important underlying assumptions to the above and these are:

1. that compartment volume remains constant;
2. that rate of passage of untreated food particles is the same as that of chromium-treated particles.

While comminution during ingestion and rumination will have the same effect on treated or untreated particles, chromium-mordanting renders particles completely indigestible and thus they will not be fragmented by microbial action.

The *in situ* technique has been modified for use in the horse cecum as described by a number of authors (Applegate and Hershberger, 1969; Miraglia et al., 1988; Drogoul et al., 1995). While the different procedural aspects that influence the results obtained using this technique in ruminants have been extensively studied and were recently reviewed by Huntington and Givens (1995), these aspects have not been fully evaluated in equids. However, Hyslop et al. (1999a) have recently reported a methodology based on the use of 6.5 x 20 cm, *in situ* bags, containing 16mg of feed per cm<sup>2</sup>, incubated in the ceca of mature pony geldings (mean weight 285 kg). A complete exchange method (Paine et al., 1982) was used to evaluate two different incubation sequences, forward (3, 5, 16, 8, 24, 48h) and reverse (48, 24, 8, 16, 5, 3h). Degradation profiles were shown to be sensitive to incubation sequence in contrast to the findings of Huntington and Givens (1995), who showed no effect of incubation sequence on the degradability of hay, soya or fishmeal in cattle and sheep.

Digesta reaching the cecum of the horse may be more homogeneous than that entering the rumen because it will have been thoroughly chewed and exposed to the digestive influences of both the stomach and small intestine. However, the small size of the cecal pool means that variation within it, due to outflow to the ventral colon and inflow from the terminal ileum, could account for some of the sensitivity measured by Hyslop et al. (1999a). The equine cecum accounts for only about 16% of the total gastrointestinal tract in contrast to the rumen, which may represent up to 70% of the total tract (Argenzio, 1993). Additionally, digesta outflow from the cecum has been measured at 20% per hour ( $k = 0.20$ ) (Hintz, 1990) which is much faster than that measured from the rumen; the latter has been assessed at between 2 and 8% per hour ( $k = 0.02$  to  $0.08$ ) (Agricultural and Food Research Council, 1992). Rapid outflow from the cecum may lead to a less stable microbial environment. Furthermore, the microbial ecosystem within the cecum has to cope with two (Goodson et al., 1988) to fourfold (Argenzio et al., 1974) changes in cecal volume which may arise from changing the nature of the diet or in relation to the time of feeding. Clearly, the foregoing factors could have a major impact on gut activity, digesta mixing, microbial populations and their activity, thereby affecting the *in situ* degradation of foodstuffs.

### Mobile Nylon Bag Methods

The concept of enclosing feed in a container and then allowing the container to pass through the digestive tract is not new. In 1782, Spallanzani enclosed bread and meat in linen bags and then swallowed them; the bags were recovered in the feces within 24 h and the contents had “disappeared” (cited by Sauer et al., 1983). Petry and Handlos (1978) orally administered small nylon bags containing feed to pigs, but unfortunately they were retained in the stomach. This problem of gastric retention was overcome by Sauer et al. (1983) who inserted 25 x 40 mm monofilament nylon bags (50  $\mu\text{m}$  pores) through a cannula directly into the duodenum. These authors concluded that the technique could be used for the rapid determination of protein digestibility. Hvelplund (1985) used 60 x 60 mm polyamide bags (9 and 22  $\mu\text{m}$  pores) and introduced them via a cannula into the duodenum of dairy cows. Some bags were recovered from an ileal cannula and the rest from the feces. Hvelplund (1985) concluded that the technique showed promise as a tool to predict digestibility in the small intestine of ruminants. Independently, Macheboeuf et al. (1996) and Hyslop and Cuddeford (1996) used mobile nylon bags to study nutrient disappearance throughout the digestive tract of horses and ponies respectively. The latter authors tested a range of bag sizes and showed that large bag sizes (19 x 110 or 19 x 55 or 45 x 45 mm) resulted in transit times in excess of 100 h and as a result, there were large feed constituent disappearances. Bags which gave transit times and feed disappearances in accordance with expectation measured 10 x 60 mm; the pore size was 41  $\mu\text{m}$ . This was the size of bag used by Macheboeuf et al. (1996), Moore-Colyer et al. (1997), Hyslop et al. (1998) and McLean et al. (1999). Moore-Colyer et al. (1997) used the mobile nylon bag technique to partition fiber

degradation in the digestive tract of cecally fistulated ponies. Bags were filled with 350 mg of a dietary fiber source (unmolassed sugar beet pulp or hay cubes or soya hulls or an oat hull:naked oat mixture-50:50) ground to pass through a 1mm screen. On two consecutive mornings, 20 bags were administered directly into the stomach of each pony using a nasogastric tube. A magnetic capture device was placed in the cannula just posterior to the ileo-cecal valve. Each mobile bag contained 2 x 100 mg steel washers so that as the bags entered the cecum, they were captured. Ten to 16 bags were captured in this manner and the balance allowed to continue through the gut and were collected in the feces. Bags that were recovered entering the cecum, 1 to 8 h after dosing, provided data on disappearance following a range of incubation times in the prececal part of the gastrointestinal tract. Degradation profiles were fitted to the DM losses from the mobile bags using the same models that were applied to *in situ* disappearances (Orskov and McDonald, 1979; Dhanoa, 1985). This experiment also allowed the calculation of both prececal and total tract losses of non-starch polysaccharide (NSP).

The results of this study contradicted the widely held view that dietary fiber is only degraded in the large intestine of the horse. Prececal losses of NSP were 84, 111, 127 and 164 g/kg of the total tract NSP disappearance for the mixture of oat products, hay cubes, soya hulls and beet pulp. Allowing mobile nylon bags to pass through the entire length of the digestive tract of the horse and then recovering them from the feces over an extended period of time yields data that reflect a range of incubation times in the whole tract (Hyslop et al., 1998). Degradation profiles can be fitted to the losses from these bags using the same models as before.

### **Marker Methods**

Continuously dosed markers can be used to measure digesta flow while the residence time of food particles in the tract from a discrete meal can be estimated by using a “pulse dose” of marker. Owens and Hanson (1992) have reviewed the use of markers for determining the site and extent of digestion in ruminants; no comparable information is available for equids.

External markers are preferred because they will be unique to the “pulse dose;” they should remain associated with the undigested nutrient of interest and the flow and digestion of marked fragments should be the same as that for unmarked fragments. Rare earths such as ytterbium, samarium, lanthanum and europium are alternatives to the historically popular chromium. While they are appropriate flow markers for ruminants, rare earths can be displaced from their foodstuff-binding sites by protons at low pH. This displacement is of little consequence in ruminant studies because ruminal digesta is the primary source of variation in the flow of particles; any effect of passage of rare earth-treated foods through the stomach of the horse on marker displacement is unknown. Bertone et al. (1989) assumed that a high proportion of ytterbium would be unbound in the stomach of the horse. However, they considered that this was not a “big concern”

(*sic*) because, provided the marker was delivered to the cecum, it would bind to particulate matter with high affinity at the higher cecal pH.

Internal markers are ideal flow markers but since they are not unique to a given meal, flux and compartmental mass must be independently determined. Acid-detergent lignin (ADL), indigestible neutral detergent fiber (INDF) and alkanes are examples of internal markers that have been used in different species.

Markers can be dosed *per os*, directly into the stomach, using a stomach/nasogastric tube or via a cannula, which can be located in any of the different segments of the gastrointestinal tract. Liquid and solid phase markers may be administered together *per os* in whole animals in order to measure digesta passage rates; feeds can be marked using the methods of Uden et al. (1980). The major advantage of this approach is that there is no need to surgically interfere with animals. Following a pulse dose of marked feed, fecal output must be sampled over an extended period of time (about 100 h). Subsequent drying of fecal samples and determination of marker concentration yields a data set that can then have a model fitted to it in order to estimate mean compartmental residence times. The purpose of using a compartmental model is to subdivide total gastrointestinal time into residence times within those particular segments or compartments of the total gastrointestinal tract that are associated with distinct modes of food breakdown. For example, in the ruminant gastric fermentation is followed by hydrolytic digestion and then post-gastric fermentation. In contrast, in equids hydrolytic digestion is followed by a significant, post-gastric fermentation although it is acknowledged that some fermentation occurs within the stomach. To reliably determine passage rate through sub-segments of the gastrointestinal tract requires that the region be cannulated so that markers can be introduced directly. Hyslop et al. (1999b) pulse dosed chromium-mordanted feeds into the cecum of cecally-fistulated ponies and withdrew cecal digesta samples by suction at regular intervals over a 10 h period. The chromium concentration in cecal digesta samples was measured and cecal outflow rate ( $k$ ) was determined by fitting simple exponential relationships of the form:

$$[Cr]=A \exp^{-kt} \quad (3)$$

to the chromium concentration data. Cecal outflow rate ( $k$ ) varied between 0.240 and 0.387 depending on the type of feed marked while the  $R^2$  of the exponential relationship ranged from 0.717 to 0.948.

### **Models: the Mathematician's Dream and the Nutritionist's Nightmare!**

The 1980s saw the development of a number of models that could be used to fit ruminant data based on the use of markers. The classic two-compartment model of Grovum and Williams (1973) was followed by the development of a multicompartamental model proposed by Dhanoa et al. (1988). Pond et al. (1988) discussed the applications and limitations of several models in expressing and

interpreting digesta flow, and more recently, France et al. (1993) have incorporated diffusion and viscosity concepts into compartmental models. Models have increased in complexity although it is questionable whether they explain the biology of the animal any more clearly than before (for example, see Holland et al., 1998).

Models can be formulated deterministically or stochastically. The former relies on fixed input parameters and no account is taken of uncertainty, predominantly random variation, whereas the latter describes this variation so that the outcomes occur with a probability (Thrusfield, 1997). Deterministic models are preferred (J France – personal communication) because it can be argued that the variation in stochastic models could hide the inadequacies of data acquired through poor experimentation.

One of the questions that has to be addressed when deciding which model to apply to horse data is whether or not the flow of digesta is time-dependent or time-independent. Briefly, the simplest situation is the time-independent paradigm where:

1. there is complete and instantaneous mixing of influxing particles with those resident in the compartment;
2. there is an equal opportunity for escape of all particles from within the compartment;
3. there is constant inflow, outflow and compartmental mass (Ellis et al., 1994).

The above could describe the situation in the stomach, cecum, ventral and dorsal colon of the horse. However, laminar flow probably occurs in the small intestine, small colon and rectum of the horse with little mixing of particles taking place. It could thus be argued that a time-dependent model would be more appropriate for these regions of the horse's gastrointestinal tract, since the probability of passage will be greater, the longer the particles reside in these particular segments of the gut.

Time-independent passage in the horse's gut is best illustrated by the outflow of chromium-mordanted particles from the cecum which follow an exponential distribution (Hyslop et al., 1999b). Excretion of ytterbium-labelled particles in the feces of horses followed a unimodal distribution when the animals were given an oral pulse dose of marked feed (Hyslop, 1998); the data were fitted using the models of Grovum & Williams (1973) and Dhanoa et al. (1985), which rely on an exponential relationship. Hyslop (1998) has proposed that small intestinal passage rate in the horse is time-dependent and to this end, McLean et al. (1999) have modelled mobile bag data assuming a Gamma 2 time dependency (Ellis et al., 1994).

## Application of Models

Hyslop (1998) has suggested that degradation profiles obtained from *in situ* studies can be combined with those from mobile nylon bag studies to provide an overall impression of feed degradation in the horse. Data from marker experiments can be fitted using time-independent models which enable the calculation of  $k$ , the rate constant for the exponential rate of digesta passage. With this information, ED can be measured as follows (Orskov and McDonald 1979):

$$ED = a + \frac{bc}{c+k}$$

Time-dependent rates of passage can be accounted for in the calculation of ED by using  $l$  values or rate constants appropriate to this type of passage. Ellis et al. (1994) describe these time-dependent Gamma functions which vary from Gamma 2 to Gamma 6, the Gamma 1 function representing the time-independent, exponential relationship. ED can be calculated using the Gamma 2 function as follows:

$$ED = a + \frac{bc}{c + [(2/MRT)0.59635]}$$

Hyslop (1998) has combined knowledge of degradation profiles with estimates of digesta passage rate using different models to partition digestion throughout the digestive tract of the horse. Assuming MRT of 3, 4 and 41 h in the small intestine, cecum and colon respectively, he calculated the loss of dry matter from sugar beet pulp (SBP) and hay cubes in different parts of the gastrointestinal tract by using the above ED equations. These losses are shown in Table 1.

**Table 1.** Proposed partition of dry matter (DM) degradation (%) of two feeds throughout the digestive tract of ponies.

	SBP	Hay Cubes
ED in:		
Small intestine <sup>1</sup>	17	31
Cecum <sup>2</sup>	41	13
Colon <sup>2</sup>	12	8

<sup>1</sup> time-dependent passage; <sup>2</sup> time-independent passage.

## Conclusion

The techniques reviewed above provide a means by which feed degradation in the horse can be described. A major weakness appears to be that of accurately quantifying digesta passage rates in order to be able to weight degradation data appropriately; these rates will be affected by both the nature of the diet and the level of feeding. A further problem is that of knowing whether passage rate in different segments of the digestive tract is time-dependent or time-independent. However, the magnitude of the MRT will affect the relevance of this distinction. In order to define the partition of digestion of food entities between different parts of the horse's gastrointestinal tract, more work must be done with cannulated animals. In contrast to this approach, compartmental analysis, based on total tract marker studies, can dispense with the need for surgically modifying animals although the interpretation of this analysis, together with choice of model, are still issues that demand further examination.

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