Improving the Nutritional Representation of Horse Feeds in South Africa.

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MSc Agric

Submitted in partial fulfilment of the requirements of the degree of
Doctor of Philosophy of Science in Agriculture

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School of Agricultural Sciences and Agribusiness
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2011

Abstract

Protein has been identified as a major reason that people purchase a horse feed, with anecdotal explanations offered for the poor prediction of response of horses to their rations, particularly in the sport horse market. The current research identifies through hierarchical cluster analysis that the myriad of riding feeds offered on the South African market fall into only four simple categories on the basis of wet chemistry. Feeds were subjected to the *in vitro* gas production technique (IVGPT) described by Pell and Schofield (1993), using equine faecal inoculum. Gas profiles, corrected for control fermentation profiles in the absence of substrate, were fitted to the model described by Campos *et al*. (2004) to derive GP kinetics. Gas production kinetics, and information in respect of pH, degradation efficiency, lag time and apparent and true digestibilities were obtained. The feeds were tested for glycaemic response in miniature horses using the hexokinase method with deproteinization using an auto analyser (Roche Diagnostics). Blood glucose parameters of feeds (mean, peak, slope and time to peak and area under the curve) in each group were compared by analysis of variance and regression with covariates. *In vivo* analysis of rates of passage and digestibility using using post-prandial percentages of acid insoluble marker collection was used to study the gastrointestinal process, to indicate foregut and hindgut compartmental flow. The need to balance nitrogen levels with a proportional supply of fermentable carbohydrate contradicts widely used protein intakes in the horse. *In vitro* fermentation was used in an analysis of nutrient synchrony, to identify optimal fermentative capacity for utilization of horse feeds. The characteristics of horse feeds were related to requirement and were composited in an analysis of the representation of horse feeds that would best reflect optimal utilization in the horse, to produce a method of feed characterisation that would lead to the optimal prediction of response of horses to feeds offered to South African horses.

Candidate's Declaration

I, Marion Belinda Young, declare that

The research reported in this thesis, except where otherwise indicated, is my original research.

This thesis has not been submitted for any degree or examination at any other university

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Supervisor's Declaration

I hereby release this dissertation for examination in my capacity as supervisor.

Prof M. Laing

School of Agricultural Science and Agribusiness

March 2011

Signed : _____

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ACKNOWLEDGEMENTS

This thesis is dedicated to all my equine compatriots that have walked the walk with me so that I can talk the talk now. I can thank Browny first for bucking me in the haystack when I was 3 years old, and then Tibbles, Famie, Squirrel, Sicko, Rosie, Legacy my heart, Pea, Fox, Squiblet, Jam, Jed Freshuss, the yard at Ukulinga (including Zimbo, Budgie, Applemac, Harry, Gambler), and all my little minnies that have behaved so well in all our research trials, especially Toto. Also the special ponies in my friends hearts, like Vitesse and Innes and Warrior, Bella and Krypton and Girlie. Also all my horse mates, like Minnet, Shaun, Brian and Mel and Megan, as well as Janine and Elana and Shirley, all of whom will be a part of my book that I am writing next!! Their experiences and those of all the horses and owners at the competitions and the training have contributed to the body of knowledge that is presented here.

I would like to acknowledge the following people and institutions:

The financial support of the NRF, the Competitive Grant UKZN, and Spurwing Horse feeds for making some of this work possible.

Prof Mark Laing. You said you could manage the unmanageable, and supervise the unsupervisable. You can! What we have done in 8 months has taken me ten years to do on my own. You also said that life starts after this PhD and I am going to believe you in that too. Thank you so much.

Sincere thanks are due to Sam Khumalo at Ukulinga Research farm for his assistance in the running of the trials, and also Shadrack Dlamini. Ngiyabonga kakhulu ukungisiza!

The organization and assistance of Sue van Malsen and especially Fabian Fon for helping me set up the gas chromatography so that I could do it, is gratefully acknowledged. Marianne Hundley is acknowledged for the ammonia and protein results. Debbie Davies is thanked for her kind assistance in compiling laboratory results on the feeds, and Nasreldin Basha and Fabian Fon and Jack Ouda for support in the laboratory.

Prof Ignatius Nsahlai for help with aspects of the SAS analysis.

Frances Salisbury and Ann Kinsey for assistance at Ukulinga Research Farm.

Colleagues including Sheryl Hendriks for creating invaluable opportunities for me to work, and Animal Science colleagues for academic support while this was being done.

My post grads. When I think of the rite of passage each of you has walked, I am once again humbled by your grace to follow where I tried to lead, and only now am I realizing what you actually did. You guys are stars. To Elaine and Jean and Tarryn, who did so much of the pilot research that just consolidated what I have been able to conclude in this thesis and to Christelle, Shanonne, Sarah, Alex and Shaun, I salute you. Thanks Shaun for reviewing drafts and being the late night family in the completion of this. The Equine Office. Let us raise our…mugs… of tea!

My mates Jo, Minnet, Gaye for prayers and tea and encouragement.

This thesis is dedicated to Mom, Keith and Glen and Weeti. Cos we Youngstas finish what we start! Mom never doubted that this would be done, and although I could never tell the sticks from the carrots, whatever was eventually in the tea that you always kept bringing me, it worked.

And I would like to thank God that we got this far. 'To be made new in the attitude of your minds; and to put on the new self, created to be like God in true righteousness and holiness" Eph4v23.

Don't be the rider who gallops all night and never sees the horse that is beneath him.

~ Jelaluddin Rumi

In Praise Of The Horse

Ronald Duncan

Where in this wide world can man find nobility without pride,
friendship without envy, or beauty without vanity?
Here where grace is laced with muscle and strength by gentleness confined.

He serves without servility; he has fought without emnity.
There is nothing so powerful, nothing less violent;
there is nothing so quick, nothing more patient.

England's past has been borne on his back.
All our history is in his industry.
We are his heirs;
He is our inheritance

Ladies and Gentlemen - The Horse!

INTRODUCTION

"IF A HORSE IS RECEIVING WHAT SHOULD BE AN AMPLE ALLOWANCE OF FOOD AND DOES NOT SEEM TO THRIVE, SHORTEN HIS ALLOWANCE OF FOOD RATHER THAN INCREASE IT. ONLY THE FOOD WHICH IS BEING PROPERLY ASSIMILATED CAN DO THE HORSE GOOD, THE SURPLUS IS NOT ONLY DOING NO GOOD BUT IS DOING A GREAT DEAL OF HARM"

SIR M.R.BURREL (1930)

It is sobering that Sir Burrel in 1930 articulated in his treatise on the breeding and management of light horses, a fundamental understanding of horse nutrition that appears to elude us in this age of scientific research. Provision of nutrients is one issue, but the consequences of the provision of excess nutrients is another issue altogether. Perhaps the most significant change from that century to this is in the use of manufactured commercial feeds for horses, the first of which was sold in the UK in 1958 (Harris and Bishop, 2007). The horse feed market, certainly in South Africa, has been consumer-driven for decades. The departure from scientific principles since the institution of commercially manufactured feeds is now beginning to manifest itself in an alarming increase in a range of metabolic disorders (Kalck, 2009). The fact that speed records on the race track have altered little over the last decades (Gardner, 2006), while productivity in cattle and poultry have doubled in less time, indicates that horse feeds have been products of "fads, foibles and trade secrets" (Ensminger, 1971).

The horse industry has managed to maintain a fairly low profile with respect to new pollution control laws. This represents an opportunity for a body of research to address environmental issues. A herd of horses of 150 animals, confined for any 45 day period throughout the year, can be regulated as a Confined Animal Feeding Operation (CAFO), with concomitant regulations for comprehensive nutrient management plan for solid wastes, a strict inventory of nutrients in and out, and a no-effluent policy (Topliff, 2002). Interest in eutrophication (Wilson *et al.,* 2006) and increased global pressure to reduce phosphorous sinks, predominantly in Europe (Lawrence *et al.*, 2003), has prompted research into a new concept of nutrient synchrony, precisely to address the inventory of nutrients in and out of animals. Nutrient synchrony in the horse has not been coined as a phrase yet in this country, but it forms the bulk of this dissertation. Synchronous supply of nutrients is important for environmental issues, but for physiological issues in animal digestion as well.

The African Horse Sickness Trust reported that the horse population in South Africa in 2006 and 2009 was about 300, 000 animals (Gerdes, 2006; AHST, 2009). In addition, the South African National Equestrian Federation (SANEF) reports that 4750 individuals are registered with provincial bodies for the purpose of competition (SANEF, 2010). Horses in SA are used for pleasure, draught purposes (Pearson, 2003), transport, hacking, hunting, showjumping, eventing, dressage, tentpegging, polo, polocrosse and horse racing. The fact that the majority of horses in SA support a lucrative racing industry has important implications for the breed and type of horse present in the turnover industries. In fact, many of the riding disciplines are supported by the rapid turnover of athletes from the race track, and it is the idiosyncrasies of the modern Thoroughbred racing horse that have spurred on much of the research into the nutritional management of the horse.

Horses are companion animals and as such they enjoy the indulgence of their owners. As athletes, this tendency is more marked. Livery costs at present are preclusive to the "pet" owner, and the horse-owning public is becoming increasingly discerning on matters of competition, nutrition, health, management and farriery, as well as Eastern and Western medical treatments (Dr Kerry Ridgeway, *pers comm*). A result of this awareness and interest in the treatment of the horses, is that concern about horse nutrition has increased, with concomitant attention to nutrition research, and more research is being conducted into performance aspects of horse nutrition (Hodges and Pilliner, 1991; Hintz, 1994; Bojer, 2004). The cost of keeping horses has increased (AFMA, 2006) and this has altered the focus in the husbandry of these animals. Furthermore, the sheer density of horses maintained in selected areas has made changes in management systems necessary.

That horse owners want to know about the feeding of their horses is important. Horses are expected to perform across a range of activities, whatever that definition of performance is. Owners pay thousands of Rands for the feeding and upkeep of their horses, with an expectation of good performance by their horses. The role of nutritionists is to provide the scientific platform for the optimal performance and welfare of the horse. Winston Churchill maintained that "there is something about the outside of a horse that is good for the inside of the man", but in extension, there is something about the inside of the horse that is good for the outside of him as well. The choice of feeds by horse owners is largely anecdotal and unscientific, and horse owners are seduced by well marketed, good looking feeds. For the purposes of this dissertation, the daily nutritional intake (the "ration") will be made up of the concentrate "feed" and the "roughage". The feed and roughage are usually provided in a ratio appropriate to the work rate of the horse and will be specified. By default, the Thoroughbred horse is considered under conditions of an intensive

management system where the horses are stabled and grazing is minimised. It is to be noted that the forage component in the South African context differs from options abroad. In Sweden, for example, the concentrate fraction of the daily ration is minimal, given that the forage is of such a high quality and quantity. In stabled horses in South Africa, dried hays of low protein content are made available as a supplement to limited or low quality grazing. A scientific framework for the objective evaluation of the real nutritive value of each horse feed, and an effective way to communicate this to the horse owner, is crucial to the horse industry. The Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act 36 of 1947 is limited in its requirements for equine feeds, requiring that the levels of crude fibre (CF), crude protein (CP) and calcium:phosphorous ratio (Ca:P) are displayed. There are laws governing the level of inclusion of certain raw materials (for example, meat and bone meals) in feeds, but none governing the raw materials or their the processing, used in the equine feeds.

Palatability is a major factor in South African rations and this precludes the use of many ingredients used for other species. CF, CP and Ca:P may be pertinent in the registration of feeds, but their relevance to the equine as a hindgut fermenter is not simple. The challenge exists for the Registrar of Feeds to regularly update the specifications of horse feeds, as the National Research Council in the USA did in 2007, producing the 6th Edition of the NRC Nutrient Requirements of Horses (NRC, 2007).

Horses are not monogastric as are our cats and dogs, and because they perform work, are not entirely companion animals either. Consumerism creeps into the horse feed market when horses are viewed as pets. What may be best for the horse is superseded by what the owner deems as aesthetically pleasing. Feed companies employ various marketing strategies to promote their product. The competitiveness of the market has increased because the volume of horse feed being sold in SA is decreasing because of increasing costs (AFMA, 2006). The horse owner has many feeds to choose between (Table 1).

Table 1 Numbers of feeds available from each of the major horse feed manufacturers in South Africa

Company	Number of Feeds	Website
Meadow	2	www.meadow.co.za
Romix	5	www.romixfeeds.co.za
Alzu	5	www.alzu.co.za
Equus	8	www.equus.co.za
Capstone	10	www.capstonehorsefeed.com
Spurwing	11	www.spurwingfeeds.co.za
Epol	11	www.epol.co.za
Vuma	13	www.vumafeed.co.za
Equifeeds	20	www.equifeeds.co.za
Total of recognised brands	85	Excluding the myriad of small scale feed manufacturers that in the KZN area, for example, produce more than 20 additional feeds.

The SA feeds are distinguished on the basis of a crude protein percentage (CP%), in contradiction to the nutritional significance of protein to the horse. The feeds fall broadly into the following categories (Table 2), with the riding feeds produced for horses which hack, jump, event or do endurance-type riding. The label of CP% is not helpful, and it misrepresents the relative nutritional significance of CP and other nutrients for the hindgut fermenter. For the studies that follow, many of these feeds were purchased locally from leading feed manufacturers, and homogenous samples of the feeds were used in subsequent studies.

Table 2 Basic description of all horse feeds sold, including the crude protein percentage which horse owners use to choose a feed

Feed	Basic description	Protein %
Stud	Yearling	14, 16
Riding	Grain	9,10,12,13,14
	Grain free	10, 11, 12,14,15
Race	Pre-train	16
Veteran	Veteran	13,14
Supplements	Balancer	15, 25

Feeds on the overseas market fall into one of two categories. They are either very limited in choice, in for example, Costa Rica, where the equine market is limited to rural animals that are not fed manufactured feeds, or show animals fed a high fat feed (Figure 2).

Figure 1, 2 High fat show pellets fed to show horses in Costa Rica, where the maize is imported and the feeding of concentrate feeds to equines is considered only by the wealthy

The other end of the scale is the British equine market, where intense consumerism is practiced, and a highly competitive market has developed. The economy of the country plays a role in the provision of feed and competitiveness of the horses in those areas. The issue is whether the demographic of horse feeds matches the needs and demographic of horses.

Figure 3 A small range of horse feeds from Badminton Horse feeds, a single company, on display at the Badminton Horse Trials, in Little Badminton, UK

Hierarchical cluster analysis was employed in this study to differentiate between horse feeds on the basis of their nutritional characteristics. Clusters obtained statistically are intended to demonstrate that feeds are being sold as "different" when there is no nutritional basis for saying so. These feed clusters are not intended to accommodate the myriad of permutations of roughage and

concentrate feed that would necessarily make the ration more appropriate. The cluster analysis in Chapter 2 demonstrates that the feeds that are sold are not sensible categorised, and this would serve merely to confound, rather than assist, the consumer. Permutations of roughage and concentrate in the daily ration of the horse are simulated in Chapter 7 and the significant classifying variables in that analysis would be influenced more by the concentrate feed than by the hay in the ration.

Nutritive value of horse feeds depends on the chemical composition, intake, digestibility and the rate at which the nutrients are released. The proportion of fore- and hindgut utilization as influenced by feeding management is important. Unscientific feeding principles and management in horses can lead to a decreased capacity of the hindgut to process raw materials, and an oversupply of rapidly absorbed, grain-based feeds. The capacity of the hindgut to process cellulosic feeds is widely disregarded in horse feed formulations. Nutrient synchrony is not yet applied in the field of equine nutrition but is critical in the optimal utilization of feed ingredients, so that the performance of the horse on the various feeds can be explained.

Several methods have been developed to determine the nutritive value of feeds. Biologically based methods are recommended as providing a more meaningful representation of the rate and extent of *in vivo* digestion than are chemical methods (van Soest, 1994). The *in vitro* gas production technique (IVGPT) can be coupled with residue determination (Blümmel *et al.*, 1997), which together with gas fermentation kinetics (Campos *et al.,* 2004) can generate diverse information regarding the fate of feed undergoing hindgut fermentation in the horse. Complemented by *in vivo*, glycaemic and chemical analyses, the fate of ingested ingredients can be used to model the extent to which feeds meet requirements of the horse and to what extent nutrient synchrony optimizes caecal microbial capacity.

At each end of a ration continuum for horses are the idle horses eating 100% forage, and the horses that would (unrealistically) be consuming 100% concentrate. Strategic feeding guidelines for horses in work necessarily require that the forage be replaced proportionately with concentrate to meet nutrient demands of work. Hence the strategies of feeding the light hacks 20% of their ration as concentrate, while the racehorses are fed up to 80% of their ration as concentrate. One can generalize in the South African context that working horses (not racing) will receive at least 50% of their ration as concentrate feeds. It is this concentrate feed that is the focus of the current study. The sport horses that are receiving the concentrates reflect the greatest financial investment in feed and valuable assets. Every possible permutation of hay and concentrate proportion has been

generalized and specified within this study to focus on the effects of feeding the concentrate. Empirically it is not possible to address the infinite permutations of roughage: concentrate ratios between different forages and mixed grazing programmes as well. The value of modelling is that it allows such permutations to be addressed where the constraints have been clearly defined. Clearly, the concentrate feed is offered with a sensible amount of forage. In the South African context, where sport horses for the most part are kept in intensive management systems, and grazing is limited or non-existent, the forage is made available as hay. Unlike European countries (for example Sweden) where the inverse is true, grazing is considered 'bonus' in the calculation of rations for horses. Were South African horses to receive rye grass, and extra lucerne and extra lush grazing, it would necessitate a reformulation of the ration and the consequences of this for the parameters measured in this study would have to be elucidated in that context. The protein in the forage is important and exacerbates the overprovision of protein in the concentrate feed. In Chapter 7, standard idle (80:20) and working (50:50) horse rations are compared in the ability of the feeds to contribute to the daily nutrient requirements of the horses. Hay and lucerne diets form a basis for comparison as well. In Chapter 4, glycaemic parameters were tested at 20 and 40% concentrate of the ration. All the feed parameters were incorporated in the ration appraisal in Chapter 7, and several ideas for future research have been generated as well.

The objective of the current study was to determine whether measurements of the nutritive value of a range of SA horse feeds derived from IVGPT, glycaemic, *in vivo* and chemical analysis could be used to consistently and meaningfully classify feeds into homogenous groups based on the functional nutritive characteristics. It is about the concentrate feeds, and the integrity of their formulation, as well as the effective characterization of them using new parameters to improve a prediction of response to the rations. As a hindgut fermenter, foregut (*x*) and hindgut (*y*) capacity and requirement determine a value for nutrients (*z*) that if oversubscribed (*intake >z*) mean that there is consequence for the gastrointestinal environment of the horse. The current study seeks to determine how this value "*z*" may be described. A most recent review by Santos *et al*. (2011) emphasizes as well the "uncoupling" between the fore- and hindgut mechanisms of nutrient utilization, where *x* and *y* need not necessarily equal *z*, but that *y* would be intrinsically linked to the processes associated with *x*.

The goals of this thesis, therefore, include the following:

1. An integrated approach to the effective representation of the nutrient quality in horse feeds;
2. The evaluation and combination of several effective means of nutrient evaluation;
3. The development of a platform for the scientific delineation of horse feeds in South Africa;
4. To create a scientific framework for the evaluation of horse feeds so that they can be accurately presented by equine nutritionists and feed representatives selling horse feed products;
5. To provide a scientific baseline from which horse feed companies can develop horse ration formulations that:
    a. Optimize nutrition for the health and performance of horses in different states of activity;
    b. Concurrently, reduce the costs of feed formulations, and reduce competition for feed resources with other agricultural activities. Horses have evolved to utilize primarily low value grassland plants, with a small contribution by broadleafed plants such as lucerne.
6. To investigate biological assays into the usefulness of common feed ingredients, and nutrient synchrony, based on the digestive capabilities and needs of both the horse, and the consortia of hindgut fermentative bacteria.
7. To develop an integration of the information captured in the above research, as an initial step towards the development of a more rational approach to the formulation of horse feeds.

What Sir Burrel expressed in the 1930's was that feeding of horses should be optimal, not maximal. While the role of the horse in traction, sport, police work, pleasure and breeding may have altered over time, its digestive physiology and capacity for utilization of forages still reflects its evolutionary history. This thesis aims to produce an integrated approach to the effective representation of the nutrient value in horse feeds by evaluating and combining several means of nutrient evaluation, to develop a platform for the scientific categorization of horse feeds in South Africa. This should provide a rational framework for horse feed companies to refine ration formulations to optimize nutrition for the continued good health of the horse, with regard for the environment, and competition for feed resource between other agricultural activities. It should also stimulate further research into similar biological assays of the usefulness of feed ingredients and

nutrient synchrony. Most importantly, the proposed framework will provide the horse owner with a sound basis for their selection of horse feeds.

South African horses have entered the international arena with South Africa's event riders qualifying for the World Equestrian Games in 2010 for the first time. Scientific equine nutrition is integral to competitiveness at this level.

CHAPTER 1: LITERATURE REVIEW

"to sum up the matter, we may say that the alimentary canal of the horse is not suited for the digestion of concentrated food; in other words, that unless the nutritive matter of a horse's food is diluted by a more or less inert vehicle, of which vegetable fibre is the natural and best form, disturbance of the digestive organs will ensue."

Captain M.H.Hayes (1900)

1 THE HORSE

All modern Thoroughbred (TB) horses are line bred individuals from the late 17th Century of foundation stock from the Byerley Turk, the Godolphin Arabian and the Darley Arabian (Wall, 1949). They have evolved from a primarily fight or flight creature into an athlete honed for the exigencies of competitive racing. They are typically referred to as "hot blooded", describing their temperament as much as the lands of their origin. Their origins in the deserts, for the purposes of speed and travel, have left them a heritage of endurance and of speed. These characteristics have been specially selected for in TB breeding programmes, with the result that race horses maximise these characteristics. Having been bred to be fed as well, the TB exhibits acute responses to changes in nutrition and the production system.

The domestic horse is derived from wild populations, distributed from Europe to the Middle East (Figure 1.1). They remain as the three forms in Europe (Coldblood, Warmblood, and Tarpan), and then as a fourth from North Africa to the Middle East (Afro-Turkic).

The combination of the hot blooded and the cold blooded horses of Central and Eastern Europe (Bennett, 2007) had meant that a composite Warmblooded horse has entered the sporting precinct. The hot blooded race horses that have been bred for speed and for endurance also possess a fleetness of mind, which renders them unsuitable for the more precise, accurate sports of dressage, for example. The Warmbloods possess higher proportions of slow twitch fibres and a slowness of mind which is useful in repetitive training exercises and thus, temperamentally, they are more appropriate to disciplines that require slow, controlled muscle control and repetitive training, as in the lateral work required in the high school movements of *Haute Ecole* and dressage, show jumping and vaulting. The Warmbloods possess a generosity of bone. The nutrition of origin in Warmbloods lends itself to smaller amounts of concentrates, good grazing and colder climates.

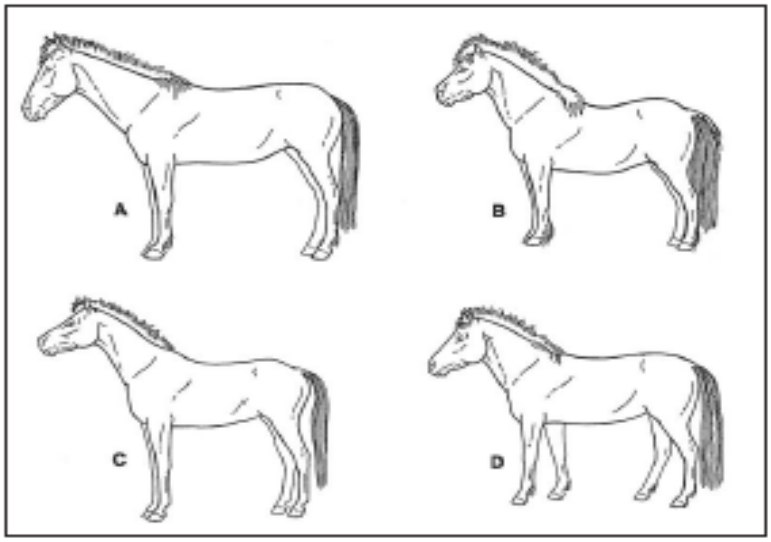

Figure 1.1 Reconstructions of four subspecies of Equus caballus, based on fossil remains, old photographs, and appearance of probable domestic dependents from the same geographic areas (Bennett, 1992). A. central European horse (*E.c.mosbachensis*); B. northwestern European horse (*E.c.caballus* -the dwarfed insular pony is illustrated); C. Afro-Turkic horse (*E.c. pumpei*); D. Tarpan (*E.c.ferus*).

In the sub-tropics of SA, this translates to well conditioned horses that are able to respond well on most feeds available in SA. Typically named according their region of origin, Warmblood breeds in SA include the Hanoverians and the Dutch Warmbloods, but also include the Friesians and the Irish Draught Sport Horses.

Some traditional composite SA breeds such as the SA Boerperd and the Basutu pony are able to maintain condition on little, if any, concentrate feed, having evolved under the harshest of conditions (Cothran *et al*., 2001). The Appaloosa breed falls into the category of "good doers" (i.e. horses that are able to retain a body condition score in excess of 6 (moderately fleshy; Henneke *et al*., 1983) in the SA environment. The Quarter Horse makes up a growing contingent of Western riding disciplines with horses of American influence. Bred true for thousands of generations, the Arabian horse retains the loyalty and the intelligence, as well as the endurance and economy of stature that made it a legend in the deserts, as well as the ability to survive on the frugal feed provided by the environment from which the breed came.

Differences in muscle fibre composition (Clayton, 1997), countries of origin (Bennett, 2007), temperament, and discipline, as well as the previous nutritional support (Pearce, 1975) to these equines will combine to influence the response of the breed to nutrition, as outlined in the following chapters of this thesis. The idiosyncrasies or characteristics of the breed provide the capacity to excel in different disciplines. The Thoroughbred is fleet, and while stayers and sprinters possess different muscular architecture, as a breed, the Thoroughbred is suited to disciplines where their

athleticism and speed can be exploited, such as three phase eventing, polo and polocrosse. A combination of the TB and the Warmblood has produced the Sport Horse, which is becoming increasingly popular in the competitive arena in SA. Arabs, Appaloosas and the Quarter Horse are excellent performers in the sports of endurance, show jumping, tent pegging, and polocrosse.

Irrespective of the differences of their origins and purpose, *Equus caballus* is a hindgut fermenter and the digestive physiology particular to the breed defines its management and feeding strategies.

2 DIGESTIVE PHYSIOLOGY

The horse is a hindgut fermenter or monogastric herbivore. The multi compartmental arrangement of the digestive tract means that there are areas for specific metabolic activity and transport of nutrients, but also, that there are physiological implications relating to feeding regimes because of that arrangement. Effective prehension and grinding depends on the occlusal surface (Ellis, 2004), and for this reason, teeth should be attended to routinely by a registered equine dentist. The foregut comprises the stomach and small intestines and is relatively small in volume (Table 1.1).

Table 1.1 Volume in litres and proportion of the total digestive tract length in horses (after Wolter, 1984; in Ellis and Hill, 2005)

Total GIT volume (L)	Stomach		Small intestine		Caecum		Colon and rectum	
Volume(L)	Volume(L)	%	Volume(L)	%	Volume(L)	%	Volume(L)	%
230	15	7	70	30	30	13	115	50
	Foregut = 37%				Hindgut = 63%			

The main functions of the foregut include the processing and absorption of soluble or easily degradable nutrients (including amino acids, certain carbohydrates, and fatty acids). The hindgut (which includes the colon and the caecum) accounts for 63% of the gut (Figure 1.2) and is the major site of microbial fermentation (Mackie and Wilkins, 1988) and the absorption of products of fermentation (Table 1.2). The rate of passage determines the time that structural carbohydrates reside at the site of fermentation (Kern *et al*, 1973; Cuddeford, 1996; Hyslop, 2006). In the caecum, roughly 20% of the digesta pass out per hour (depending on the diet) whereas in ruminants, only 2-8% of the digesta pass out of the rumen per hour (van Soest, 1994). Mixing occurs in the caecum and in the right ventral and right dorsal colon (Moore Colyer *et al*., 2003).

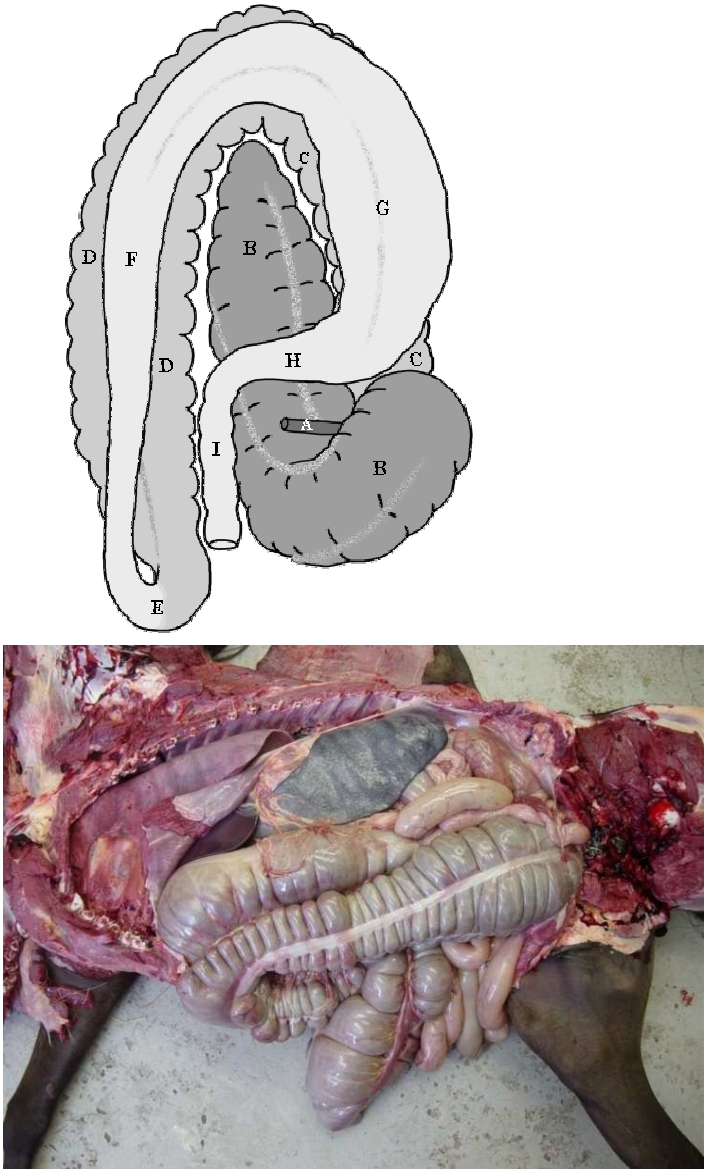

Figure 1.2 Above, the ileum (A), caecum (B), right ventral colon (C), left ventral colon (D), pelvic flexure (E), left dorsal colon (F), right dorsal colon (G), transverse colon (H), and descending colon (I). (http://www.vetmed.wsu.edu/Van308/equine.htm) and below, ventral view of a miniature horse to show arrangement of the gastrointestinal tract *in situ* (Author's own)

Hindgut passage kinetics depend on time and not always on the feed particle size (Moore-Colyer *et al*., 2003), therefore hay and oats have comparable passage rates (Rosenfeld *et al*., 2006). Horses will increase their intake of lower quality roughages in compensation. The control of voluntary intake still requires much research. Physical appetite-regulating mechanisms are not apparent in horses and perhaps sensory (organoleptic) processes control VFI in horses (Dulphy *et al*., 1997a). Other studies have also shown that ponies do not regulate their intake according to energy

requirements (Ralston, 1992; Cuddeford, 1996; Hyslop, 2006), but nutrients in the gastrointestinal tract affect subsequent feeding responses (Ralston and Baile, 1983; Ralston, 1984).

Table 1.2 The major structural sections, capacity and function of the digestive system of a 500kg horse (Kohnke, 1998).

Organ	Volum	Length	Passage Time	Digestive Activity
Stomach	7.5-15	0.25m	Water: 75% in 30 mins	Some protein digestion by acid
Small Intestine	40-50L	15-22m	Water: 2-8 hours	Major fat and protein digestion
Large Intestine: Caecum	25-30L	0.9-1.2m	Water: 5 Hours	Fibre
Large Colon	50-60L	3.0-3.7m	*Relative Passage Times*	Fibre
Small Colon	18-19L	3.0-3.2m	Fresh Grass: 24-36 h	Fibre
Rectum	2-3L			Faecal storage
Total	143-177L		Total Transit Time 78h	

Horses are social, non-territorial, nomadic animals that usually live in hierarchical groups, and little of their cognitive or motivational ethology has changed with domestication. Their development as a trickle feeder, even from the Eocene (Ellis and Hill, 2005), has meant that behaviourally and physiologically, they have to maintain intake of cellulosic forages for the proper functioning of the GIT.

A hindgut fermenter or monogastric herbivore may display a lower apparent digestibility of feeds when compared to a ruminant (Ellis and Hill, 2005). However, the ability to digest and absorb non-structural carbohydrates, followed by fermentation of cellulose and hemicellulose fibres, and the reduced loss of carbon from acetogenesis, are the primary reasons that horses can compete with grazing ruminants (Figure 1.3).

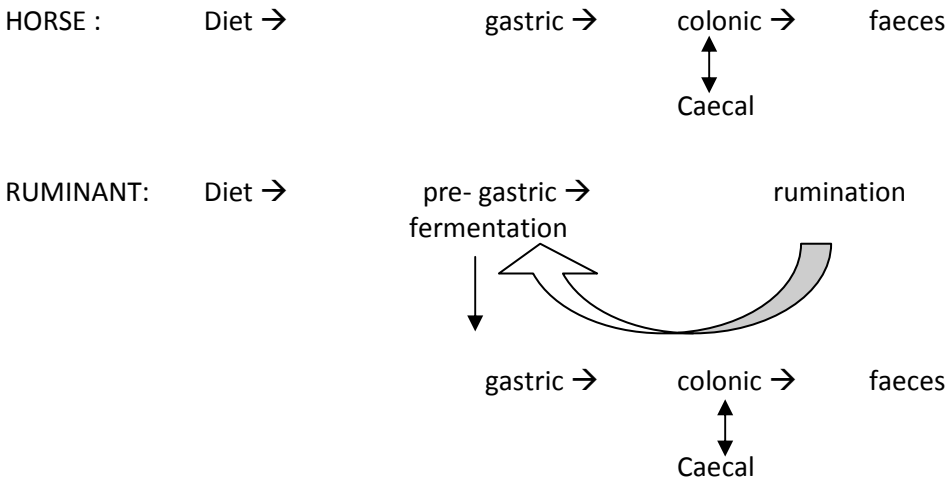

Figure 1.3 Sequence of digestion in horses and ruminants (after van Soest, 1995; in Ellis and Hill, 2005).

Our improved understanding of the equine digestive tract has allowed the improved modelling of the tract, with the caecum and colon as the main sites of fermentation. The convention of using ruminant models to explain nutrient utilization in the equine has been challenged (Ellis and Hill, 2005; Lindsay, 2005) as failing to provide appropriate and adequate models for equine nutrition.

3 DIGESTA FLOW MODELS

Digesta flow kinetics have been modelled in order to determine residence times in compartments of the tracts, and as a result, the digestibility of feed materials. Castle (1956) determined the passage rate of feed through the digestive tract of a goat, and produced an effective means of calculating the foregut digestion (5%) and hindgut digestion (95%) and mean retention time (MRT). This was incorporated into models by Blaxter *et al*. (1956) and Grovum and Williams (1973). This formula is used as the basis for most of the recent herbivore digestibility studies (Austbø and Volden, 2006; Goachet *et al*., 2009) as:

$$MRT = \frac{\sum miti}{\sum mi}$$

m_i = the amount of marker and t_i is the centre time point of the sampling interval.

Mathematical modelling of faecal excretion data using indigestible external markers is a non-invasive method that can be used to obtain digesta passage rate and mean retention time (MRT) in animals. Mathematical models can be categorised into time-independent models (Grovum and Williams, 1973; Dhanoa *et al.* 1985) and time-dependent models (Moore-Colyer *et al.* 2003). Time-

independent models are deterministic in nature and assume that digesta flows irreversibly through a fixed number of sequential compartments according to first order kinetics (Lalles *et al*., 1991). Time-dependent models are based on assumptions of probability (stochastic models) and use γ-functions (non-exponential residence time distributions) to describe the time-dependent passage of digesta through different segments of the gastrointestinal tract. Due to the flexibility of this model, it has allowed it to be successfully fitted to ruminant animal faecal excretion data, where the more rigid time-independent models have failed (Pond *et al*., 1988).

Moore-Colyer *et al.* (2003) concluded that the pattern of faecal marker excretion could be accurately described by time-dependent models, which use non-exponential residence time distributions to describe the time-dependent passage of digesta through different segments of the GIT. Time-dependent compartmental models fit equine faecal excretion data much better than time-independent models (Dhanoa *et al*., 1985 and 2000; Pond *et al*., 1988; Moore-Colyer *et al*., 2003), as digesta passes through the caecum and large colon through narrow flexures between the right and left ventral, and left and right dorsal chambers in the horse. Furthermore, polynomial models of post-prandial percentages of marker (as a percentage of the total marker) excreted from the faeces against time (Thielemans, 1976) have yielded foregut (5% excretion) and hindgut (95%) transit times. In the absence of needing to compartmentalise digesta flow in faecal marker excretion data, this is more appropriate than time-independent representations of marker kinetics, such as those developed by Grovum and Williams in 1973 (Lindsay, 2005). Murray *et al.* (2009) concluded that 'equine-specific models are required to conclusively determine digesta MRT within the different segments of the equid gut'.

Several factors affect the passage rate, including species, size, age, activity, state and the health of the animal. Feed properties such as the forage: concentrate ratio and fibre content have an effect on passage rate of the digesta through the gut and the extent of its digestion (Pearson *et al*., 2006). The physical form of the diet can influence the rate of passage. Pelleted diets have a faster rate of passage than chopped or long hay (Hintz and Loy, 1966). Fresh grass moves more rapidly through the tract than does hay. Particle size will also influence the rate of passage in a horse (Olsson and Ruudvere, 1955).

3.1 Markers

Markers must fulfil certain criteria. They should be non absorbable, neither affect or be affected by the GIT nor by its microbial population, not be similar to the fraction they represent, and be easy to analyse (Owens and Hanson, 1992). Where external markers are used, the assumption is

made that they behave identically in passage through the GIT as the nutrient under investigation (Tamminger *et al.,* 1989). Internal markers usually fulfil these criteria, as intrinsic components of the feed of fraction under investigation. Indigestible markers recovered from faeces provide a non-invasive method of calculating passage rate and mean retention time (MRT). Acid insoluble ash (AIA) has been the most frequently used marker in equine studies (Sutton *et al*., 1977; Cuddeford and Hughes, 1990; McMeniman *et al*., 1990; Cuddeford *et al*., 1992; Barbisan *et al*., 1993; Miraglia *et al.*, 1999), and is superior to chromic oxide (McCarthy *et al*., 1974; Orton *et al*., 1985). The cost of using N-alkanes (Ordakowski *et al*., 2001) or Ytterbium (Pagan *et al*., 1998; Moore-Colyer *et al*., 2003) as markers is preclusive in many instances. Markers can be used to describe the digestibility of feed by determination of total faecal output in cases where total faecal collection is impossible, dry matter and nutrient digestibility intake of grazing animals (Miraglia *et al*, 1999; Stevens *et al*., 2002; Lindsay, 2005), the behaviour of digesta through different compartments of the gut, the faecal excretion pattern over time for determination of the passage rate and the mean retention of digesta in different compartments and the entire digestive tract (Castle, 1956; Moore *et al.,* 2002; Lindsay, 2005). The percentage apparent digestibility can be calculated from the difference between the dry matter intakes of nutrient and marker and the dry matter retrieved in the faeces (Bush *et al*., 2001):

$$AD\ \% = 100 \times \left(1 - \frac{\%Marker\ Intake}{\%Marker\ inFaeces}\right) \times \left(\frac{\%Nutrient\ in\ Faeces}{\%Nutrient\ intake}\right)$$

Length of collection period in horses, as determined by rate of passage trials, has been reported as 4 days by Vander Noot *et al.* (1967), 5 days by Paterson (1879), 7 days by Nicholson and Friend (1965), 8 days by Lindsey *et al.* (1926), and 12 days by Lathrop and Bohstedt (1938). When indicators such as chromic oxide or coloured particles are added to hay-grain diets, about 10% of the indicator is excreted in 24 hours, 50% within 36 hours and 95% within 65 hours (Alexander, 1946).

3.1.1 External Markers

External makers are added to the feed or dosed to the animal at a known rate per day and must be evenly distributed throughout the feed. Some of the external markers used in digestibility trials include Cr_2O_3, cobalt (III) ethylene diamine tetraacetate (CoEDTA), Europium, Ytterbium and n-alkanes (Schürch *et al*., 1950; Dove and Mayes, 1996; Bush *et al*., 2001; Moore-Colyer *et al*., 2003; Austbø and Volden, 2006; Goachet *et al*., 2009). These markers are administered to the feed in various ways which include administration via a cannula, orally in feed and via a nasal-oesophageal tube.

Chromic oxide is solid phase marker and it is widely used in the digestion studies. However, there is uncertainty in its reliability as an inert marker. Schürch *et al*. (1950) used Cr_2O_3 in rats and the results were comparable to the total collection method. However, Furuichi and Takahashi (1981) reported that Cr_2O_3 faecal recovery was variable and this may contribute to errors such as underestimating digestibility. Bush *et al*. (2001) found that chromic oxide results were negative and had a high variance due to incomplete recovery. Holland *et al*. (1998) also found that there was about 79% coefficient of variation in faecal concentration of Cr_2O_3. The results emphasised that there was a need for thorough mixing of the faeces after collection. When using external markers in free range horses, the dosing must be as frequent as possible. The faecal collection must be done at a constant rate (Schürch *et al*., 1950; Holland *et al*., 1998).

Table 1.3 Mean retention time (MRT) in hours (h) of digesta in horses fed various diets and measured with different markers

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

3.1.2 Internal markers

Internal markers can be defined as natural substances occurring in plants that are completely recoverable in faeces. Some of the markers that have been used in equine digestibility studies are acid insoluble ash (AIA), acid detergent lignin (ADL), also known as lignin, and n- alkanes (Furuichi and Takahashi, 1981; Glade, 1984; Callum *et al*., 1996; Bush *et al*., 2001; Bergero *et al.*, 2004; Pereitti *et al*., 2006; Goachet *et al*., 2009). According to Varloud *et al*. (2004) natural markers

do not influence the gastro-intestinal secretion, digestion and absorption. This makes them suitable for use in digestibility trials.

3.1.2.1 Acid insoluble ash

Acid insoluble ash (AIA) can be administered orally as a marker, and has been used in a number of equine digestion studies (Glade, 1984; Delobel *et al*., 2008; Bush *et al*., 2001; Varloud *et al*., 2004). The AIA of the feed and the faecal samples is determined as described Van Keulen and Young (1977), modified by Furuichi and Takahashi (1981). The feed and the faeces are heated in hydrochloric acid (HCl) to obtain the ash which is insoluble. The same AIA results are produced by 2N HCl, 4N HCl and 6N HCl (Furuichi and Takahashi, 1981; Bergero *et al*., 2009). There was variability in the outcome of the two methods because hay was used in one of the diets. There were also no recoveries in AIA for maize used in all the treatments. This was due to the fact that AIA is a cell wall component and therefore will be found in smaller quantities in concentrates (Van Soest *et al*., 1991).

Varloud *et al*. (2004) successfully used the AIA method to determine the apparent digestibility OM, starch and sugars, neutral detergent fibre (NDF) and acid detergent fibre (ADF). The horses were fed two diets with starch and sugar contents of 211g.kg^{-1} and 416g.kg^{-1}, respectively. Glade (1984) used AIA in a study on the effect of fibre on the nitrogen requirements of mature horses (Glade, 1986), and Bergero *et al*. (2002), to measure apparent digestibility of haylages in ponies at work and in maintenance. Goachet *et al*. (2009) found that when AIA was used as marker, there was an over-estimation of the apparent digestion coefficient (ADC). Overestimation means that there is more AIA in the faeces than in the feed. The overestimation could be due to faeces contamination because they were collected from the ground. AIA is preferred over a chromium-mordanted marker for apparent digestibility coefficients (Cuddeford and Hughes, 1990).

3.1.2.2 Acid detergent lignin

Acid detergent lignin (ADL) is a widely used internal marker in herbivore nutrition studies (Fahey and Jung, 1983). AIA is considered superior to ADL in horse digestibility studies because the latter was not fully recovered in the faeces (Miraglia *et al*., 1999). Lignin is a cell wall component and it can be determined by methods described by Van Soest *et al*. (1991). Concentrations of lignin in forage increase with maturity (Ragnarsson and Linberg, 2008). A problem is that legumes contain less lignin than grasses, and lignin levels vary from season to season. High variability in lignin faecal recovery was found in ruminants suggesting that lignin could be digested by rumen microbes (Fonnesbeck, 1968). However, this does not stop the use of lignin as an internal marker, as long as one determines the ratio of the lignin to the compound under study. Miraglia and Bergero (2006)

used ADL as an internal marker, while Varloud *et al*. (2004) showed that the use of ADL as a marker gave results similar to the total collection method for all the digestibility coefficients for a low sugar starch diet. Acid detergent lignin can underestimate the apparent digestion coefficients (except for DM). This could be due to problems with faecal recovery and dietary lignin. Furthermore, a proportion of the lignin could be digested by microbial activity in the caecum (Goachet *et al*., 2009), but this is questionable. Lignin is a naturally occurring marker in feedstuffs for horses (Miraglia *et al.*, 1999) which is why it is still used.

3.1.2.3 n-Alkanes

Long chain n-alkanes of uneven chain length (C21-C37) can be used as internal markers (Mayes and Dove, 2000; Stevens *et al*., 2002) and are increasingly used in horse digestibility studies. These are found in the cuticular wax of plants and their concentration is found to vary with plant species. In general, they are found in higher concentration in forages than in concentrate feeds. By estimating the proportion of different alkane chain lengths in plants, one can identify the concentration range of these markers. The recovery of n-alkanes increases with chain length and therefore a long-chain n-alkane occurring in high concentration is the most suitable marker (Gudmundsson and Thorhallsdottir, 1998). Similar results were observed by Martins *et al*. (2002) who used n-alkanes to determine the utilisation of complex feeds by rabbits. They focussed on faecal recovery of carbon chain lengths of C21 to C35.

n-Alkanes have been used successfully in digestibility studies in horses (Pereitti *et al*., 2006). Apparent digestibility and feed intake can be measured simultaneously by using internal markers and external markers (Gudmundsson and Thorhallsdottir, 1998), who compared n-alkanes to the *in vitro* method. N-alkanes underestimated the AD, while the *in vitro* method overestimated the AD. The coefficient of variation was also high for the feed and the faecal recovery of n-alkanes.

In contrast, Stevens *et al*. (2002) used n-alkanes as internal and external markers to determine intake and AD in horses fed kikuyu and ryegrass. N-alkane C32 was used as an external marker, while C31, C33 and C35 were used as internal natural markers. There was an underestimation of apparent digestibility based on the n-alkane method compared to the total collection method. The dry matter intake was comparable to digestibilities obtained using the conventional method. As a result, Stevens *et al.* (2002) concluded that n-alkanes gave unreliable results and will not provide accurate estimations of apparent digestibility in horses.

4 DIGESTIBILITY STUDIES IN THE DETERMINATION OF NUTRIENT RETENTION

Digesta kinetics can be modelled *in vivo*, *in vitro* and *in silico*. Hence, the utilization of raw materials and compound feeds in the horses can be evaluated using these methods, in addition to proximate and Van Soest analysis. *In vivo/in situ* (including *in sacco*, fistulated and cannulated) techniques are widely used to study gastrointestinal processes, providing information about the nature and sensitivity of horses to various changes (France *et al.,* 2000). These overcome many of the limitations in the routine use of *in vivo* trials (Broderick and Cochran, 2000). *In vivo* trials remain the gold standard, particularly in digestibility experiments. Simultaneous *in vivo* digestibility trials in horses and wethers were used by Martin-Rosset *et al*. (1984) to develop prediction equations so that *in vivo* ruminant data could be used to generate values for horses. The R^2 value for legumes was 0.71 compared to a grass value of 0.96, indicating that a closer correlation was found between sheep and horses for grasses than legumes. Vander Noot and Trout (1971) used *in vivo* studies in steers to predict values for horses. They found that crude fibre was the best predictor of dry matter digestibility (DMD) (R^2 = 0.81). However, predictions based on *in vivo* studies applied to different species are prone to error, and the INRA (French Net Energy System) model is heavily dependent on such extrapolation (Cuddeford, 2000). To yield useful data, *in vitro* and *in situ* techniques must somehow mimic specific *in vivo* digestion processes in each herbivore. Ideally, *in vitro* and *in situ* estimates of rate and extent of digestion should be quantitatively similar to those obtained *in vivo*. Estimates that are only correlated with *in vivo* values are also useful, indicating that important, perhaps limiting, characteristics of *in vivo* digestion can be simulated by the experimental system (Broderick and Cochran, 2000).

Digestibility is the proportion of feed that has disappeared in the digestive tract following ingestion and which is not excreted in the faeces (Dryden, 2008). It is commonly expressed as a coefficient or percentage. There are two measures of digestibility coefficients, namely the apparent digestibility (AD) and the true digestibility (TD). The AD is the preferred estimate because it is less complicated. It does not take into account the endogenous substances (Glade, 1984). Digestibility in animals has been estimated using *in vivo* and *in vitro* methods. The *in vitro* method is lab based. A feed sample, a buffer and a sample of digestive microbes obtained from the caecal fluid are used to estimate feed digestibility (Dreyden, 2008). This is not a true estimate because it does not represent the movement and digestion of feed through the entire gastrointestinal tract (GIT).

In most herbivore studies, especially in ruminants, the *in sacco* method using a nylon bag suspended in a cannulated animal is the most popular (de Fombelle *et al*., 2004). This method has also been used in horses, where horses were fitted with ileum or caecal cannula (Bush *et al*., 2001; de Fombelle *et al*., 2004) to determine the dry matter disappearance. This method provides a poor estimation of digestibility because it takes into account only feed particles degraded in a specific compartment and not the entire gut. The process of cannulating an animal is also very expensive and may impose health issues on the horse. Peloso *et al*. (1994) found that ponies fitted with ileal fistula experienced a mild colic and partial anorexia. Swelling and leakage at the surgical site were also observed by Lopes *et al*. (2010). There is also a risk of breakage of the cannula due to rubbing and biting. De Fombelle *et al*. (2004) also found that it is difficult to keep cannulated horses in good health. Furthermore, it is labour intensive since only few samples can be fitted at a time. There are also difficulties associated with human safety when obtaining feed samples from the ileal cannulas. In contrast, the *in vivo* method provides an estimation of digestibility based on a live healthy animal and is therefore considered a superior method. Caecal cannulas do not separate caecal and colonic activities, which represents a further limitation to their use. The mobile bag technique has been used in horses (de Fombelle *et al.,* 2004; Silva *et al*., 2009). Compartmental analysis, based on total tract marker studies, can dispense with the need for surgical modification in horses, but interpretation must be carefully considered (Cuddeford, 1999).

Factors which affect digestibility of feeds and nutrients are linked to animal factors (species, breed or subspecies differences, variation between animals, age, exercise and health status) and feed factors (chemical composition, ration composition (associative effects), effect of feed processing, level of feed intake). Digestibility is the product of the retention time and the degradation characteristics of a foodstuff (Forbes, 1996). Rates of passage are a measure of how long digesta is retained in the gut for mechanical mixing, digestion, microbial fermentation and absorption (Warner, 1981). Along with rate of fermentation, the mean retention time (MRT) of feeds within the gastrointestinal tract (GIT) is important in determining the extent of feed digestion and efficiency of microbial metabolic activity in herbivores.

4.1 *In vivo* Systems (Digestibility Experiments)

In situ and mobile bag technologies are applicable in the horse (Hyslop, 2006). The *in vivo* method commonly used is the faecal collection method. This method gives a true measure of the total tract AD because it takes into account the whole gut (Miraglia *et al*., 1999). A total faecal collection method involves feed intake and the total faecal output. This method should take place

over a 20 d experimental period, divided into a 14 day preliminary period and a 6 day faecal collection period. In some studies less than 14 d preliminary periods have been used and satisfactory results were still obtained (Kane *et al*., 1953; Lindsay, 2005). All the normal activities of the horse such as movement and exercising must be accounted for to ensure good health and non-interference with digestibility throughout the experiment. The test group of horses can either be stalled in single pens or stalled as a group, in which case horses should be fitted with a harness to collect faeces (Crozier *et al.*, 1997). The feed intake must be monitored constantly by recording the amount of feed given and the amount of feed refused at the end of the day. The refused feed and the faeces collected is then stored before analysis. The faecal sample must be dried to constant weight or frozen (Bush *et al*, 2001) to avoid moulding and changes in nutritional composition during the storage period. The apparent digestibility (AD) of the feed is then calculated using the following formula (Schürch, *et al*., 1950; Holland *et al*., 1998; Pereitti *et al*., 2006).

AD% = [(Intake–faecal output)]/intake) x100

Digestibility coefficients such as crude protein (CP), crude fibre (CF), fat, ash, organic matter (OM), nitrogen detergent fibre (NDF), and acid insoluble detergent fibre (ADF) can then be determined from the above equation, which is modified as:

Nutrient AD% = {(nutrient intake – nutrient in faeces)/nutrient intake] x 100

The total collection method may be difficult to implement in cases where the total faeces collection is not possible, for example where horses are grazing, in competition and racing (Goachet *et al*., 2009), because this interferes with their routine training. It also requires more labour for frequent collection of faeces and it is very expensive. Faeces left for too long will be contaminated if it is collected from the ground or may cause stress in the horse if faecal collection bags or horse diapers are used. However, in these cases, markers can be used.

Certain feeds cannot be fed as a sole component of the diet. Therefore, in order to determine their digestibility, they must be mixed with another basal feed which is usually roughage. The digestibility of the basal feed is known and it is assumed that the nutrients in this basal feed will have the same digestibility as they did when they constituted the entire diet. One assumes that in mixing the two feeds neither one would alter the digestibility of the other one. The level of inclusion of the test feed must be high enough to ensure that differences between the test and basal feeds are observed. The smaller the proportion of the test feed to basal feed, the greater the number of animals required per test. A series of digestion trials can be conducted in which different levels of

the test feed are added to the basal feed. The digestibilities of these mixtures can then be plotted on a graph and by extrapolation to zero basal level, the digestibility of the test feed can be calculated (Schneider and Flatt, 1975), provided there is no interaction effect between the basal and the test feed.

Digestibility by difference is another method (Palmgren-Karlsson *et al*., 2000; Lindsay, 2005) for estimating the AD. This method is used to determine the value of supplements or feed that are not used alone as complete diets, and it is therefore able to account for the associative effects of feeds. The basal diet and the basal diet plus the feed in question (e.g. concentrate) is fed to horses. The digestibility of nutrients in the concentrate is calculated as:

$$NDconcentrate = \frac{\left(TDN - \left(\frac{\%\,N forage}{\%\,N\ concentrate} \times NDforage\right)\right)}{\frac{\%\,N\ conc}{TDN}}$$

ND= nutrient apparent digestibility in the concentrate or the forage, % N is the amount of that nutrient and TDN is the total apparent digestibility of the nutrient.

4.2 *In vitro* Systems

Applegate and Hershberger (1969) were probably the first to use *in vitro* digestion techniques to investigate forage digestibility in horses. Since that time a number of different approaches have been developed. There is a continuing request from the compound feed industry for a rapid *in vitro* method capable of assessing the nutritional quality of both the raw materials, which make up the formulated compound feeds fed to horses, and the formulated compound feeds themselves. *In vivo* techniques are expensive and time-consuming to carry out and also require skilled personnel. Concerns over animal welfare and ethics associated with experimentation, specifically regarding animals kept in metabolism crates, and the use of surgically modified animals, makes *in vitro* experimentation more attractive.

In vitro techniques with rumen fluid or substitutes have been routinely and extensively used for evaluation of ruminant feed, and in monogastric nutrition using pig gut inoculum (Lowgren *et al*., 1989). Applegate and Hershberger (1969) showed that the *in vitro* rumen fermentation technique can be successfully adapted to *in vitro* hindgut fermentation studies.

4.2.1 Pepsin-cellulase

In this technique, feed samples are incubated at 37°C for 48 hours in an acid-pepsin mixture, either at the start of the experiment (monogastrics/hindgut fermenters), or after a 48-hour ruminal fermentation stage in the case of ruminants. This is then followed by the incubation of the samples in a cellulase and buffer solution for a further 48 hours. This method was used with 52 forages whose organic matter digestibility (OMD) had been determined *in vivo* by Martin-Rosset *et al.* (1996). Using multiple regressions, the following equation was derived:

$$OMD\ (\%) = 29.38 + X + 2.30315Y - 0.01384Y^2\ (R^2 = 0.927)$$

X = 4.12 for green forages, 0 for grass hays and –2.61 for legume hays

Y = cellulase dry matter digestibility (DMD) %.

This relationship has been shown to be more reliable than that which depended on chemical composition. Therefore, for any feeding system, this enzymatic method deserves further study for the purpose of evaluating horse feeds.

4.2.2 Gas Production and Fermentation Kinetics

The *in vitro* gas production technique (IVGPT) is an *in vitro* technique that has received much attention in ruminant research (Rymer, 1999; Dhanoe *et al*., 2000; Ouda, 2007). The two-stage method was used by Tilley and Terry (1963), and subsequently modified by Minson and McLeod (1972) and used by others (Lowman *et al*., 1996; Murray *et al*., 2003; Hussein *et al*., 2004; Hackland, 2007). It was developed by Menke *et al*. (1979) and refined by Theodorou *et al.* (1994). The method was modified to mimic the digestive system of the horse, with microbial fermentation following acid digestion, while vitamins and micronutrient support for fermentation are provided. Murray *et al.* (2006) used the method of France *et al.* (1993) to fit curves to experimentally derived gas accumulation profiles, to which Campos *et al*. (2004) was able to derive a model for two compartment fermentation kinetics.

Macheboeuf *et al*. (1998) used the Menke *et al.* (1979) method with caecal fluid inocula to ferment the same feeds as used previously in the pepsin-cellulase method. Two relationships were obtained, one for alfalfa hays and the other for green forages and grass hays. In the former, gas production after 24 hours was the best predictor of OMD ($r^2 = 0.76$), whereas in the latter case, gas production at 25 hours, together with crude protein (CP) were the best ($r^2 = 0.87$). Using faeces as the source of inoculum improved the prediction of OM for alfalfa hays ($r^2 = 0.96$) from the rate of gas

production (Macheboeuf and Jestin, 1998). The prediction for green forages and grass hays was as good with faecal inocula as with caecal inocula using the same parameters ($r^2 = 0.86$).

Lowman *et al.* (1999) used the method of Theodorou *et al*. (1994) with faecal inocula to predict dry matter digestibility values based on the *in vivo* values of 16 diverse feeds. The best predictive equation that was obtained used gas production parameters:

DMD ($g.kg^{-1}$) = 155 + 6209 FRGP + 1.505 GP ($R^2 = 0.72$)

FRGP is the fractional rate of gas production estimated when 50% of the gas has been produced

GP is the total gas production.

It is clear that gas production methods can provide useful data although, so far, the enzymatic method seems to be more reliable (Cuddeford, 2000).

Fermentability is an indicator of the ease at which this happens and depends in part on the length of fermentation time. *In vivo* this is related to the time the digesta spends in the gut (Campbell *et al*., 2002). Short chain volatile fatty acids (VFA), which are the end products of fermentation, are used as energy sources by ruminants and hindgut fermenters. Fakhri *et al.* (1998) found that the volume of gas produced during an *in vitro* fermentation procedure was a reflection of the amount of carbohydrate fermented. Highly fermentable carbohydrates produce more gas during fermentation than less soluble carbohydrates. However, when comparing feeds of similar digestibility, the feed with a lower gas production may have a higher nutritive value because more of its degraded fraction is likely to be partitioned to microbial biomass rather than to fermentation acids and gas. Gas is a nutritionally wasteful product and excessive gas production in the caecum leads to discomfort and irritability in the horse (Kohnke, 1998).

Lindsay (2005) manipulated protocols for ruminant digestibility studies for use in horse research. There are many different methods of determining fermentation *in vitro* (Rymer, 1999). Tilley and Terry (1963) mimicked gastric digestion pre-fermentation and were able to accurately predict the *in vivo* digestibility of many forages (Rymer, 1999). In this method, the feed sample is initially digested under conditions simulating rumen fermentation, followed by an acid pepsin digestion to solubilize the protein in the feed sample. Lindsay (2005) altered this method to mimic the digestive system of the horse rather than a ruminant. An initial acid-pepsin digestion (simulating the foregut) was followed by microbial fermentation as the final step (simulating the hindgut) (Lindsay, 2005). Hackland (2007) dispensed with the acid digestion step entirely. Predigestion to mimic foregut

digestion in the horse would seem essential at different forage contents in the daily ration, and will be the subject of further research by the author of this dissertation.

The gas production technique is a measure of the proportion of the feed that is fermented (Rymer, 1999). Accurate gauging of the pH and fermentation changes in the hindgut of horses on high grain diets will help towards providing an objective means of explaining the performance (and behaviour) of the horse. The *in vitro* gas production technique generates kinetic data and measures the appearance of fermentation gases, notably CO_2, CH_4 and H_2 (Adesogan, 2002). The inoculum that is used is the single greatest source of variation in this technique (Rymer, 1999), such that inoculum should be collected at the same time daily from the same animals before the morning feed (Rymer, 1999). Cone *et al.* (1996) observed that the rate of fermentation increased when rumen fluid samples were taken after the morning feed, but total gas volume was not affected. Lindsay (2005) reported no significant differences in the DE, CP, GE, DM, CF, ADF and NDF digestibilities when horse faeces and rumen fluid were used as the source of microbial inoculum.

In a trial by Murray *et al*. (2003), the source of the faecal inoculum collected from ponies that were fed diets with varying levels of starch had little effect on the results of the *in vitro* digestibility study. Gas production profiles using the *in vitro* gas production technique showed that the donor horse had no effect on the rate or extent to which the feed substrates were degraded. Murray *et al*. (2003) used three substrates: a grass hay, a low starch concentrate and a high starch concentrate, which showed showed significant differences in their individual fermentabilities. There was no difference between the total gas volume produced between the hay and the low starch concentrate but significantly more gas was produced when the high starch concentrate was fermented. Other sources of inoculum for IVGPT studies include caecal fluid, effluent from a RUSITEC, faeces and frozen or freeze dried rumen fluid, and the use of pure bacterial cultures in the future may increase the reproducibility of the technique and reduce the reliance on surgically modified animals (Rymer, 1999).

The *in vitro* technique has many advantages over *in vivo* or *in situ* methods. *In vitro* techniques are simple, inexpensive and require standard laboratory equipment. It requires only a small quantity of test feed and it can be used as a rapid screening or indexing technique for a large number of forage samples (Schneider and Flatt, 1975).

4.2.3 Factors affecting the gas production profile

The gas that is produced during an *in vitro* fermentation needs to be released from solution if it is to be detected (Rymer, 1999). Accumulated pressure can be vented (Theodorou *et al.,* 1994) or the

volume of the fermentation vessel increased (Schofield and Pell, 1995). However, allowing the gas pressure to increase can alter the gas production (GP) profile (Theodorou *et al.,* 1998; Rymer, 1999). Fermentation end product ratios can alter gas production early in the fermentation cycle (Hall and Weimer, 2007), so fermentation time is important. Agitation of the sample is also important (Wilkins, 1974; Rymer, 1999; Pell and Schofield, 1993; Stevenson *et al*., 1997) when using an automated pressure transducer.

Sample size should be large enough to reduce experimental error, but small enough such that buffering can occur and accumulated gas will not affect the pattern of gas evolution (Theodorou *et al.* 1994). Dried ground samples approximate the mechanical plant cell destruction caused by chewing (Rymer, 1999). Increasing the proportion of rumen/caecal fluid in the inoculum solution has been observed to increase the volume of gas produced (Wood *et al.,* 1998), increase the rate of gas production and decrease the observed lag time (Pell and Schofield, 1993). It is important that the microbial activity of inoculum is relatively constant between experiments and that initial microbial activity is measured in blanks (Rymer, 1999). Blanks correct for changes in atmospheric pressure and residual fermentable organic matter in the inoculum (Pell and Schofield, 1993).

The feeding programme of the inoculum donor animal is important, in that the volume of gas produced is greater when rumen fluid is taken from steers fed grain rather than hay (Trei *et al.,* 1970; Cone *et al.,* 1996). End point measurements are not strongly affected by donor species (Rymer, 1999), although fermentation kinetics may be different. Inocula should be strained carefully because mixing rumen solids with samples results in decreased lag times and increased rate of fermentation (Mertens and Weimer, 1998). Straining is traumatic for microorganisms (Rymer, 1999) and increased recovery time should be considered (Harris, 1996).

Faecal inoculum in ruminants is not the same as the rumen inoculum because it follows gastric digestion and caecal microbial additions (Kohnke, 1998). However, a greater lag time in incubations over 48 hours allow reconstituted sheep faeces to simulate rumen inoculum (Nsahlai and Umunna, 1996). In contrast, hindgut fermenter faeces can replace caecal fluid (Lowman *et al*., 1996; Lowman *et al*., 1999; Murray *et al.,* 2003; Lindsay, 2005; Hackland, 2007).

4.2.4 Factors affecting the accuracy of in vitro results

Repeatability is influenced by the accuracy of weighing, the fineness of samples, maintenance of anaerobic conditions and the correct pH and the number of samples. The composition of micro-organisms can change rapidly in a test tube to a composition adapted to the substrate, which is

different to the parent population initially collected from the animal. End products may accumulate which can inhibit metabolic activities. Extraneous reactions that do not normally take place inside the animal can occur. Therefore it is strongly advisable to include feeds of known *in vitro* digestibility as standards with each batch to ensure reliable results (Schneider and Flatt, 1975).

4.3 Near infrared spectrophotometry (NIRS) and GLC

NIRS is a routine laboratory procedure that is used extensively to evaluate forages for ruminants. The calibration curve should be determined in the machine in which the samples are to be tested. Andrieu *et al.* (1996) applied the NIRS method to 52 forages that had been evaluated *in vivo* and obtained a prediction of OMD with an r^2 = 0.96. They concluded that the NIRS method was as reliable as the enzymatic method. Thus, two techniques are available for determining OMD, which is a crucial component in the calculation of UFC values (Cuddeford, 2000). Gas liquid chromatography can be used to determine non-starch polysaccharides (NSP) (Englyst *et al*, 1992).

5 FLOW OF ORGANIC MATTER IN THE HORSE

The horse has a requirement for energy, CP, amino acids (AA) and fibre as well as vitamins and minerals. The site of digestion of the raw material strongly affects the breakdown of the feed ingredient, and rates of passage and fermentability of substrate in the hindgut are important in the horse. For this reason, not only should the feed ingredient be assessed, but also its metabolic fate in the horse, as related to how it is fed to the horse. To describe or predict the performance of a horse, an effective feed evaluation system is required, in order to formulate diets of optimum quality. Feed evaluation systems should be simple, which is a characteristic that is often in conflict with accuracy of prediction of response over a wide range of rations. Most feed evaluation systems used on a large scale are a compromise between simplicity and accuracy of prediction (Birkett, 2001). In the future, NIRS will allow for greater speed, lower costs and far greater complexity in a routine analysis of horse feeds.

5.1 Nutritional evaluation

Nutritional evaluations of feedstuffs are undertaken for many different reasons:

1. To measure the extent to which one basic feed can replace another to support an animal function (i.e. the relative values of primary feed ingredients);
2. To relate feed characteristics to animal function;
3. To allow the prediction and/or control of animal performance through nutrition

(Emmans and Oldham, 1988).

Farmers throughout the world have developed intuitive and often quite elaborate methods for assessing the feeding value of available feed resources. Typically, this knowledge became established within a community because a relationship with production could be demonstrated and used to predict and manipulate performance (Chesson, 2000). Interestingly, subsequent attempts to relate a farmer's historical understanding of nutritive value to current laboratory-based proximate analysis have often proved unsuccessful (Thapa *et al.,* 1997). The development of laboratory methods to characterize feedstuffs was an attempt to formalize this traditional knowledge, allowing feed evaluation to be put on a numerical basis, as required by the feed industry. Tables of feeds ranked by nitrogen content were available by the middle of the 19th century (Bossingault, 1843), with total digestible nutrients following (Wolf, 1874). However, it was not until the turn of the 20th century that work underpinning the present concepts of digestible, metabolizable energy and the net energy of feeds for farm animals was published (Armsby, 1903). Recently there has been a greater urgency to refine the methods used to characterize feed resources. Changes in production methods and feed formulation, the introduction of new feed resources and animals with greater genetic potential, together with falling profit margins for feed producers have shown the need to define feeds and their ingredients more accurately, in order for more accurate predictions to be made (Chesson, 2000). Existing tables of feed composition are still based on the crude nutrient fractions of the proximate analysis scheme and the cell wall fractions of the detergent fibre system (Van Soest, 1994). Additional information on fatty acid, amino acid, mineral and vitamin contents may also be included. The proximate analysis of feeds, an arbitrary and empirical series of tests, which allowed some prediction of animal performance, was first described by Hanneberg and Stohmann (1864) in the 19th century. Under this scheme, five fractions: moisture, ash, ether extract, crude protein (N x 6.25) and crude fibre, were determined directly and the sum of all five was expressed as grams per kilogram, subtracted from 1000 to generate a sixth fraction, this being the

nitrogen free extract (NFE). Severely criticized for its imprecision over the years, proximate analysis has been replaced by other methods in most laboratories. Crude fibre and nitrogen free extract (NFE) have been found to lack any consistent relationship with recognizable components of crop plants. Crude fibre contained some, but not all, of the polymers which constitute the plant cell wall, while NFE encompasses water-soluble carbohydrate, starch, organic acids and much of the pectin fraction of cell walls. These measures have now been replaced by the direct determination of the water-soluble components and of neutral detergent fibre (NDF). Acid detergent fibre (ADF), which provides a measure of only the cellulose and lignin content of the cell wall, has found value in the description of forages because of its statistical relationship with degradability (Chesson, 2000). The limitations of these chemical analytical methods of feed description applied to predicting nutritive value are well recognized and documented (Hoffman, 2003).

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

Chemical analysis is the starting point for determining the nutritive value of feeds, but the value of a feedstuff does not depend entirely upon the amounts of the different nutrients it contains. The value of a feed depends upon the amounts of these nutrients that the animal can digest and use. The chemical composition alone of any feeding stuff is a very imperfect standard by which to judge its nutritive value. The true standard should be the interaction between chemical composition and the digestion, because the factorial of these two aspects will determine the quality of the feed to the horse. It is only the digestible portion of the feed that can serve to maintain the vital functions and is of value for energy and the formation of animal products, and to ensure animal performance. However, the chemical composition and the percentage digestibility are not all that determine the value of a feed. Two feeds may be equal in composition and equally digestible, yet one may be more valuable than the other because its digested matter can be used to better advantage by the body (Schneider and Flatt, 1975). Furthermore, two feeds, such as hay and lucerne, may be synergistic in the correct balance.

Feed evaluation involves the use of methods to describe animal feedstuffs with respect to their ability to sustain different types and levels of animal performance. Subsequently, the acquired data

can be used, with appropriate animal indices, in feeding systems comprised of empirical equations, to determine whether a desired level of animal performance can be achieved from specific diets. The ultimate goal of feed evaluation is to optimize the efficiency of feed utilization and animal performance at a cost most affordable to the animal owner. It is important to establish the potential of major feedstuffs for horses, and the need for appropriate supplements, in order to overcome nutritional deficiencies and raise the level of performance (France *et al.,* 2000). Although the horse does not supply a tangible animal product of which the animal producer can receive financial return, feed evaluation is still vital so that nutritionally induced illnesses, such as colic, can be reduced, and performance in the various equine sports can be improved. Horse racing, for example, does not supply a tangible product as such, but the financial returns an owner can receive from a winning racing performance, provide a powerful incentive for proper feed evaluations.

5.2 Flow of Nutrients

Emmans (1994) described the flow of organic matter in the monogastric above maintenance (Figure 1.4). Instead of a single site of digestion and absorption, principally in the small intestine using a single array of enzymatic hydrolytic products (as in pigs and chickens), the hindgut fermenter processes different nutrient classes, and has several absorption zones and utilizes both enzymatic hydrolysis and microbial fermentation (Figure 1.5).

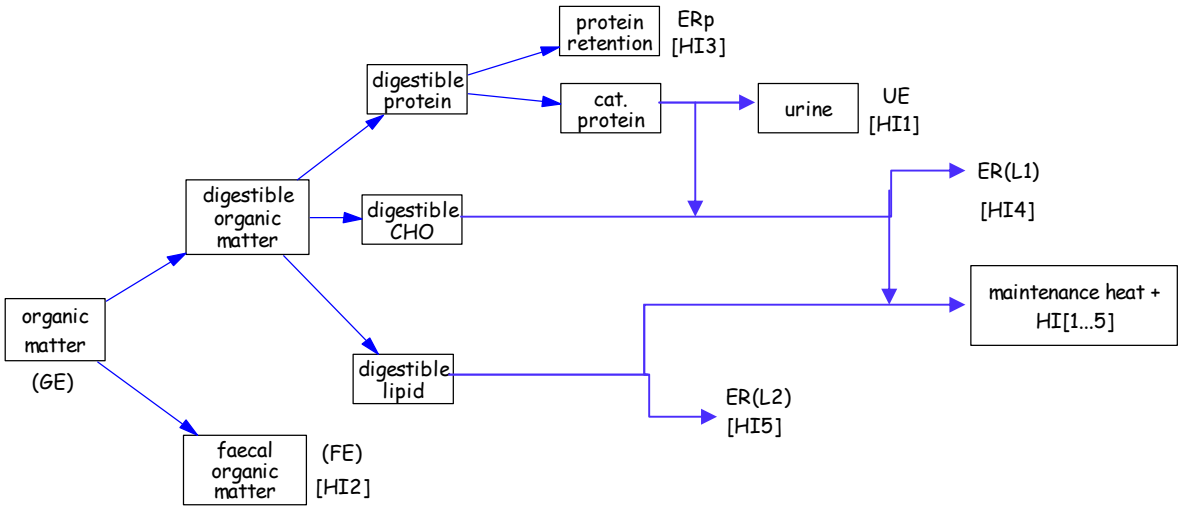

Figure 1.4 Flow of organic matter in the monogastric, with no caecal activity, above maintenance (Emmans, 1994), where CHO = carbohydrate, GE= gross energy, FE= faecal energy, ER_p= energy retained as protein, UE=urinary energy, ER_L= energy retained as lipid, HI= heat increment.

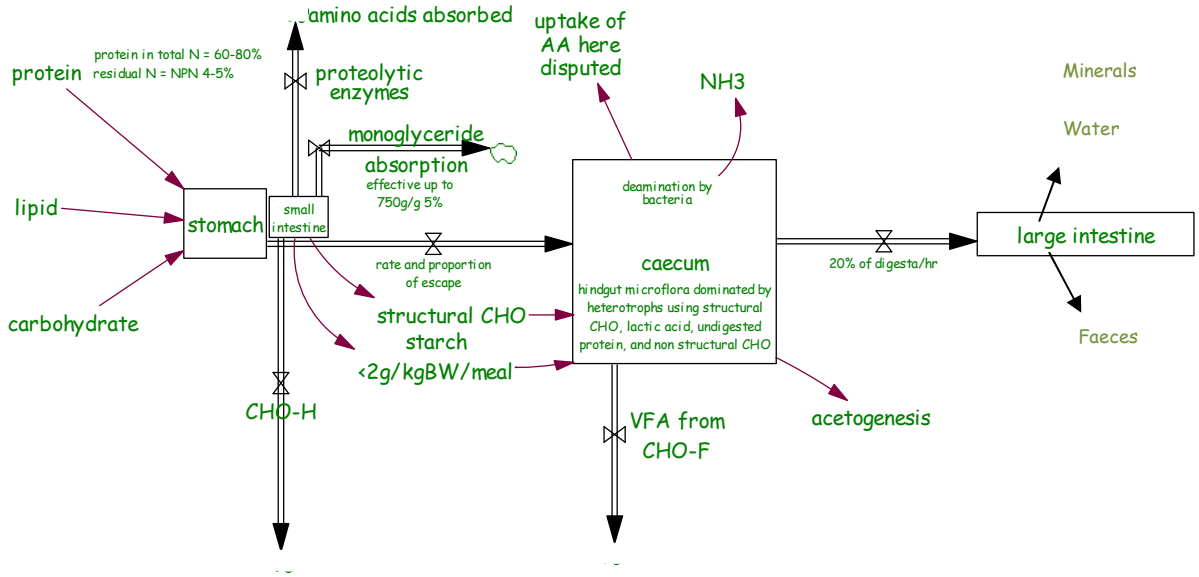

Figure 1.5 Schematic representation of nutrient flow in the horse, with monogastric and caecal activity (Author's own). Abbreviations: N=nitrogen, NPN=non-proteinic nitrogen, CHO=carbohydrate, CHO-H=hydrolysable carbohydrates, BW=body weight, AA=amino acids, NH_3=ammonia, VFA=volatile fatty acids, CHO-F=fermentable carbohydrates,

5.2.1 Fat

The balance or synchrony between nutrients is important as well, especially for fats (Geelen *et al.*, 1999). Dietary lipids can be an alternative source of energy in horses and provide essential fatty acids to improve skin and coat condition (Oldham *et al*., 1990). The replacement of fermentable carbohydrates with lipid in the diets of sport horses has been proposed for glycogen sparing (Geelen *et al*., 2001), energy provision without heat increment (Kronfeld, 1996), and conditioning (Ellis and Hill, 2005), as well as temperament moderation (Holland *et al*., 1996). Positive effects of adaptation to fat as a fuel in athletic training in horses have been identified (Kronfeld *et al*., 1994; Pagan *et al*., 1995). Fats are not supposed to have any long term negative effects (Zeyner *et al*., 2002), although effects of fermentation effects have not been considered. The depression of the activity of especially cellulolytic bacteria (Jenkins, 1993; Jansen *et al*., 2000; Jansen *et al*., 2007a) in the hindgut cannot be disregarded if optimal use of fibre cellulose is to be made (Figure 6.9). Reduced fibre digestibility also occurs at levels of 25% and 35% NDF (Bragal *et al*., 2008). Antimicrobial effects of fat on rumen fermentation is well documented (Onetti *et al*., 2001), and in cattle, the ammonia-N was higher when 4% fat was fed. Fat levels of about 5% tend to be negatively related to fermentative capacity and certainly fibre digestibility (Jansen *et al*., 2007a/b). This problem provides a good reason to evaluate the antimicrobial effects of fat and fat sources in horse diets. Low levels of fat (100ml.day^{-1}) do not affect nutrient digestibilities *in vitro* and *in vivo* (Bush *et al*., 2001). No direct requirement for fat in horses could be found in the literature.

Fat-adapted horses can potentially meet energy demands better on a fat adapted diet in endurance training (Treiber *et al*., 2006). Fats delay fatigue and reduce the incidence of digestive disorders related to high carbohydrate diets (Hambleton *et al*., 1980). Horses exhibit aerobic endurance when fed a high (10%) fat diet (Oldham *et al*., 1990), by increasing muscle glycogen storage in fit horses, and increasing the amount of muscle glycogen utilized during an anaerobic workout. Metabolism of fats provides pyruvate to feed the aerobic pathway. It delays blood glucose and glycogen depletion, maintaining higher muscle reserves during extended exercise (Frape, 1986; Hyyppa, 2005). Addition of 12% DE as fat has been reported to increase resting glucose concentrations by approximately 26% (Kohnke, 1998). To avoid post-prandial glycaemic and insulinaemic responses, feeding strategies should reduce starch rather than add fat (Vervuert *et al*, 2009b). The reduction of gut fill on fat-boosted diets may lower hindgut fluid reserves trapped in the cellulose and lignin fibre structures of grains and hays. Fats and oils used in horse feed are high grade and therefore expensive. Oils added to horse feeds must be properly stored to avoid rancidity (Schumaker *et al*., 1997). Commercially produced feeds with high levels of fat deteriorate quickly in storage (McDonald *et al.*, 1995).

Lipids are a class of hydrocarbon- containing organic compounds, which are cleaved into monoglycerides by lipases. Lipids are used for energy storage and as structural components of cell membranes. Fat can reduce the protein: energy ratio and allows for a reduction in the dietary starch content by making the feed nutrient dense (Hambleton *et al*., 1980). Fats contain 2.25 times more energy per unit weight than carbohydrates and proteins (Hambleton *et al*., 1980). The main reason for feeding high fat diets to performance horses is to provide the horse with a high-energy, easily digestible diet (Oldham *et al*., 1990). Fats have a digestibility ranging from 76 to 94% (Pagan, 2000). Even at high levels, dietary fat is digestible (Pagan, 2002). Bowman *et al.* (1977) in Hambleton *et al.* (1980), tested up to 20% dietary fat which was 90% digestible by the horse.

Both fats and proteins can be converted to glucose to fuel the horse's energy requirements (Kohnke, 1998). Protein does so through the conversion of the carbon chains of amino acids to intermediate chains and some of the carbon chains to glucose (Frape, 1986). Fats are hydrolysed to fatty acids and glycerol. The glycerol is then converted to glucose and the fatty acids broken down by beta-oxidation in the mitochondria, producing ATP and acetyl-CoA (Frape, 1986).

5.2.2 Protein

The level of protein in a feed has been identified as a major reason why people purchase a specific horse feed (Hiney and Potter, 1996). While digestion of protein occurs in the small intestine

(Slade *et al*., 1971; Wootton and Argenzio, 1975), deamination of excess amino acids can take place in the hindgut, where exogenous (bacterial) D-amino acid oxidases convert amino acids to keto acids, H_20_2 and NH_3. The H_20_2 is immediately converted to water and oxygen by catalase. The crucial event is that the three major pathways of amino acid metabolism all lead to the release of ammonia which must be cleared rapidly from the metabolism, usually via the formation of urea in the liver (Ellis and Hill, 2005). Nitrogen supplied to the large intestine will be utilized by the microflora or be utilized in the synthesis of non-essential amino acids from ammonia (Potter, 2002; in Ellis and Hill, 2005). However, protein supplied in excess of requirement (as frequently happens) (Patterson *et al*., 1985; Householder, 1994), may exceed the ability of the horse to synthesize urea efficiently, being beyond the capacity of the urea cycle (Miller-Graber *et al*., 1991), which will then be detrimental. No ergogenic potential can be identified in the excess provision of protein (Snow, 1994; Harris and Harris, 2005). The small intestine usually contains little ammonia, but in a slaughter experiment, Hecker (1971) confirmed more ammonia in the large intestine, particularly in the rectum, as did Mackie and Wilkins (1988). Excess protein intake increases the burden of unusable nitrogen (Frape, 1994), and contributes to heat stress (Ott, 2005). N retention also plateaus after 0.202g N.kg BW^{-1} or at 1.26g CP. kg BW^{-1} for horses at maintenance (NRC, 2007). Excess protein also increases the times to complete in racehorses (Glade, 1983). Excessive N intake increases water intake and NH_3 excretion (Hintz, 1994), which in a stabled horse can induce respiratory problems (Nielson, 2001). Frank *et al*. (1987) found that there were no consistent benefits or detrimental effects to feeding 20% as opposed to 10% protein to young horses in intense training. Thoroughbred (TB) racehorses typically consume in excess of 80:20 concentrate to roughage ratios (Hackland, 2007). The consequences of excess protein appear not to have been considered, nor are recommendations available for the equine nutritionist on the optimal provision of protein in the horse. However, the incidence of metabolic disorders and gastrointestinal disturbances in horses is increasing (Kalck, 2009).

N forms other than ammonia are required for optimal microbial growth and fibre digestion by caecal bacteria (Maczulak *et al*., 1985; Carro and Miller, 1999; Hainze *et al*., 2003). Ruminal and caecal bacteria ferment structural and non-structural carbohydrates, affecting the extent of NH_3 absorption and urea N recycling and excretion (Reynolds and Kristensen, 2008). Bacteria utilizing non-structural carbohydrate (NSC) produce less ammonia when carbohydrate fermentation is rapid. Ammonia production is moderated by amino acid and peptide uptake, but 34% of ammonia production is insensitive to the rate of carbohydrate fermentation (Russel *et al*., 1992). The fate of N and the production of ammonia is potentially damaging to microorganisms, given an adequate

supply of energy for the microbes (Mair *et al.,* 2002; Medina *et al*., 2002). The inability of 79.5% of caecal isolates in culture to grow with ammonia as the sole nitrogen source, indicates that caecal bacteria need N in forms other than ammonia or urea for growth (Medina *et al.,* 2002). A balanced dietary NDF/starch ratio and probiotic support (*S. cerevisiae)* can limit the extent of undesirable changes in the intestinal ecosystem of the horse (Medina *et al.,* 2002).

The horse's daily protein requirement is increased through work. Protein deficiency in horses is rare. However, under stabled conditions, protein excesses are more likely to occur (Pagan, 2002). Provided sufficient energy is available to racehorses, they can perform adequately on feeds containing 12-14% crude protein. According to Pagan (2002), the present NRC recommendation is reasonable for a 500kg horse. An increased intake of good quality protein may be beneficial during early training because muscle mass and blood cell production is increased. The day after a heavy race, a higher protein level in the feed may help repair of muscle tissue (Kohnke, 1998). NPN (non-protein nitrogen) urea can be tolerated in the horse. Bacteria in the caecum break down the urea to form protein, but no protein absorption occurs in this region (Schmitz *et al*., 1991). Excess nitrogen as urea in the blood can be converted to ammonia, which is toxic and can result in nervousness and airway irritation, ultimately affecting athletic performance (Slade *et al.,* 1971). The association between primary hyperammonemia and antecedent or concurrent signs of gastrointestinal disease, raises suspicion that excessive ammonia production in the large intestine is a possible etiology (Mair *et al.,* 2002).

5.2.3 Carbohydrate

Variable proportions of the energy requirement of the horse are provided by carbohydrates (CHO) as well as lipid and protein. The nutritive value of a feed depends on the proportions of soluble, insoluble but degradable, and undegradable fractions of the feed (Getachew *et al*., 2005). CF, ADF and NDF measure fibre levels in horse diets, with a consistent under-estimation of non-starch polysaccharides (NSP) by the NDF (Ellis and Hill, 2005). These are intended to meet requirements for maintenance and work (Harris, 1997), which are often overestimated in riding school horses (Dekker *et al*, 2007). Requirements in the NRC (2007) are now reported per kilogram body weight.

The source of the carbohydrate will affect pre-ileal starch digestibility (Meyer *et al*., 1993). Each kind of grain has a different structure of starch molecule. Oats contain the most digestible form of starch, followed by sorghum, maize and barley. These differences can be explained on the basis of the differences between the starch granules contained in the different plant materials; oat starch

granules are small and easily digested. In work done by Meyer *et al*. (1993), it was found that pre-ileal digestibility of oat starch (80-90%), regardless of preparation, was significantly higher than that of whole or crushed maize (30%) or barley (26%). The nutritive value of oats can be increased by dehulling (Sarkijarvi and Saastamionen, 2006). Similarly, grinding of corn in this particular experiment increased pre-ileal digestibility from 30 to 51%. Fombelle *et al*. (2004) found that oat and wheat starch had a very high digestibility of above 99%. These results show that these cereals can be safely fed to horses as only a very limited amount of the starch will reach the large intestine (Fombelle *et al*., 2004). The utilization of carbohydrates in grains that are not highly digestible is improved by processing of the grain. Grinding maize can increase digestibility from 30 - 45% and extruding maize improves digestibility to 90% (Kohnke, 1998). The effect of processing does not seem to influence hindgut fermentation of starch though (Jullian *et al*., 2006).

As starch intake increases, small intestine starch digestibility declines (Potter *et al.,* 1992; Kienzle *et al.*, 1994). Recommendations for maximum starch levels in feed are 3.5–4g starch.kg BW^{-1}.$meal^{-1}$ (Potter *et al.,* 1992), 3.5g of starch. kg BW^{-1} (Medina *et al*., 2002) and <1.1g starch. kg BW^{-1}.$meal^{-1}$ (Vervuert *et al.*, 2009c). Non-structural carbohydrate (NSC) can be 32-36% for a high performance horse, while idle horses need little NSC in their diet (Coleman, 1999). Some unlikely sources of NSC indicate that control of this for laminitic ponies requires special management on the part of the horse owner (Watts, 2005). It is the activity or the ability of the equine α-amylase to degrade starch which limits its small intestinal digestion, which can be altered through the exogenous administration of amylolytic enzymes (Kienzle *et al*., 1994; Richards *et al*., 2004). The order of the feeding of roughage and starch can increase pre-caecal digestion time (Vervuert *et al.,* 2009b). Longer lag times have been found when purified starch sources are incubated *in vitro* (McLean *et al*., 2005). Addition of starch to *in vitro* caecal fluid over a 24-hour incubation period led to a significant decrease in pH (1.5±0.2) (Bailey *et al.,* 2002), a principal cause of laminitis and behavioural problems. Increased caecal activity and narrowed acetate:propionate ratio associated with an all-concentrate diet, significantly influenced the horse's desire to chew wood and practice coprophagy (Willard *et al*., 1977). Behavioural changes characterised by chewing wood, eating bedding and wind-sucking were higher in horses fed high grain diets (Rowe *et al.,* 1995).

Grain fermentation by amylolytic bacterial species increases lactate production, decreases hindgut pH, fibre digestion and volatile fatty acid production (Hussein *et al.,* 2004). However, Radicke *et al.* (1991) showed that different grains vary in the amount of lactate produced during caecal fermentation. It is important to avoid or decrease levels of grain that may be the cause of digestive or metabolic disorders due to their rapid starch fermentation (Goer, 2007; Frank, 2006;

Frank, 2009). Improper management of grain feeding (Goodson *et al*, 1988) can predispose horses to health risks, such as colic, laminitis or post-feeding acidemia (Hussein *et al.,* 2004). In a study done by Medina *et al.* (2002), the addition of a live yeast culture to a high grain feed, led to a reduction in the pH drop and a decrease in lactic acid production usually experienced in the hindgut after a starch overload. Virginiamycin can also assist in controlling the accumulation of lactic acid in the hindgut in horses fed high grain diets by killing lactic acid bacteria (Rowe *et al*., 1995). NSP in raw materials, for example, in sugar beet, promotes good degradation, and increase the nutrient value of concurrently fed hay and lucerne (Harris and Bishop, 2007). In addition, sugar beet pulp stimulates the conversion of ammonia into urea (Olsman *et al*., 2004).

The proportion of roughage in the total diet may diminish the consequences of soluble carbohydrates reaching the hind gut. It is widely reported and acknowledged that carbohydrate is provided in excess to both the exercising and the idle horse but some of the consequences have yet to be established. Roughage will decrease the rate of passage to increase exposure to digestive enzymes in the small intestine, and it is reported (Willard *et al.,* 1977; Karlsson *et al.*, 2000) that feeding long stem roughages will slow rate of passage to maximise grain digestion before it reaches the hindgut. On the basic assumption of South African horse owners and managers feeding in excess of 50% of the ration as concentrate feed, little amelioration of the effect of soluble carbohydrate in the hindgut is offered by feeding hay, and the feeds needs to be directly compared for the effect of their providing soluble CHO for fermentation in the hindgut.

An effective scheme for the representation of the usefulness of CHO to horses would include three main fractions (Figure 1.6): 1. A hydrolysable group (yields mainly glucose absorbed in the foregut); 2. A rapidly fermentable group (lactate and propionate, metabolised as 3C and 6C units mainly via glucose; and 3. A slowly fermented group (yields primarily acetate and butyrate, metabolised as 2C and 4C units through acetyl-coA (Hoffman, 2003); fractions 2 and 3 absorbed in the hindgut.

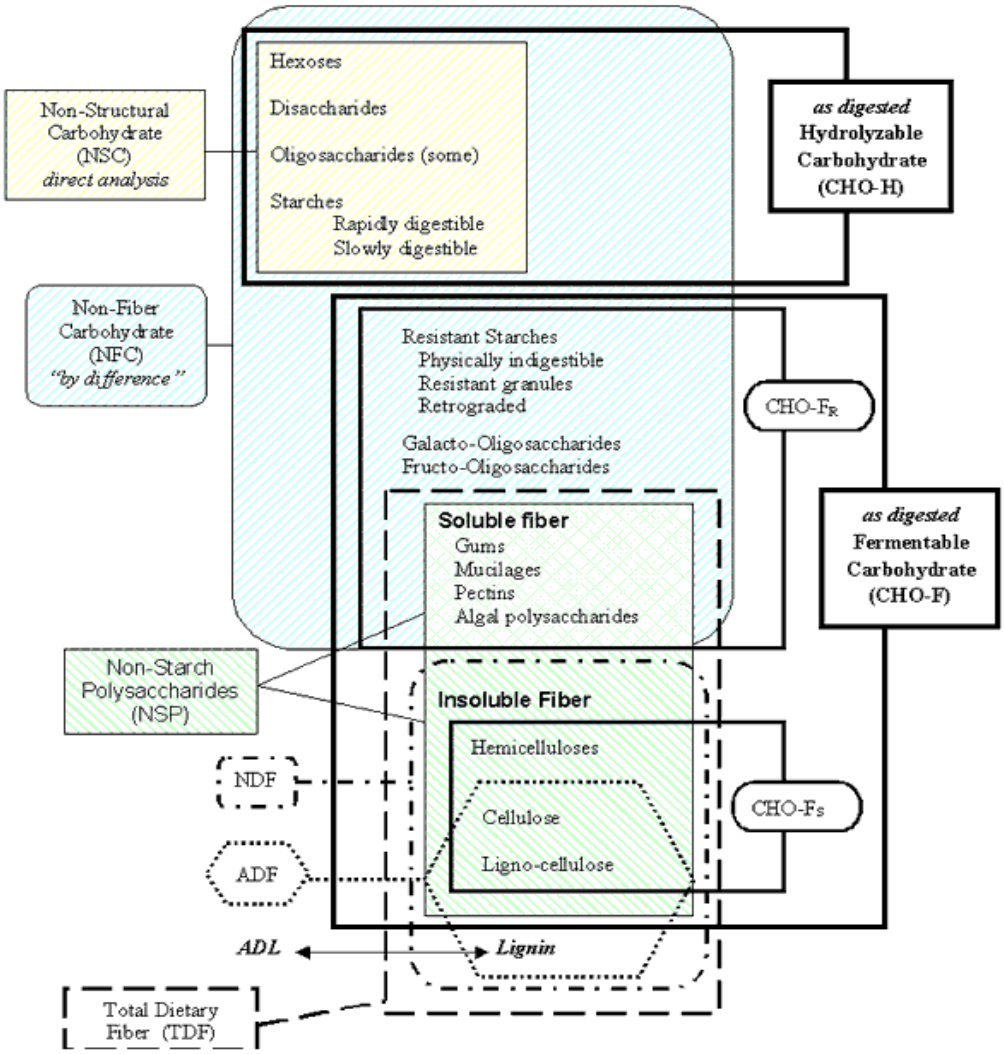

Figure 1.6 The scheme of carbohydrate fractions for the horse, as a comparison of proximate analysis fractions (left) with fractions as digested (right). Abbreviations include ADL, acid detergent lignin; ADF, acid detergent fiber; NDF, neutral detergent fiber; CHO-FS, slowly fermentable carbohydrate; CHO-FR, rapidly fermentable carbohydrate. (Adapted from Hoffman *et al.*, 2001; in Hoffman, 2003).

In the horse, hydrolysis of polysaccharides to simple sugars occurs in the small intestine and involves the enzymatic hydrolysis of α-1,4 linked molecules (Hoffman, 2003). Disaccharides (sucrose, maltose and lactose), some oligosaccharides, and starch are hydrolysed by small intestine enzymes to produce free 6C sugars, glucose, galactose, and fructose. CHO escaping enzymatic hydrolysis in the small intestine are then subjected to bacterial fermentation in the caecum where the horse obtains energy from the production of VFA's. The cellulose β-1,4 links are are broken by cellulase. Hemicellulose, cellulose, lignocellulose, soluble fibes and oligosacharides (galactans and fructans are implicated in laminitis) and starch, when fermented by the action of the microbial ecosystem, produce acetate, propionate, butyrate and lactate and valerate to a lesser extent. The

transmucosal pH gradient in the large intestine facilitates the passive diffusion and absorption of acetate, over proprionic, butyric and lactate (based on MW). VFA's also function as nutrient stimuli in the control of meal frequency (Ralston and Baile, 1983b).

5.2.3.1 Fermentable carbohydrate

The main source of energy for the horse comes from carbohydrates since approximately 75% of all plant matter is comprised of carbohydrates (Hoffman, 2001). Carbohydrates are simple molecules that are straight chain aldehydes or ketones, with many hydroxyl groups added. In the horse, one can distinguish between the sources of carbohydrate that can be processed by mammalian enzymes and those that need to be fermented by microbes (Hoffman, 2001; Ellis and Hill, 2005). Hydrolyzed carbohydrates yield mainly glucose, while fermented carbohydrates yield acetic, propionic and butyric acids, the VFAs. Digestive and metabolic processes are more rapid for hydrolyzed carbohydrates than for fermented carbohydrates, which have to be fermented by microbial populations largely in the caecum of the horse (Ellis and Hill, 2005). Fermented carbohydrates can be further classified according to their rate of fermentation (Hoffman, 2001), i.e. slowly fermented CHO and rapidly fermented CHO. It is important to distinguish between the two because rapidly fermenting forms of CHO tends to give rise to lactate rather than acetate. Lactate is responsible for reducing caecal pH and the subsequent development of caecal acidosis and laminitis. Hindgut pH changes through rapid fermentation have also been connected to behavioural disorders, which result in excessive gas production in the hindgut, irritating the horse (Willard *et al.,* 1977; Ellis and Hill, 2005).

High energy diets can affect the digestion of structural CHO in the hindgut (Julliand *et al*., 2004). Cellulose is composed of β-1,4 linked, unbranched, D-glucose units. Cellulases cleave the glycosidic linkages. Hemicellulose is made of D-pentose sugars, especially xylose. Its levels can be estimated from the difference between the NDF and ADF fractions in the feed.

The increase in the grain ratio in the feed will increase the proportion of propionate:lactate, at the expense of acetate, suggesting the support of rapid over slow fermentation. This favours the proliferation of lactobacilli, which decrease the pH. A pH of 6.0 indicates subclinical acidosis, with less than 6.0 indicating clinical acidosis, which is a common disorder amongst race horses (Richards *et al*, 2006). An excess of 0.4% of BW per meal (or as low as 0.2% of BW) of CHO-H overload will lead to this occurring (Hoffman, 2003).

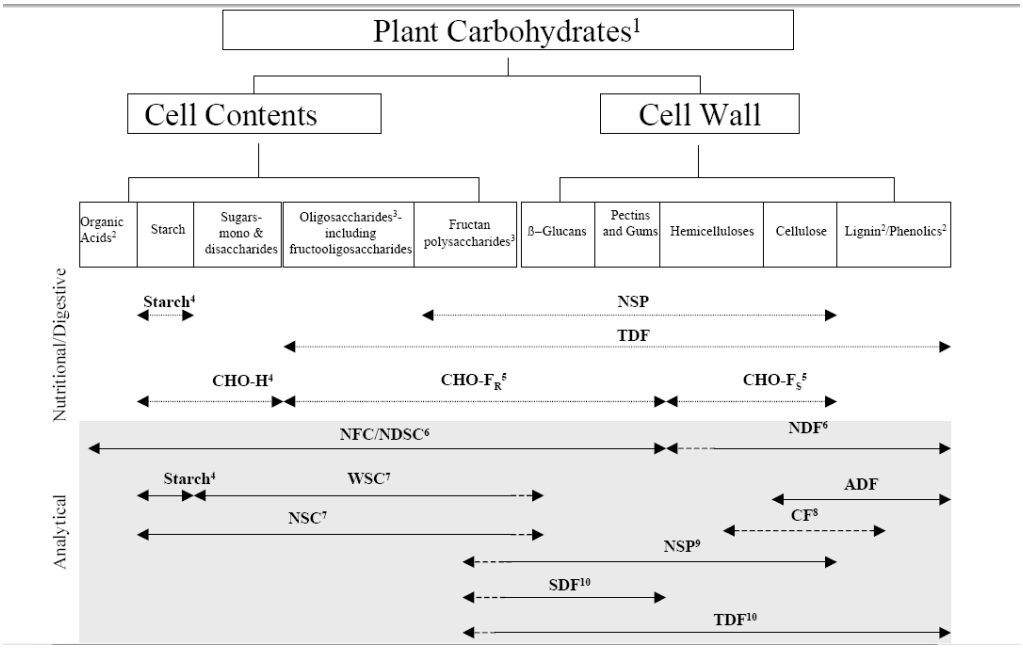

Figure 1.7 Fractionation of plant carbohydrates (Adapted from Hall (2003) and Hoffman *et al*. (2001), in the 6th revision of the *Nutrient Requirements of Horses*, 2007). Current and proposed systems for partitioning dietary carbohydrates based on current analytical methods (lower/shaded bracket; solid/dashed lines) and nutritional or physiologic definitions (upper bracket; dotted lines) are relative to equine digestive function. In the analytical bracket, dashed lines indicate that recovery of included compounds may be incomplete.

Abbreviations: ADF= acid detergent fiber; CF= crude fiber; CHO-H= hydrolyzable carbohydrates; CHO-Fs= slowly fermented carbohydrates; CHO-FR= rapidly fermented carbohydrates; NDF= neutral detergent fiber; NDSC= neutral detergent soluble carbohydrates; NFC= non-fiber carbohydrates; NSC= non-structural carbohydrates; NSP= non-starch polysaccharides; SDF= soluble dietary fiber; TDF= total dietary fiber; WSC= water-soluble carbohydrates

1. Major categories of carbohydrates and associated substances are shown. These categories may not include all carbohydrates produced by plants.

2. Some non-carbohydrate components are included here as they are components of the specific analytical fractions.

3. Specific fructans can be categorized as either fructooligosaccharides or fructan polysaccharides depending on degree of polymerization.

4. A variable fraction of total starch can be resistant to enzymatic hydrolysis and thus some starch may appear in other nutritional fractions.

5. Fermentability of gums may be variable.

6. Some hemicellulose may be soluble in neutral detergent and thus recovered in the NFC/NDSC fraction, rather than the NDF fraction.

7. Recovery of compounds in the analytical WSC fraction (and thus the NSC fraction when NSC is approximated as starch + WSC) may depend on methodology used.

8. Amount of cell wall constituents included in CF analysis varies by feed.

9. From a nutritional perspective, NSP includes all polysaccharides except starch. However, the analytical method for NSP may recover a variable amount of fructan polysaccharide.

10. From a nutritional perspective, TDF includes all carbohydrates resistant to mammalian digestion. However, the analytical method for TDF (and SDF) does not recover oligosaccharides and may recover a variable amount of fructan polysaccharides.

5.2.3.2 Hydrolysable carbohydrate

Non-structural carbohydrates are those carbohydrates that occur as simple sugars in the feed and can be broken down by enzymes produced in the stomach and small intestine of the horse. These carbohydrates are nearly non-existent in hay but form a large part of high grain diets. This category of carbohydrates is made up of: glucose, fructose, lactose and starch. Structural carbohydrates are components of the cell wall and consist of cellulose and hemicellulose. They are resistant to the horse's foregut enzymes. These carbohydrates need to be fermented by bacteria in the hindgut of the horse. Their digestion is dependent on the rate of fermentation and the retention time in the caecum (Harbers *et al.,* 1981). Carbohydrates can only be absorbed in the small intestine as monosaccharides. Starches are broken down into the disaccharide maltose by the enzyme amylase. Maltose, sucrose and lactose are split into their monosaccharide sub-units by the enzymes maltase, sucrase and lactase that are produced by the brush border of the small intestine. These disaccharides are completely digested in the small intestine of a healthy horse. However, the horse's ability to produce amylase is limited, resulting in a proportion of starch escaping digestion in the small intestine and passing into the hindgut. However, the type of forage and the time it is fed relative to grain intake can have a large effect on pre-caecal starch digestibility (Pagan, 1997), as can the amount of grain fed. Meyer *et al*. (1993) in Pagan (1997) found that feeding grass hay instead of ground lucerne meal resulted in a decrease in the digestibility of pre-caecal starch of ground maize from 45% to 16%. This was probably because ground lucerne meal was not as effective as the hay at slowing down the rates of passage of the ground corn because of its fine structure. This emphasizes the importance of feeding good quality, long stem roughage to slow rates of passage and maximize grain digestion before it reaches the hindgut (Willard *et al.,* 1977; Karlsson *et al.,* 2000).

Much of the literature on processing of raw materials is concerned with methods of increasing the pre-caecal digestion of starch (Meyer *et al.,* 1993). Glycaemic responses have been used to test the extent of starch digestion (Hoekstra *et al*., 1999). A high CHO meal is sometimes beneficial to horses after training as this has been shown to increase replenishment of muscle glycogen post-exercise (Lacombe *et al.,* 2004), although Jose-Cunilleras *et al.* (2006) found this not to be the case in exhaustive exercise. Glucose tolerance in younger horses seems to be higher (Ott *et al*., 2005).

5.2.3.2.1 Glucose

Glucose is the main soluble CHO absorbed by the foregut when horses are fed high grain diets. In contrast, VFA are the main energy source in horses fed high roughage diets (Roberts, 1975; Ellis and Hill, 2005). Horses given high grain diets will experience higher peaks and lower troughs of blood glucose than horses maintained on mainly roughage diets (Roberts, 1975; Lawrence *et al*., 1993). Feed type and intake will affect the glycaemic response (Pagan *et al*, 2001). A grain-fed horse will be more energetic when blood glucose peaks and less energetic in the blood glucose trough (Frape, 1986). By increasing the feeding frequencies the cyclic changes in the blood glucose can be smoothed out (Frape, 1986). Provision of excess sugars can make a horse over-energetic if it is an excitable horse, or overweight if it is a quiet horse (Kohnke, 1998).

Carbohydrate digestion is the most energy efficient method of producing glucose (Blaxter, 1956). Glycogenic (proprionic) and lipogenic (acetic, butyric) volatile fatty acids are produced from CHO degradation. Glucose and propionate are stored in the liver as glycogen. Acetate and butyrate are stored as fat. After a feed the concentration of blood glucose will rise above a basal level (Argenzio and Hintz, 1972). Insulin is released as a result of the increase in blood glucose, and aids in the storage of excess glucose as depot fat or muscle glycogen (Argenzio and Hintz, 1972; Trieber *et al.,* 1995), but also inhibits lipolytic activity (Hyyppa, 2005). This prevents excess glucose levels in the blood, and prevents the loss of glucose in the urine (Frape, 1986). Blood glucose peaks about three to eight hours after meal feeding and returns to its basal level within two hours (Argenzio and Hintz, 1972). Insulin levels peak shortly after the glucose peak occurs. The time from glucose peak to the return to the basal level is called the tolerance time. Thoroughbreds are more sensitive to insulin and the insulin activity of the blood than ponies that have a longer tolerance time. This means that ponies can go without food for a longer period than Thoroughbreds and accounts for why Thoroughbreds become more excitable after eating (Frape, 1986). Horses accustomed to high starch diets have high levels of insulin activity and are more inclined to hypoglycaemic shock when fasted (Williams *et al*., 2001). The insulin status of a horse can be determined by measuring glucose tolerance, which is the concentration of glucose that can be absorbed without causing glycosuria. Glycosuria is the presence of abnormal amounts of glucose in the urine. This condition can arise from diabetes mellitus, fasting or high starch diets (Frape, 1986). Thoroughbred horses have a delayed insulin response following a high starch feed (Kohnke, 1998). This can increase the chances of hot and nervy behaviour within four hours of eating a concentrated grain based feed, which may increase as the size or bulk of the concentrate meal is increased (Kohnke, 1998).

There are two classes of glucose carrier proteins. SGLT1 carrier proteins have a high affinity and low capacity for absorption of D-glucose/galactose absorption across the intestinal lumen membrane and in the kidney. There is a lag time in SGLT1 production in mice, and the hypothesis is that, if in the horse the activity is similar, then abrupt changes to CHO exacerbate CHO overload because of the 12-24 hour lag time in the production of SGLT1 production for its absorption in the small intestine. The GLUT proteins are facultative, and three classes exist. Fructose is absorbed by GLUT5 (of GLUT 5-12) and represents a small fraction of the absorption of simple sugars, which are mainly as glucose (Hoffman, 2003). Fructose substitution for glucose does not improve glucose metabolism in animal studies (Gerrits and Tsalikian, 1993), but is well absorbed and rapidly converted to glucose in endurance horses (Bullimore *et al*., 2000). Of the pentose sugars, xylose is the most abundant, and while it has been demonstrated to be largely non-nutritive in the small intestine, overflow to the hindgut will also yield VFA's by its microbial degradation (Ralston and Baile, 1983b).

The development of a glycaemic index (Jose-Cunilleras *et* al., 2004; Munro, 2005) was followed by the glycaemic load (Ludwig, 2003) and the GGE (glycaemic glucose equivalent; Monro, 2002; Liu *et al.*, 2003). GL is the product of the GI and its carbohydrate content, while the GGE is the number of grams of glucose that it would take to produce an equivalent glycaemic response. The measurement of the area under the curve is a valid measure of the glycaemic response (Wolever, 2004). The jury is out on the effectiveness of the glycaemic response in elucidating all of the effects of feeding CHO/high starch feeds (Menzies-Gow, 2009). There is a lack of standardization of responses in the equine literature, with the baseline for relative responses changing between oats, and whatever reference standard feed is used in any particular trial. Glycaemic response is useful in determining the extent of precaecal digestion and the contribution of hydrolysable carbohydrate of the carbohydrate ingested, but needs to be linked to the metabolic needs of the horse after eating.

6 NUTRIENT SYNCHRONY

Anaerobic fermentation in the soil, composting or in ruminant ecology, requires a ratio of nutrients to be available to them, where environments are conducive to the propagation of microorganisms that break down substrate to utilizable fractions. Interest in eutrophication (Wilson *et al.,* 2006) and increased global pressure to reduce phosphorous sinks, predominantly in Europe (Lawrence *et al.*, 2003), has prompted this research. The concept of nutrient synchrony in respect of the supply of carbon and nitrogen and their influence on digestive microbial systems has been evaluated most recently by a few authors (Cole and Todd, 2008; Hall and Huntingdon, 2008; Reynolds and Kristensen, 2008). An excessively high C:N ratio causes an increase in acid formation (Ghasimi *et al*., 2009), an issue which could be extrapolated in the hindgut. Maximum performance occurs when the non-lignin-carbon to nitrogen ratio of feed mixtures is between 25 and 32, utilizing data generated by the anaerobic digestion of dairy manure and field crop residues (Hills and Roberts, 1981). The C_{biodeg} : N ratio can be calculated (Richard, 1996; cited in Richard, 2005) and this should be less than the total C:N ratio. Richard (1996) calculated the amount of biodegradable carbon (C_{biodeg}) as = C_{total}(NDF%/100)(1-0.054(lignin%/(NDFx0.01))$^{0.76}$) + C_{total} (1-NDF/100). Richards *et al.* (2003) also calculated the (Cellulose+Hemicellulose)/Lignin (CHL) ratio as a tool to evaluate the degradation potential of feeds. There was good correlation between this ratio and the gas production of anaerobic waste degradation.

While the pregastric fermentation of protein in the ruminant may make them more adaptable to asynchronous nutrient provision (Reynolds and Kristensen, 2008), the caecal ecosystem of the horse is much more demanding of nutrient synchrony in respect of carbon and nitrogen. Synchrony between other nutrients is important as well, especially fat (Geelen *et al.*, 1999). The replacement of fermentable carbohydrates with lipid in the diets of sport horses has been proposed for reasons stated above. However, the depression of the activity of especially cellulolytic bacteria (Jenkins, 1993; Jansen *et al*., 2000; Jansen *et al*., 2007a/b) in the hindgut cannot be disregarded if optimal use of cellulose is to be made.

Reduced fibre digestibility also occurs at levels of 25% and 35% NDF in horses (Bragal *et al.*, 2008). Because of a favourable fibre: energy balance in feeds tested in Miraglia *et al.* (2006), cellulose digestibility was found to be good, but the author suggested more work on the fibre energy ratios to understand why the functionality of the hindgut seemed to be improved. The [(Cellulose+Hemicellulose)/Lignin] (CHL) ratio indicates a good way of elucidating structural, fermentable substrates for the horse, while the C:N ratio must be investigated.

Nutrient synchrony is the goal of much of the research done on fat/fibre, as opposed to sugar/starch combinations (Harris and Bishop, 2007), which are involved in metabolic flexibility (Trieber *et al.,* 2005), as well as indicated for laminitic, insulin-resistant or PSSM horses (Zeyner *et al.*, 2006; Harris and Geor, 2009), and improved behaviour (Houpt, 1986; Nicol, 2000; Nicol *et al*., 2005). Fat/fibre as opposed to sugar/starch diets also enhanced insulin sensitivity in young horses (Duarte *et al.,* 2005). In heat stressed environments, increasing fat, but decreasing protein is beneficial (Ott, 2005).

Digestive synchrony, which provides optimal nutrition simultaneously to the fore-and hindgut systems is also a major factor in the investigation of for example, fat and fibre synchrony in the horse. The horse, as a monogastric herbivore, has requirements for both monogastric digestion and fermentation of substrates in the hindgut. Therefore synchrony between nutrients and the location of their digestion influences the efficacy of their assimilation in the horse. Optimising nutrient synchrony for microbial health and therefore optimal equine nutrition must be evaluated in horse feeds, and an holistic appraisal of the digestive capacity and nutrient requirements requires a fresh approach to optimal equine nutrition.

7 RECOMMENDATIONS FOR NUTRIENTS

The NRC was mandated to review all pertinent summaries of the equine literature in terms of maintenance and exercise requirements for life stages and body weights in the horse. By 2007, the NRC fresh recommendations reflected the body of research that had developed in the interim since the previous NRC. However, little faith existed in anticipation of these recommendations, and rations formulated on their recommendations “just will not sell!” was the opinion of Topliff (2002). The relationship between actual nutrient requirement and feed intake is often poorly correlated in that horses are usually oversupplied with starch or protein, or requirements are overstated. The horse owner generally resorts to increasing meal size to compensate. The recommendations may not always be correct (Hallebeek *et al.*, 2000). The new NRC (2007) recommendations attempt to improve on the calculation of requirements on a per kg BW basis, but derivation of requirements from empirical data with mechanistic modelling is still required. For this reason, the 2007 NRC recommendations can only provide a baseline for future research on the mechanistic modelling of nutritional requirements in the horse. The baseline values taken from NRC (2007) have been used in several instances in the body of this current research in order to evaluate the profiling of current

feeds to meet these recommendations. A paradigm shift in the relationship of requirement to intake is required for many feed components.

8 FEED FORMULATION

The full spectrum of nutrient requirements which would create reliable feeding goals, is not on the feed bag label (Duren and Watts, 2004). Confidence intervals in digestible energy requirements and nutritional needs per life stage or discipline still need to be determined (Harris, 1998). At the 56th meeting of the EAAP in 2005 it was resolved that there should be harmonization of feed evaluation systems for farm animals, including horses. In particular, their goal was to improve the quality of information provided by the feed industry on the chemical composition and nutritive value to buyers of concentrate horse feeds (Miraglia and Martin-Rosset, 2006).

"The psychology of feed buying decisions by horse owners is not the primary focus of the NRC committee, but this consideration is a significant factor in determining feeding strategies, feed forms and nutritional products that horse owners are willing to offer their horses. The adequacy of dietary energy, protein, vitamins and minerals in a horse's diet may receive considerably less conscious thought from the horse owner than the expression of diet sufficiency as a glossy hair coat, vigorous attitude, hearty appetite and soundness of limb and wind. The extent to which today's horse owner wants her horse to be not merely healthy, but also fully contented, can be a great motivating force that drives feed-buying decisions. Consumers will pay a premium price for high-quality products that directly acknowledge their view of the horse as both a livestock equine partner and a companion (Kline, 2004).

The digestive system of the horse does not vary by breed, and yet horse owners request "Warmblood" feeds. Instances of special feeds per discipline (endurance vs dressage) or temperament ('hot' vs relaxed) can be supported from a scientific point of view, but the equine nutritionist has to, from an educational standpoint, focus on meeting the horse's nutrient requirements (Topliff, 2002), irrespective of the "fashions and popular interests". Herein lies the competitive aspect of the business.

8.1 Feeding Management

The development and growth in the sport horse industry is evidenced by the increasing demand for Warmblood and other horse semen being brought into South Africa. The performance of the horse has become paramount. Consistency in performance is demanded by both the hacker and the

competitive rider. It becomes imperative therefore that the nutritional requirements of the horse in a management system in violation of its historical habitat be addressed. This involves the management system as well as the nutrition of the horse. Not every problem in the yard can be laid at the door of the nutritionist (Jackson, 2003). Consider the behavioural strategy of the horse in the historical context: the horse is a social creature, with a hierarchy defined by its herd, and a time budget defined by its nomadic, unterritorial existence. Even domestication has failed to change this, and cognisance should be taken of the frustration of specific motivational systems that underlie the development of stereotypic behaviours (Kiley-Worthington, 1983; Sweeting *et* al., 1984; Luescher *et al*., 1991; Mason, 1991; Marsden, 1993; Rushen *et al.* 1993; Winskill *et al.*, 1995; Marsden, 2002). These are behaviours that are deviant, repetitive and with no apparent function (Arkins, 1999), but most can be attributed to the frustration of a foraging behaviour (Cooper and Nicol, 1993; De Leeuw *et al*. 2004). The work of several authors (Hackland, 2005; van Weyenberg *et al*, 2006), has indicated that the quality and balance of nutrients provided to horses is mostly to blame.

Nutrient requirements of the horse are the sum of the requirements for maintenance, work, growth, reproduction and lactation. There is an increment for work that will be quickly exceeded where the animal is maintained on natural forages only. In the wild, the maintenance requirement would have included a foraging distance, and would have included a small measure of "work". In the modern context, the walk, trot, canter and gallop at the collected and extended gait, interspersed with jumping and work on different surfaces, certainly constitute an increase in work and hence nutrient requirements, especially where it becomes necessary to do this without a compromise in body condition score (Henneke *et al*., 1983). As far back as 200BC, Xenophon (200BC) was an advocate of the provision of some oats for energy per day to the working horse. Some energy systems, for example the Scandinavian Feed unit developed from this notion that "a cup of oats a day will keep the vet away" (Martin-Rosset, 1994). However, Lindsay (2005) evaluated these energy systems, and determined that some of the proposed energy systems are fundamentally flawed in their approach, specifically when using ruminants to evaluate the equine system.

Diet and feeding management are core contributory factors in pathophysiology such as laminitis (King and Mannsman, 2004), respiratory health, rhabdomyolysis syndrome, insulin sensitivity, and developmental orthopedic disease (Waltham, 2006). In addition, the frustration of a foraging reflex in horses has led to the development of stereotypic behaviours. Environmental enrichment (Waltham 2006), feeding frequencies (Hackland, 2007) and flavour choice (Waltham 2006) have all been tools used to simulate the horses's preferred natural feeding pattern.

Requirements in work relative to gut capacity dictate that forage be substituted for concentrate feeds, and permutations of provision of concentrate and forage have been studied in order to reduce the incidence of stereotypes (Hackland, 2007). Many diet-related problems will be expressed as either stereotypic behaviour or physiological problems in the stabled horse (Frape, 1994). Most common ailments and problems associated with feeding result from boredom and confinement, irregular feeding times, restriction of social interaction between horses at feed times, the feeding of highly concentrated rations that are quickly consumed, inadequate roughage and the lack of opportunity for self-exercise or regular exercise (Kohnke, 1998; Harris and Bishop, 2007). There are a number of serious disorders associated with horses consuming high levels of grain and soluble carbohydrates (Kalck, 2009) and while the negative consequences of grain feeding are diverse, a major cause is the development of acidic conditions in the hindgut (Willard *et al.,* 1977).

The objectives of feeding management, incorporating the roughage: concentrate ratio (Kienzle *et al*., 2002) and the stalk length of forage, and meal frequencies, can be reflected by rate of passage, glycaemic response and fermentation kinetics as well as performance parameters that have been defined for that class of horse (Gordon *et al*., 2007).

8.2 Objective Functions

Horse feed companies will advertise that they will not use least cost objective function ration formulation. This is considered a selling point when purchasing their feeds, because the customer must believe that no money has been spared in the "optimal' formulation of the feeds. However, they do not inform the consumer what the alternative objective function is. Performance in the horse is elusive to enumerate, although it can be done (Bojer, 2004). Performance of the feed in terms of nitrogen wastage, for example (Warren, 2006), can also be enumerated. What is commonly overprovided is the NSC content of the feeds, which is not displayed on the feed bag label and is not optimized as an objective function in feed formulations (Duren and Watts, 2004). One cannot compromise on the amount of structural CHO, but NSC can be refined, and this provides a useful objective function for formulations. Fashions and psychologies of the horse owning fraternity should have no place in the science of optimal horse nutrition (Topliff, 2002; Kline, 2004).

8.3 Nutritional Modelling in the Horse

The way horse owners choose feeds for their horses is poorly informed (Honore and Uhlinger, 1994). Deterministic models have been used for the only branch of equine nutrition that has received any modelling attention: rates of passage (Cuddeford, 1999). Relatively few empirical models exist for growth rates (Staniar *et al*., 2004). The NRC (1989) provided recommendations for nutrients that

were all related to energy requirements, which were empirically determined. Fortunately the new NRC (2007) Requirements for Horses updates this, but only partially, by providing three energy levels to choose from, to which the requirement for other nutrients is related (NRC, 2007). It has been determined that protein requirements do not increase *pro rata* with energy requirements in the working horse. However, the NRC recommendations (2007) do exactly this (van Saun, 2007). Harris and Bishop (2007) noted that there has been very little change in the absolute requirements for horses, even in the new NRC recommendations. Little research has targeted the determination of core nutritional requirements, especially as the dietary composition is dependent on the level of intake provided under a given feeding management system (van Saun, 2007). In contrast, nutrition modelling in poultry has seen major advances in the last thirty years. One of the most important of these was making feed intake an output from, as opposed to an input to, the growth model (Gous, 2007).

Instead of testing a multitude of empirical diets for broiler growth or egg production, Gous adopted the reciprocal approach. He calculated the nutritional requirements of poultry according to growth and egg production models, and determined stochastically the absolute requirements for nutrients to produce the response curve, and hence the level of the first limiting nutrient. In this way, he could accurately predict, *a priori,* the feed requirements of broilers, for example. The simulation optimiser models that he has developed have successfully combined nutritional, genetic and environmental constraints to predict feed intake, and therefore performance based on the requirement of the broiler for maximal protein deposition. A similar approach is needed with respect to horse feeds for horses of different ages and with different work levels and performance expectations. The nutritional demands need to be determined first, and responses in performance can be determined from the provision of nutrients relative to requirement, given the substrate and site of digestion.

Van Saun (2007), a member of the committee responsible for new NRC (2007) for horses, acknowledges that there is a need to move to a level of predicting available nutrients from feeds, and data are needed to incorporate microbial fermentation into prediction of nutrient provision for consumed feeds in the horse. Emmans and Oldham (1988) proposed in the monogastric that a desire for the satisfaction of the first limiting nutrient would drive feed intake until the physical constraints of the digestive tract constrained it (Figure 1.8). Feed intake is then an output and not an input in the system (Gous, 2007). Presently, feed intake is an input in models of horse nutrition. The issue was raised previously about adopting a reciprocal approach to equine nutrition. Nutrient requirements should be determined for the horse at a particular age and stage. Along with nutrient

synchrony and the several other parameters necessary for optimal performance, nutrients should be balanced in rational feeding management programmes, and should inform the feed intake. What happens now is that nutritional inadequacies in horse feeds are corrected by increasing feed intake, forcing the ingestion of high levels of concentrate feeds, with a concomitant increase in metabolic disorders in the horse (Kalck, 2009). This paradigm can be shifted with the use of empirical modelling to determine requirements and response curves, and hence, with nutritional constants for defined performance parameters, in order to improve horse ration formulations.

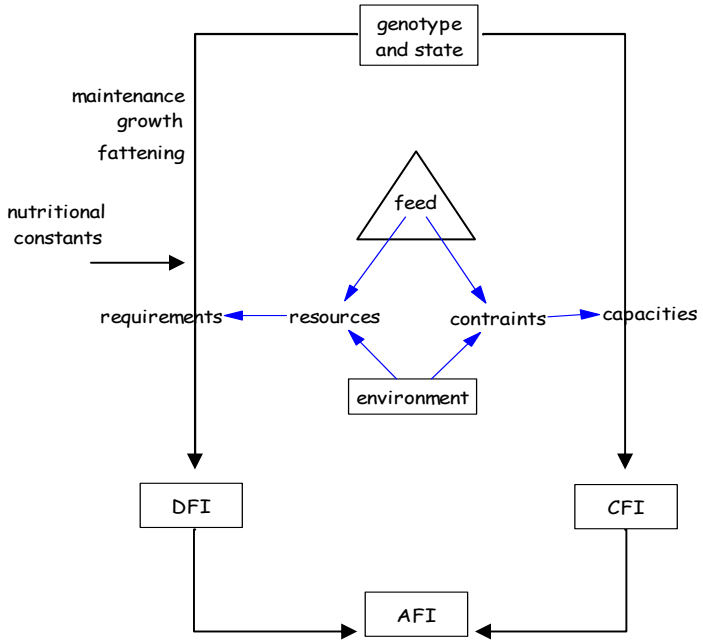

Figure 1.8 A scheme for the prediction of feed intake in the monogastric (after Emmans and Oldham, 1988), where actual feed intake (AFI) is determined as smaller of the desired feed intake (DFI) and the constrained feed intake (CFI).

Mechanistic models are required to consolidate the bodies of research that industry produces. Vermorel *et al.* (1997b) calculated efficiencies of use for utilization and modelling the partitioning of nutrients in the hindgut fermenter (after Emmans, 1994), which means that energy requirements could be accurately modelled for prediction of feed intake and performance. Harris and Bishop (2007) identify that very little research has targeted core nutritional requirements. Where these nutritional constants (Vermorel *et al*., 1997b) can be incorporated into, for example, effective energy models (Emmans, 1994). A more precise effect of thermic load and utilization could be determined to improve specifications and raw material selections in feed formulation.

The use of classifying variables that are identified and defined from nutritional physiology is a start in the mechanistic approach to the modelling of requirements in the horse. Nutritional principles

should govern the provision of nutrients to the horses, and a paradigm shift towards that of requirement: →predicting intake →predicting performance, is required. The strategic use of nutritional determinants that represent partitioning and fermentative capacity has been used in this study to demonstrate that horse feed formulators in South Africa are subscribing to the incorrect paradigm in their formulations. Refining the characterisation of equine diets, with respect to and cognisant of colleagues in monogastric and ruminant nutrition, will advance equine nutrition science, towards the modelling of performance parameters that can be defined in the equine (Bergero and Valle, 2007). Improved nutrient profiling of horse feeds will reposition companion animals to the status of the performance animal, where expectation of their performance will match their potential through optimum nutrition.

9 DISCUSSION AND CONCLUSION

A paradigm shift is required in the way nutrients are provided to the horse. Fashions and foibles have no place in optimal equine nutrition. There are a number of methods to determine the nutrient profile of feeds. When proper classifying variables have been identified, they can be integrated into effective feed formulations, where the definition of objective functions in ration formulation is important and is discussed in Chapter 7. Nutrient synchrony and the supply of the principal nutrients to the horse as a hindgut fermenter (and not a monogastric or a ruminant) need to be identified, to serve as a starting point for proper feed formulation. Feeds should be evaluated relative to their ability to meet requirements of the horse. Requirements of the horse should be related to nutritional consequence in meeting defined performance objectives for all classes of horse.

CHAPTER TWO: USING HIERARCHICAL CLUSTER ANALYSIS TO CATEGORISE HORSE FEEDS INTO GROUPS

Abstract

The objective of this trial was to classify several conventional equine feeds into categories based on nutrient characteristics and then to test the *in vitro* gas production characteristics of feeds in these categories as a means of improving the nutrient profiling of feeds. Hierarchical cluster analysis yielded four distinct categories of feeds. Apparent degradabilities and microbial mass were different ($P<0.01$) between feeds, although gas fermentation kinetics using equine faecal inoculum *in vitro* failed to identify significant differences between the four feeds representing the clusters. Gas fermentation kinetics should be used to inform the feed categories rather than vice versa. The *in vitro* gas production technique is an effective tool for rapid assays of feed characteristics for horses.

Keywords: horse feeds; *in vitro*; gas production

Introduction

The horse is a monogastric herbivore that is suited to the digestion and utilization of high fibre diets due to continual microbial fermentation, primarily within the hindgut (Harris, 2001). Proportional increments in the provision of energy/protein concentrate feeds are necessary as work rate in the horse often surpasses the capability of forage to provide nutrients. The breed of horse, temperament, purpose and body condition also inform the choice of raw material and feed, as do the season and weather (Harris, 1997). For example, a Thoroughbred eventer needs stamina and athleticism for the three-phase event, but is usually leaner in condition, and is less tractable in temperament for the dressage phase of competition than a European Warmblood horse imported into the country.

Digestion in the small intestine tends to vary with starch origin, grain processing, starch supply, forage processing and animal (Vermorel and Martin-Rosset, 1997; Coleman, 2001). The decrease in hindgut pH due to lactic acid build up may increase the likelihood of "hot" or nervous behaviour in many horses (Willard *et al.*, 1977; Krzak *et al.*, 1991). Grains have been implicated in the production of excess gas (McGreevy *et al.*, 1995a/b; Nicol, 1999), which causes a distension of the caecum and concomitant discomfort. The fermentation of grains will also provide volatile fatty acids, rapidly

transformed into energy (Ellis and Hill, 2005). Both these situations are linked to unpredictable behaviour in horses (Willard *et al.,* 1977; Johnson *et al.*, 1998; Hanstock *et al*., 2004). Oilseeds can replace maize and grains in low carbohydrate diets to reduce unpredictable behaviour and increase body condition. In some horse riding disciplines, for example polo and horseracing, a high energy to protein ratio with fermentable carbohydrates is preferred, or, as in stud animals, is not an issue.

The *in* vitro gas production technique (IVGPT) is a useful research tool for predicting digestibility of feeds, effects on rumen microbial activity, fermentation kinetics, associative effects of feeds, effects of feed additives on rumen fermentation and partitioning of fermented substrates (Ouda, 2007). *In vitro* gas production techniques generate kinetic data and measure the appearance of fermentation gases (Adesogan, 2002). Artificial rumen fermentation techniques can be utilized to study the digestibility of forages and feedstuffs by caecal microflora (Vallance, 1966; Applegate and Hershberger, 1969). Much nutrient evaluation in horses has been modelled using ruminant data. The donor horse has little effect on *in vitro* digestibility determinations of horse feedstuffs (Murray *et al.,* 2005), but a difference in fat digestibility between horse faecal inocula and rumen fluid has been observed (Lindsay, 2005). *In vitro* gas production profiles (Schofield and Pell, 1993; Hackland, 2007) can elucidate responses in the hindgut. A hierarchical cluster analysis (a multivariate analysis technique) can be used to classify feeds into homogeneous groups (clusters), based on *in vitro* gas production and parameters derived from other *in vitro* and *in vivo* studies (Ouda, 2007).

Horse feeds are sold on the basis of crude protein percentage (CP%). There is no documented rationale for this. It serves merely to classify the feeds into low, medium and hard work categories, although the literature has shown such a classification is unnecessary (Hintz, 1994; Marlin and Nankervis, 2002). This analysis is intended to show that analysed percentages and digestibilities of protein are equivalent between feeds that are described as being different. Hoffman (2003) explained how a hydrolysable group, and a rapidly and slowly fermentable group (approximated by the NSC, (NFC-NSC) and NDF portions of the feed, respectively) make the crude fibre incompletely represent the digested/fermentable fibre in the horse. Similarly, crude protein as a basis for the classification of horse feeds incompletely represents the response in the horse (Hiney and Potter, 1996; in Ellis and Hill, 2005). The contribution of protein to energy supply for working horses is minor (maximum 10% in long-term medium exercise levels) (Ellis and Hill, 2005). It has therefore been concluded that horses in work do not need a higher protein concentration than those at maintenance (Marlin and Nankervis, 2002). Horses have a requirement for amino acids that may limit nitrogen balance or protein synthesis at the level of the first limiting amino acid. But other than the provision of the ten essential amino acids, the over-provision of protein is more likely to do harm

than good (for example in an elevated thermogenic effect, hypocalcaemia, or increased acidogenic effect). Because of this and the replacement of grains with oilseeds, horse feeds should be reclassified into heterogeneous groups of life stage and work.

Categorization of a range of horse feeds (irrespective of their proportion in the ration) into distinct groups could assist SA horse owners in their selection of feeds. Correct categorization should also provide the horse owner with some indication of how the horse should perform on that feed. This study used a cluster analysis technique based on nutrient characteristics to categorise feeds. Feeds from each category were subjected to *in vitro* fermentation. The hypothesis was that feeds could be more accurately grouped and marketed on the basis of fermentation characteristics than on current method of CP% used by horse feed companies in South Africa. In Chapter 7, the cluster analysis is extended over nine classifying variables that are identified in the following chapters.

Materials and methods

Feeds and chemical composition analysis

The trial compared the gas production characteristics of 17 widely available South African horse feeds in the categories of riding feeds, grain-free, pony and breeding feeds, across a range of advertised protein percentages (Table 2.1). The feeds were dried at 50˚C overnight and were milled through a 1 mm screen. Total dry matter (DM) was determined by drying the samples in a fanned oven at 100˚C overnight. Total ash was determined by igniting samples in a muffle furnace at 500˚C overnight. Nitrogen was measured by the micro-Kjeldahl technique and CP values were calculated as N x 6.25, according to the method of AOAC (1995). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) determinations were used as described by van Soest *et al.* (1991), where NDF and ADF are expressed inclusive of residual ash (Editorial, 2005). Soluble ash was determined by mixing total ash with hot water followed by filtration using a crucible with a sintered glass filter, heating at 500˚C overnight and weighing the residue to determine insoluble ash. Soluble CHO (CHO_{sol}) was calculated by the method of Harris (1970), where CHO_{sol} ($g.kg^{-1}$) = 1000 – (NDF + crude fat + CP +soluble ash). A hierarchical cluster analysis was employed to segregate feeds into heterogenous groups, and a representative from each of these groups was chosen for the *in vitro* gas production analysis.

Gas production analysis

The *in vitro* gas production technique (IVGPT) described by Pell and Schofield (1993) was used. A total of 1.0 ± 0.0010g DM of each feed was weighed into 250ml Duran glass bottles for incubation. The incubation was replicated three times for 72 hours with all treatment feeds and controls represented each time. A buffer solution was prepared using the method of McDougall (1948) whereby 4ml of Solution B (5.3g $CaCl_2.2H_20$ in 100ml distilled water) was added drop wise to 4L Solution A ($NaHCO_3$ + diNaH_3PO_4(anhydr) + KCl), to make a complete buffer solution, which was warmed to 37˚C. 67ml of buffer solution was added to the 1g DM of sample in the Duran glass bottles and left to equilibrate at 37˚C in an incubator.

Equine faecal inoculum was prepared from freshly voided faeces from Thoroughbred horses maintained on pasture and fed 0.6% of body weight of concentrate feed in two meals. Every effort was made to maintain anaerobic conditions. The faeces were collected as they were produced, into a sealed packet, and sealed in the flask that had been infused with CO_2. Faecal samples were collected before the morning feed into a flask maintained at 37˚C and were transported within 15 minutes to the laboratory. Half a litre of faeces was mixed with 500ml buffer solution and stirred with a glass rod and squeezed through four layers of cheesecloth into a glass beaker flushed with CO_2. Inoculation was done by adding 33ml of faecal fluid to the sample and buffer under a stream of CO_2. The bottle lids were tightened and the pressure sensors fitted. Samples were allowed to settle for 20 minutes before pressure logging started at 20 minute intervals for 48 hours. The pressure data was converted to gas volumes (in ml) using a predetermined calibration equation where 1kPa=2.2489ml gas determined in our laboratory (Nsahlai and Ummuna, 1996).

The two compartment model of Campos *et al.*, (2004) is supported by the research of Rosenfeld *et al*. (2006) for the representation of flow kinetics of digesta in horses. The model was fitted to gas profiles, corrected for control fermentation profiles in the absence of substrate, to derive GP kinetics as follows:

$$Y= A/[1+\exp(2+4a_1(C-t))] + B/[1+\exp(2+4b_1(C-t))] \quad \text{Equation 1}$$

Where y is the total gas volume (ml) at time t, A and B are the gas volumes (ml) from the fast and the slow degradable fractions, a_1 and b_1 are the degradation rates (h^{-1}) for the fast and the slowly degradable fractions and C is the colonization or lag time (h).

Partitioning factor as a measurement (degraded substrate:gas volume mg/ml), as described by Blümmel *et al.* (1997), has been used to describe the efficiency of microbial protein synthesis. In the

horse, it indicates the relationship between substrate entering the hindgut and its fermentation. What is entering the hindgut is exactly what we wish to provide a parameter for, and the partitioning factor (PF) of the IVGPT provides this.

The degradation efficiency factor (Ouda *et al*., 2006) is calculated from the partitioning factor as follows:

$PF = deg/V$ and $DEF = deg/[T_{½} \times V_{½}] = 2PF/T_{½}$ Equation 2

Where deg = true degradability (defined below; mg), V = total gas volume (ml), $T_{½}$ = time taken to produce $V_{½}$, and $V_{½}$ = half of V (ml).

Microbial activity is close to maximum at half maximum gas volume (France *et al*., 1993; Davies *et al*., 2000; Makkar, 2004, in Ouda; 2007), and $T_{½}$ can therefore be used to improve the prediction of nutritive value (Murray *et al*., 2006).

Determination of degradability

After incubation, terminal pH was determined, and the samples were centrifuged at 18000G for 15 minutes. The supernatant was discarded and the pellet residue (R) was dried in a fanned oven at 100˚C for 48 hours until constant weight was attained. The difference in weight between R and 1g DM was regarded as the apparently degraded fraction (AppDeg). R was refluxed with neutral detergent solution (NDS) and the residue (NDF) was dried. The weight of the NDF was subtracted from the 1g DM to yield the truly degraded fraction (degradability). The difference between the true and the apparent degradability is the microbial yield.

Statistical analysis

A correlation analysis was done on the analysed compositions of the feeds. A multivariate, hierarchical cluster analysis technique (SAS, 2005) was used to segregate feeds into homogeneous groups (clusters) on the basis of classifying variables (CP%, NDF%, NFC% and fat %), derived from proximate analysis. The variable reduction ACECLUS procedure summarized the multivariate set of data into orthogonal canonical variates. Ward's method (William, 1994) was used to ensure that within-cluster differences and between-cluster linkages were minimized. A feed was selected from each cluster and clusters were replicated in a randomized blocks design to generate gas production data by *in vitro* gas production (Pell and Schofield, 1993). SAS (2005) software was used to fit the gas production model by Campos *et al*. (2004) and analysis of variance (Genstat, 2007) was used for predetermined comparisons amongst feed types. ANCOV, using protein percentage as the covariate

was performed, in order to establish differences between the four feed types. Means were compared by least significant difference at $P<0.05$.

Results

Table 2.1 provides the chemical analysis of the feeds used in this analysis. The selection of feeds provided a range of protein, fat and fibre values. The correlation between the bag label and the actual analysed percentage of protein in the feed was 62.4%, on a dry matter basis. The classifying variables of the horse feeds (crude protein, fat, neutral detergent fibre) and the calculated non-fermentable content (100-CP-fat-NDF) were used to generate four canonical variables. These were used by the cluster procedure in SAS (2005) to generate groups of feeds which were more similar. The choice of four clusters accounted for 87% of the variation in the feeds analysed (Figure 2.1) and made the most sensible classification of the feeds available.

Table 2.1 Proximate and detergent analyses on a dry matter basis of common South African riding, breeding, maintenance and grain-free feeds, showing advertised and actual protein percentages (CP%), % fat, % neutral and acid detergent fibres (NDF and ADF, respectively), and the cluster identification of the feeds following hierarchical cluster analysis.

CODE	Feed type	Advert CP %	CP (%)	Fat (%)	NDF (%)	ADF (%)	CHO_{sol} ($g.kg^{-1}$)	Cluster
67	Breeding	14	18.34	4.66	19.53	10.84	535	1
69	Maint	11	13.79	5.02	26.76	10.71	516	3
73	Grain free	14	17.62	5.53	28.14	13.01	469	2
75	Riding	12	15.17	9.23	24.27	12.49	467	4
77	Grain free	14	17.40	4.98	27.45	14.31	439	2
68	Riding	13	15.21	4.54	19.94	11.63	560	2
70	Breeding	14	17.97	4.45	17.06	9.86	555	1
71	Riding	12	15.12	4.78	22.53	11.61	534	2
72	Riding	12	15.84	4.03	25.46	10.82	519	2
74	Breeding	14	18.01	4.40	18.53	9.73	531	1
76	Riding	12	11.40	4.62	26.37	11.62	538	3
77	Grain free	14	14.86	10.49	28.43	14.21	439	4
78	Maint	10	11.57	2.86	25.46	10.16	580	3
79	Riding	12	12.44	4.37	30.00	10.20	519	3
80	Riding	12	15.94	6.14	30.99	11.90	474	2
81	Grain free	10	14.91	3.35	34.15	13.21	480	3
82	Riding	14	13.22	3.25	50.19	23.74	333	3

where 1=breeding, 2=riding, 3=maintenance and 4=conditioning feeds; Advert CP = advertised crude protein %.

The four clusters (with feeds per cluster indicated in the Table 2.1) were named as breeding, riding, conditioning and maintenance feeds. The discerning characteristics of these clusters are summarized in Table 2.1. Group 1 (breeding) had high protein and, Group2 (riding), Group 3 (conditioning) characterized by high fat and Group 4 (maintenance) with low fat and high NDF and ADF.

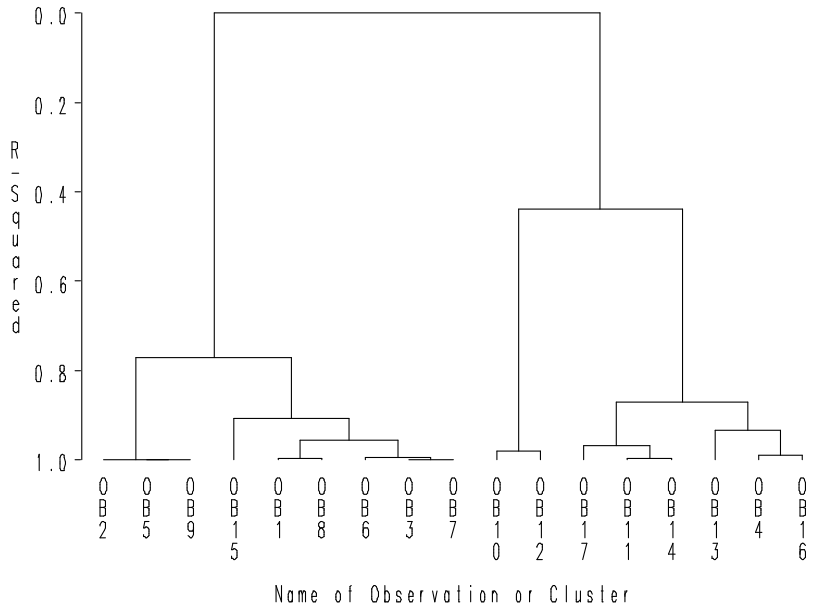

Figure 2.1 Dendrogram indicating that four groups of feeds can be derived from an hierarchical cluster analysis of classifying variables (NDF, CP, fat and NSC) which account for 87% of the variation observed between the compositions of the feeds.

Gas production parameters are presented following the incubation of the feeds with equine faecal inoculum. The measurements that are important follow from Equations 1 and 2, and include the pH of the medium, the degradation parameters, the time at maximum gas volumes, and the calculations of degradation efficiency (Tables 2.2-2.4). Fitting the data to standard curves produced the following gas production parameters (Table 2.3). Fermentation characteristics were calculated from the gas production profiles of the feeds (Table 2.4).

Table 2.2 Effect of 72 hour incubation on final pH, apparent and true degradability (AppDeg; TruDeg; g.kg^{-1}) and microbial mass (g.kg^{-1}) of feed samples incubated in faecal inoculum (after the method of Pell and Schofield, 1993*)*

Feed Cluster	Final pH	AppDeg (g.kg$^{-1)}$	TruDeg (g.kg^{-1})	Microbial mass (g.kg^{-1}*)*
Breeding (1)	6.62^{b}	436^{a}	825	257^{c}
Riding (2)	6.65^{b}	333^{b}	829	401^{ab}
Maintenance (3)	6.72^{b}	371^{b}	841	493^{a}
Conditioning (4)	6.68^{b}	310^{b}	834	321^{bc}
Control	7.01^{a}			
OVERALL MEAN	6.79	363	832	368
SED	0.04	36.2	16.5	59.4
LSD (1%)	0.15	135.9	34.4	124

^{a,b} Means in same column bearing different superscripts differ significantly. SED = standard error of difference of mean, LSD = least significant difference

Table 2.3 Mean gas production parameters obtained by fitting the model of Campos *et al*. (2004) to gas production data of common SA horse feeds fermented *in vitro* for 72 hours using equine faecal inoculum

Feed	Max gas volume (ml)	gas vol for rapidly degradable fraction (ml)	gas vol for slowly degradable fraction (ml)	degradation rate of rapidly degradable fraction (h^{-1})	degradation rate of slowly degradable fraction (h^{-1})	Lag time (h)
Breeding (1)	124.4	65.8	62.5	0.114	0.0482	7.25
Riding (2)	110.3	66.0	74.3	0.141	0.0358	7.87
Maintenance (3)	129.8	62.3	76.3	0.142	0.0412	9.41
Conditioning (4)	91.8	49.4	61.4	0.157	0.0506	12.22
MEAN	114.1	60.9	68.6	0.138	0.0439	9.18
SED	16.73	13.37	18.06	0.0511	0.0106	2.63
LSD	34.89	28.21	38.09	0.1066	0.2235	5.53
Significance	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

^{a,b} Means in same column bearing different superscripts differ significantly.

SED = standard error of mean, LSD = least significant difference

Table 2.4 Mean gas production parameters of common South African horse feeds fermented using equine faecal inoculum *in vitro*.

Feed		$T_{½}$ (h)	Part factor ($mg.ml^{-1}$)	DEF
	Breeding (1)	21.89[b]	7.32[a]	0.695[a]
	Riding (2)	17.89[ab]	7.08[a]	0.791[a]
	Maintenance (3)	20.41[ab]	7.14[a]	0.636[a]
	Conditioning (4)	17.40[a]	5.53[a]	0.723[a]
MEAN		19.40	6.77	0.712
SED		2.060	0.914	0.140
LSD		4.347	1.921	0.2942
Significance		P>0.05	P>0.05	P>0.05

[a,b] **Means in same column bearing different superscripts differ significantly.**

$T_{½}$ = time taken to half maximum gas volume; part factor = partitioning factor; SED = standard error of mean, LSD = least significant difference

Discussion

This study was designed to test whether a cluster analysis technique based on nutrient characteristics could categorise feeds more astutely than the current method of CP% used by horse feed companies in South Africa. Whether this categorization is able to inform some of the nutritive and fermentative characteristics of the feed was tested by choosing representative feeds for each category and subjecting these feeds to *in vitro* fermentation. It is apparent that the fermentative characteristics of the feed were possibly more informative of the differences between feeds for horses than the chemical characteristics per se. Clusters were produced between the feeds, segregating them

The protein percentage advertised on the feed bag is used in this country as a basis for selection of horse feed. The low correlation between the bag label and the actual analysed percentage of protein in the feed indicates that objective functions in the formulation of horse feeds are not optimization of amino acids or protein and are most likely to be least cost, with substitution of feed ingredients to achieve least cost. While the Breeding feeds had a significantly higher actual CP% (15.96%) than the Riding and Maintenance feeds in the samples analysed ($P<0.01$), each feed is about 10.9% different from its advertised CP%.

Using a cluster analysis, including the CP, fat, ADF, NDF and NSC contents of 18 feeds, produced four distinct feed categories. The discerning characteristics of Cluster 1 (Breeding feeds) were more CP and lower ADF values, and higher mineral contents, while both Breeding and Riding feeds (Clusters 1 and 2) tended to have more CP. The Conditioning feeds (Cluster 4) contained the most fat. The Maintenance feeds (Cluster 3) contained less fat, less protein and more ADF than the other feeds. So these categories inform a better selection of feed for horses than the CP percentage alone. UK based feed companies (for example Dodsen and Horrel, Badminton Feeds, Dengie Feeds, Winergy, *inter alia*) market feeds on the basis of life stages, which still neglects a more pragmatic approach to performance incentives in the selection of feeds. The proximate analysis of feeds as a clustering technique has not refined feed categories to include Grain-free, Warmblood or High-Energy feeds, and nor does it discern between the quality of feeds presented, which should have been elucidated through this *in vitro* investigation.

High correlations between *in vivo* apparent digestibilities and *in vitro* gas productions make IVGPT a useful research tool to extrapolate from fermentation characteristics, in order to provide some indication of the behaviour of the feed *in vivo* (Menke *et al*., 1979).

Equine faeces is a suitable source of inoculum, as discussed by Lowman *et al* (1996) and Macheboef and Jestin (1997), (cited in Abdouli andAttia, 2007), and Murray *et al.* (2006), with Bush *et al.* (2001) using equine caecal fluid for *in vitro* studies. Hackland (2007) concluded that there was no improvement in using the two stage method and simple fermentation was enough, where Abdouli and Attia, 2007 suggest that high quality feeds with low fibre fermentation should be carried out in two stages, with pre-enzymatic digestion followed by fermentation. They do concede that this approach needs to be evaluated with a large array of feeds. Their analysis was only using raw materials, and for routine feed evaluations, they suggested that a single stage method should suffice.

The addition of 1g (DM) of substrate to the faecal inoculum significantly ($P<0.001$) reduced the pH of the medium after incubation (Table 2.2). Addition of starch significantly decreases pH (Bailey *et al*., 2002), whose pH's of the control and treatment feeds concur with the present study. Bailey *et al*. (2002) obtained values of 7.2 for the control 7.2 and a rapid decrease of about 1.5 pH units over 12 hours with the inclusion of starch. The pattern of the post-prandial decrease in pH in the caecum and colon will depend on the composition of the ration (Douglas *et al*., 2000).

There was no effect of incubation on the true degradability of the feeds, although the microbial mass and the apparent degradability were different between feeds (Table 2.2). The Breeding feed had the highest apparent degradability and therefore digestibility (Hackland, 2007). The microbial masses in the Maintenance and Riding feeds were significantly higher than in the Breeding feed. The supply of readily degradable structural carbohydrate (NDF) to provide a more favourable ratio of primary to secondary cell wall material, can lead to increased microbial proliferation and hence increased degradation rates (Murray *et al*., 2006).

Gas production parameters were obtained using the model of Campos *et al*. (2004). The method of France *et al*. (1993) for ruminant feeds has been used in one other study of this nature (Murray *et al*., 2006) but the two compartment model of Campos *et al.*(2004) is supported by the research of Rosenfeld *et al*. (2006) for the representation of flow kinetics of digesta in the horse.

Gas production parameters obtained by fitting data to the model of Campos *et al*. (2004) were not influenced by the feed category ($P>0.05$, Table 2.3). Partitioning factor and degradation efficiency factor were the same across feeds as well. The clustering of feeds according to CP, fat, ADF, NDF and NSC, failed to differentiate between the four feed categories in their fermentative capacity, which means that the feeds in these categories are not significantly different enough. But yet, anecdotal and scientific responses of horses to Breeding, Riding, Maintenance and Conditioning feeds do produce different responses.

Differences between the feeds in respect of IVGPT parameters of degradation rates, gas production, lag time, PF, $T_{½}$ and DEF could be expected. The differences between degradability and the true digestibility can be accounted for by the microbial biomass which attaches itself to the feed particles (Schneider and Flatt, 1975). The rate of microbial digestion and the mean retention time determines the extent of fibre degradation in the gut of herbivores (Drougal *et al*., 2000). Grinding to increase the structural cell wall area exposed to fibrolytic microorganisms, does not significantly change the digestibility of the forage, where there is an increase in retention time for the smaller particles (Drougal *et al*., 2000). They demonstrated a lag phase of about 8 hours, similar to this study. Fermentation time will influence the apparent digestibility. Addition of concentrate to hay does not necessary increase the digestibility of hay, even where ADF % is reduced, because hay and concentrate does not necessarily spend enough time in the small intestine (Holland *et al*., 1998). Rates of passage and meal size in horses are critical, and underscore the need for quantification of fermentation characteristics of feeds.

Microbial populations respond to changes in feed composition (Ellis and Hill, 2005). High starch diets increase the populations of total anaerobic, lactic acid-utilizing bacteria, lactobacilli and streptococci in the caecum whereas cellulolytic bacteria decrease (Medina *et al*., 2002). Caecal pH will decrease 5-7 hours after the meal, and the rate and extent of this decrease will increase with starch content. This can be ameliorated with addition of probiotics such as yeasts, for example (Medina *et al*., 2002; Lattimer *et al.*, 2007). Profiles and activities of intestinal microflora are modified by the NDF to starch ratio in the diet. The increase in the concentration of lactic acid in the hindgut was twice as low as that observed when the overload of starch was not balanced with a great amount of fibre (Jullinas *et al*., 2001, in Medina *et al*., 2002). The release of peptides from slowly degraded protein fractions may stimulate microbial proliferation and enhance degradability.

The longer colonization time and the lower GP volumes in the high fat conditioning feeds may be an indication of the effect of fats on digestibility (Table 2.3). High fat rations tended to delay colonization (Murray *et al*., 2006). Fat is added to increase palatability, to decrease dustiness and to increase energy content, but does not affect DM, OM or fibre reduction in substrates (Bush *et al*., 2001). Fat digestion occurs in the foregut (Ellis and Hill, 2005). Fat supplementation does not affect the *in vitro* DM or OM disappearance of substrates (Bush *et al*., 2001). Fat increases the *in vitro* NDF disappearance of rolled oats (which has 60% starch and a low concentration of fibre). Hemicelluloses are more extensively fermented than celluloses, so feeds with a higher NDF value should be more fermentable. A high ADF content (lignins) has a negative effect on the *in vitro* NDF digestibility. Substrates that contain more soluble nutrients (CP and soluble CHO) have higher nutrient disappearance values than those with large amounts of insoluble fibre.

Maximum gas production occurred at 24h for barley and 48 h for soybean meal (SBM) (Abdouli and Attia, 2007), whereas for these composite feeds, it occurs at about 40 hours. Lag times of 12h are common (Abdouli and Attia, 2007; Murray *et al*., 2006). SBM over a 72 hour incubation yielded 111ml.g^{-1} DM, with barley up to 152ml.g^{-1} DM. Murray *et al* (2006) found total gas production for lucerne of 157.06ml.g^{-1}, and research by Ouda (*et al.,* 2006; 2007) produced similar results to this study.

No inferences can be drawn from the $T_{½}$ and the PF and DEF, except that these provided a bench mark for the behaviour of common horse feeds *in vitro*. The substrate degraded per ml of gas produced was lower in the high fat feed, which is significant in terms of the rationale behind many feeds on the market at the moment. Higher fat feeds are supposed to reduce volatility in behaviour by producing less fermentation and therefore discomfort in the caecum, which has been related to

behaviour in the horse (Willard *et al.,* 1977; McGreevy *et al.*, 1995a; Johnson *et al.*, 1998; Nicol, 1999; Hanstock *et al*., 2004).

Conclusions

The objectives of this study were to test whether a cluster analysis technique based on nutrient characteristics could categorise feeds more astutely than the current method of CP% by horse feed companies in South Africa. While the latter proved to be successful, it is not clear that those four feed categories of Breeding, Riding, Maintenance and Conditioning feeds comprehensively categorise feeds. Representative feeds in these categories failed to produce significantly different fermentative characteristics, although literature supported the integrity of the results. It is concluded that IVGPT produces useful results for evaluation of equine feeds using equine faecal inoculum, and that proximate analysis does not inform the best clustering of feeds with which to obtain parameters of cause and effect. Hence, IVGPT should be used to augment the techniques used for feed evaluation to permit more appropriate feed clustering techniques.

CHAPTER THREE: USING IN VITRO TECHNIQUES TO CHARACTERIZE COMMON SOUTH AFRICAN HORSE FEEDS

Abstract

Rapid assays are needed to determine the characteristics of common South African sport and leisure horse feeds relative to the perceived and actual benefits for horses. In the South African context, such correlations have been largely anecdotal. Horse feeds are marketed on the basis of a crude protein percentage which bears little relation to content or purpose. Feeds and feeding management can alter the fate of ingested materials in the gastrointestinal tract of the horse because it is a hindgut fermenter. Seventeen common concentrate feeds were analysed *in vitro* as part of a study of characteristics of feeds available for sport and leisure horses. Feeds were subjected to the *in vitro* gas production (GP) technique (IVGPT) described by Pell and Schofield (1993), using equine faecal inoculum. Gas profiles, corrected for control fermentation profiles in the absence of substrate, were fitted to the model described by Campos *et al*. (2004) to derive GP kinetics. The pH of digesta drops with the addition of feed. Gas production kinetics produced different parameters in respect of slow and rapidly fermentable components of the feed. There were no differences in the degradation rate of the substrates and no differences in lag or colonization time. The addition of concentrate improves the degradable portion of the feed in comparison to hay. Apparent and true digestibilities were negatively correlated to fibre content. The feeds that took longer to reach 50% of their maximum gas production were also the feeds that were formulated with more lucerne or more raw materials in an unprocessed form. The digestion efficiency factor is correlated to the rapidly fermentable portion of the feed and inversely proportional to the maximum GP, but not particularly to any proximate characteristic of the feed. IVGPT can be used to discern between feed groups more informatively than CP percentage

Keywords : *in vitro,* horse feed, gas production

Introduction

Horse feeds in South Africa are marketed, broadly on the basis of a crude protein (CP) percentage. In South Africa, consumer decisions on which feed to use for their horse are informed by functionality (for example hacking, breeding or maintenance) or temperament and

body condition (grain, grain-free or warmblood feeds), or simply cost. The link between these perceived benefits and the capacity of the feed to confer these characteristics is seldom questioned.

Raw material selection affects behaviour in horses as a function of the rate of passage of feed in the gastrointestinal tract (GIT) (Warner, 1981; in van Weyenberg *et al*., 2006). As a hindgut fermenter, proportional digestion in the sections of the GIT influences the provision of nutrients to the horse and influences behaviour of the horse (Willard *et al.,* 1977; Johnson *et al.*, 1998; Hanstock *et al.,* 2004; Ellis and Hill, 2005). The generation of volatile fatty acids in the caecum and the concomitant distension in the caecum has been blamed for undesirable behaviour in the performance of the horse. Such undesirable behaviour is also blamed on the provision of fermentable carbohydrates. Many feeds preclude the use of fermentable substrates to produce more consistent behaviour in performance horses (Zietler-Feicht *et al*, 2001). The nutritive value of horse feeds is a function of chemical composition, feeding frequency, intake, digestibility and efficiency of utilization. Several techniques have been used to determine chemical composition. Over the years, much of the nutrient characterisation of horse feeds has been extrapolated from ruminant data (NRC, 2007). While *in vivo* techniques in equines are done (van Weyenberg *et al*, 2006), the costs and sensitivity of these animals to these operations and the length of the acclimations and digestion trial periods can diminish their value in assessing horse rations.

IVGPT is an *in vitro* technique that is receiving much attention in ruminant research (Ouda, 2007). The two-stage method was developed by Tilley and Terry (1963), and subsequently modified by Minson and McLeod (1972) and used by others (Lowman *et al*., 1996; Murray *et al*., 2003; Hussein *et al*., 2004; Hackland, 2007). The method was modified to mimic the digestive system of the horse with microbial fermentation following acid digestion. The limited action of salivary amylase at higher feeding levels and previous work by Hackland (2007) supports a one-stage digestion method. It is important to discern the contribution of the foregut to the hindgut digestion, which is considered in the models presented in Chapter 7.

Hackland (2007) found that using maize and Eragrostis hay, significant differences in respect of the degradability and true digestibility can be obtained for roughage concentrate ratios from 20:80 to 80:20. No significant differences were found between the maximum gas production and the rates and lag time in the samples, so that feeds alone were compared in this study. Further exploration of the effect of roughage: concentrate ratios is included in future work. The development of rapid assays on the characteristics of common horse feeds will lead to a

comparison of their perceived and actual benefits. In the SA context, such correlations have been largely anecdotal in nature. It was the purpose of this study to demonstrate that feeds that are purported to affect temperament and performance through anomalies in their formulation can elicit differences in gas production kinetics, and that these parameters and their inferences are more informative in the description of the feeds than proximate analyses. IVGPT would then provide a more effective, rapid assay of horse feed characteristics. Breeding, riding, grain-free and pony meals were compared in terms of their gas production parameters. It was hypothesised that there should be some difference in the grain-free concentrates in this respect.

Materials and Methods

Feeds and chemical composition analysis

The trial compared the gas production characteristics of 18 common South African horse feeds in the categories of Riding, Grain-free, Pony and Breeding feeds, across a range of advertised protein (CP) percentages (Table 3.1). The feeds were dried at 50˚C overnight and were milled through a 1mm screen. Total dry matter (DM) was determined by drying the samples in a fanned oven at 100˚C overnight. Total ash was determined by igniting samples in a muffled furnace at 500˚C overnight. Nitrogen was measured by the micro-Kjeldahl technique and CP calculated as N x 6.25 according to the method of Association of Analytical Chemists (1995). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) determinations were described by van Soest *et al.* (1991), where ADF and NDF were expressed inclusive of residual ash (Editorial, 2005). Soluble ash was determined by subtracting the total ash with hot water, followed by filtration using a crucible with a sintered glass filter, ignition at 500˚C overnight and weighing the residue to determine insoluble ash. Soluble CHO were calculated by the method of Harris (1970), where CHO_{sol} ($g.kg^{-1}$) = 1000 – (NDF + crude fat + CP +soluble ash).

Gas production analysis

The *in vitro* gas production technique (IVGPT) described by Pell and Schofield (1993) was used. A total of 1.0 ± 0.0010g DM of each feed was weighed into 250ml Duran glass bottles for incubation. The incubation was replicated three times for 72 hours. Buffer solution was prepared using the method of McDougall (1948) whereby 4ml of Solution B (5.3g $CaCl_2.2H_20$ in 100ml distilled water) was added drop wise to 4L Solution A ($NaHCO_3$ + diNaH_3PO_4(anhydr) + KCl), to make a complete buffer solution, which was warmed to 37˚C. To the one gram of

sample (DM), 67ml of buffer was added, which was then left to equilibrate at 37˚C in the incubator.

Equine faecal inoculum was prepared from freshly voided faeces from Thoroughbred horses maintained on pasture and fed 0.6% of body weight of concentrate feed in two meals. Every effort was made to maintain anaerobic conditions. The faeces were collected as they were produced, into a sealed packet, and sealed in the flask that had been infused with CO_2. Faecal samples were collected before the morning feed into a flask maintained at 37˚C and were transported within 15 minutes to the laboratory. Faecal samples were collected into a 37˚C flask in the morning before the morning feed and were transported promptly to the laboratory. 500ml of faeces was mixed with 500ml buffer solution and stirred with a glass rod and squeezed through four layers of cheese cloth into a glass beaker flushed with CO_2. Inoculation was done by adding 33ml of faecal fluid to the sample plus buffer under a stream of CO_2. The bottle lids were tightened and pressure sensors fitted. Samples were allowed to settle for 20 minutes before pressure logging started at 20 minute intervals for 48 hours. The pressure data was converted to gas volumes (in ml) using a predetermined calibration equation where 1kPa=2.2489ml gas (Nsahlai and Umunna, 1996). Gas profiles, corrected for control fermentation profiles in the absence of substrate, were fitted to the model described by Campos *et al*. (2004) to derive GP kinetics as follows.

$$Y = A/[1+\exp(2+4a_1(C-t))] + B/[1+\exp(2+4b_1(C-t))] \quad \text{Equation 1}$$

Where y is the total gas volume (ml) at time t, A and B are the gas volumes (ml) from the fast and the slow degradable fractions, a_1 and b_1 are the degradation rates (h^{-1}) for the fast and the slowly degradable fractions and C is the colonization or lag time (h).

A partitioning factor (degraded substrate:gas volume $mg.ml^{-1}$) has been used to describe the efficiency of microbial protein synthesis (Blümmel *et al.*,1997). However, in the horse, the partitioning factor indicates the relationship between substrate entering the hindgut and its fermentation. The degradation efficiency factor is calculated from the partitioning factor as follows:

$$PF = \frac{deg}{V} \quad \text{Equation 2}$$

$$\text{and} \quad DEF = \frac{Deg}{T_{½} \times V_{½}} = \frac{2PF}{T_{½}} \quad \text{Equation 3}$$

Where deg = true degradability (mg), V = total gas volume (ml), $T_{½}$ = time taken to produce $V_{½}$, and $V_{½}$ = half of V (ml).

Microbial activity is close to maximum at half maximum gas volume (France *et al*., 1993; Davies *et al*., 2000; Makkar, 2004; in Ouda, 2007). $T_{½}$ can therefore be used to predict the nutritive value of horse feeds (Murray *et al*., 2006).

Determination of degradability

After incubation, terminal pH was determined, and the samples were centrifuged in a Beckman Centrifuge at 18 600 G for 15 minutes at 4˚C, with rotor JA14. The supernatant was discarded and the pellet residue (R) was dried in a fanned oven at 100˚C for 48 hours until constant weight was attained. The difference in weight between R and 1gDM was regarded as the apparently degraded fraction (AppDeg). R was refluxed with neutral detergent solution (NDS) and the residue (NDF) was dried. The weight of the NDF was subtracted from the 1g DM to yield the truly degraded fraction (degradability). The difference between the true and the apparent degradability is the microbial yield.

Statistical analysis

Feeds were randomly allocated to 24 pressure transducer channels in the incubator (Pienaar, 1994) to generate gas production data by *in vitro* gas production (Pell and Schofield, 1993), following a modified Tilley and Terry (1963) method. SASv9.1 (2005) software was used to fit the gas production responses over the control *in vitro* treatment to the model by Campos *et al*. (2004). ANCOV (Genstat, 2007) using protein percentage as a covariate was performed, for predetermined comparisons amongst feed type across the three replications. Means were compared by least significant differences and were considered different at $P < 0.05$.

Results

Horse feed companies use protein percentages as a means of describing what they think the feed is intended for, broadly as 10%, 12% and 14% feeds. These bear little relevance to the analysed composition of the feeds, which are tabulated in Table 3.1.

Table 3.1 Nutrient specifications of common South African horse feeds (dry matter basis) chosen on the basis of functionality and raw materials, and subjected to proximate analysis and *in vitro* gas production technique

Feed	Advert CP %	CP %	Ca %	P %	Moist %	Fat %	Starch %	NDF %	ADF %	CHO_{sol} %
SPURWING HI5	11	10.97	1.32	0.51	11.90	3.28	25.00	27.60	12.66	34.39
SPURWING TRANKILO	14	15.07	1.09	0.49	13.4	4.31	12.99	23.77	12.39	48.6
SPURWING BROODMARE	14	16.25	1.27	0.57	11.4	4.13	18.75	17.3	9.6	53.1
SPURWING PERFORMANCE	13	13.51	1.11	0.42	11.2	4.03	24.72	17.73	10.33	57.0
SPURWING MAINTENANCE	11	11.71	1.01	0.46	15.1	4.26	17.01	22.72	9.09	53.0
SPURWING SUPA START	14	15.78	1.27	0.42	12.2	3.91	25.06	14.98	8.66	56.7
SPURWING WARMBLOOD	12	13.55	1.04	0.58	10.4	4.28	23.73	20.19	10.47	54.3
SPURWING HACK	12	13.72	1.01	0.52	13.4	3.49	18.3	22.05	9.37	53.1
SPURWING SHOW HORSE	14	15.17	1.11	0.5	13.9	4.76	11.55	24.23	11.2	46.7
SPURWING SUPA GROWTH	14	15.85	1.2	0.52	12	3.87	20.52	16.31	8.56	54.4
EQUIFEEDS ENDURO	12	13.53	1.58	0.47	10.8	8.23	16.9	21.65	11.14	48.1
EQUIFEEDS WARMBLOOD	12	10.15	1.18	0.4	11	4.11	23.76	23.47	10.34	53.8
EQUIFEEDS TRANQUILO	14	13.17	1.03	0.5	11.4	9.29	13.21	25.19	12.59	43.9
ROMIX PONY	10	10.09	0.93	0.35	12.8	2.49	23.54	22.2	8.86	56.7
ROMIX HACKING CUBE	12	11.1	0.92	0.52	10.8	3.90	22.92	26.76	9.1	49.7
VUMA VALU RED	12	14.11	0.24	0.56	11.5	5.43	19.74	27.43	10.53	44.5
VUMA SUPA COOL	10	13.18	0.27	0.55	11.6	2.96	19.71	30.19	11.68	45.2
MKONDENI HIGH ENERGY CUBES	14	11.77	1.14	0.58	11	2.89	11.7	44.67	21.13	32.2
LUCERNE	15	16.35	1.33	0.26	11.75	1.27	2.0	45.52	29.45	29.1

Where: Advert CP = advertised feed bag label CP%; CP = crude protein, Ca=calcium, P=phosphorous, moist=moisture, NDF=neutral detergent fibre, ADF=acid detergent fibre, CHO_{sol}=soluble carbohydrates

A poor correlation exists between the advertised crude protein value and the actual protein content of the feed (54%). GP kinetics of the feeds produce significantly different parameters in respect of slow and rapidly fermentable components of the feed (Table 3.2).

Table 3.2 Mean gas production kinetics of common SA horse feeds fermented using equine faecal inoculum *in vitro*, adjusted for a covariate (CP), where A/B=gas volume from fast (cell content) and slowly (cell wall) degradable fractions, respectively; a1/b1=degradation rates (h^{-1}) for fast and slowly degradable fractions, respectively; C=lag time (h)

Feed	A (ml)	B (ml)	a1 (h^{-1})	b1 (h^{-1})	C (h)
SPURWING HI5	65.1^{ab}	72.8^{b}	0.335	0.228	4.33
SPURWING TRANKILO	97.8^{ab}	32^{ab}	0.398	0.177	10.51
SPURWING BROODMARE	71.7^{ab}	70.7^{b}	0.370	0.239	7.86
SPURWING PERFORMANCE	39.3^{a}	139.4^{c}	0.365	0.349	9.55
SPURWING MAINTENANCE	67.9^{ab}	61.4^{b}	0.329	0.190	9.47
SPURWING WARMBLOOD	113.6^{b}	47.1^{ab}	0.423	0.368	8.50
SPURWING HACK	98.2^{ab}	54.9^{ab}	0.407	0.230	8.44
SPURWING SHOW HORSE	82.2^{ab}	62.0^{b}	0.456	0.184	6.97
SPURWING SUPA GROWTH	100.6^{b}	44.4^{ab}	0.375	0.283	10.38
EQUIFEEDS ENDURO	55.3^{ab}	47.4^{ab}	0.323	0.161	6.48
EQUIFEEDS WARMBLOOD	166.5^{c}	33.4^{a}	0.316	0.001	1.09
EQUIFEEDS TRANQUILO	57.6^{ab}	47.8^{ab}	0.394	0.314	12.01
ROMIX PONY	70.3^{ab}	75.9^{b}	0.351	0.232	9.69
ROMIX HACKING CUBE	68.3^{ab}	43.1^{ab}	0.387	0.454	10.12
VUMA VALU RED	80.3^{ab}	68.7^{b}	0.456	0.227	11.15
VUMA SUPA COOL	100.7^{b}	48^{ab}	0.417	0.247	10.79
MKONDENI HIGH ENERGY CUBES	40.0^{a}	54.3^{ab}	0.338	00.238	11.96
LUCERNE	57.9^{ab}	38.0^{ab}	0.378	0.231	4.59
MEAN	69.3	54.3	0.364	0.230	7.55
SE	28.28	25.08	0.0988	0.0941	4.914
LSD	61.02	54.11	0.2123	0.2023	10.56
Significance (5%)	**	**	Ns	Ns	Ns

Means with different superscripts in the same column differ significantly (P <0.05). Ns= non-significant

Table 3.3 pH, apparent and true degradabilities (g.kg^{-1}DM), maximum gas production (ml.gDM^{-1}), partitioning factor (degraded substrate:gas volume mg.ml^{-1}) and time (hours) to maximum gas production in common SA horse feeds fermented *in vitro* using equine faecal inoculum.

Feed	pH	App Degrad g.kg^{-1}DM	True Degrad g.kg^{-1}DM	Max gas prod ml.g^{-1} DM	Part factor mg.ml^{-1}	Time half max gp (h)
SPURWING HI5	6.62^{a}	502.5^{cd}		123.1^{b}		13.42^{ab}
SPURWING TRANKILO	6.74^{ab}	504.6^{cd}	844.4^{cd}	104.1^{ab}	8.22^{ab}	9.17^{ab}
SPURWING BROODMARE	6.75^{ab}	474.0^{d}	825.0^{c}	120.6^{b}	7.32^{ab}	21.89^{b}
SPURWING PERFORMANCE	6.68^{ab}	511.7^{cd}	860.8^{cd}	91.3^{ab}	11.31^{ab}	16.33^{b}
SPURWING MAINTENANCE	6.62^{a}	431.2^{cd}	847.9^{cd}	101.4^{ab}	10.31^{ab}	17.20^{b}
SPURWING WARMBLOOD	6.65^{ab}	448.3^{cd}	848.6^{cd}	75.3^{ab}	12.83^{ab}	8.33^{ab}
SPURWING HACK	6.70^{ab}	317.8^{bd}	834.9^{cd}	45.8^{a}	36.12^{c}	20.33^{b}
SPURWING SHOW HORSE	6.79^{ab}	403.5^{c}	825.9^{cd}	122.7^{b}	6.80^{a}	17.96^{b}
SPURWING SUPA GROWTH	6.76^{ab}	441.3^{cd}	868.6^{d}	77.7^{ab}	9.73^{ab}	22.75^{b}
EQUIFEEDS ENDURO	6.68^{ab}	435.8^{cd}	847.9^{cd}	154.8^{b}	5.53^{a}	17.40^{b}
EQUIFEEDS WARMBLOOD	6.75^{ab}	425.4^{cd}	815.7^{bc}	42.0^{a}	20.14^{b}	3.33^{a}
EQUIFEEDS TRANQUILO	6.74^{ab}	248.0^{b}	800.5^{bc}	144^{b}	5.73^{a}	8.73^{ab}
ROMIX PONY	6.71^{ab}	427.2^{cd}	838.3^{cd}	90.6^{ab}	12.17^{ab}	10.13^{ab}
ROMIX HACKING CUBE	6.79^{ab}	435.7^{cd}	775.7^{b}	135.2^{b}	5.90^{a}	10.56^{ab}
VUMA VALU RED	6.86^{b}	371.0^{bc}	876.1^{d}	124.1^{b}	7.06^{a}	16.33^{b}
VUMA SUPA COOL	6.73^{ab}	474.3^{cd}	796.7^{bc}	110.9^{b}	7.18^{a}	14.83^{b}
MKONDENI HI ENERGY CUBES	6.83^{b}	265.3^{bc}	617.7^{a}	156.9^{b}	4.16^{a}	11.00^{ab}
LUCERNE	6.82^{b}	233.0^{b}		127.5^{b}		13.15^{ab}
HAY	6.85^{b}	72.2^{a}		94.0^{ab}		14.73^{b}
SPEEDY BEET	6.63^{ab}	558.8^{d}		109.9^{ab}		19.72^{b}
Control	7.13^{c}					
MEAN		425.4	824.8	109.1	9.99	15.11
SED		71.9	21.6	32.1	6.46	5.06
LSD		142.5	43.4	64.6	12.98	10.19
Significance (5%)	**	**	**	**	**	**

Means with different superscripts in the same column differ significantly (P <0.05). Ns= non-significant

In feeds containing high NDF, there was concomitantly lower soluble carbohydrate content (Figure 3.1). The feed containing the highest NDF contains a significant proportion of rice hulls, and is also incongruously sold as a high energy feed. It contains the lowest soluble carbohydrate

content, and as such is a valuable indicator of the nutrient content that is being formulated for sale to our horses. This feed also accurately represents the decline in apparent degradability that will be experienced (Figure 3.2) where quality is compromised in the delivery of concentrate rations for horses.

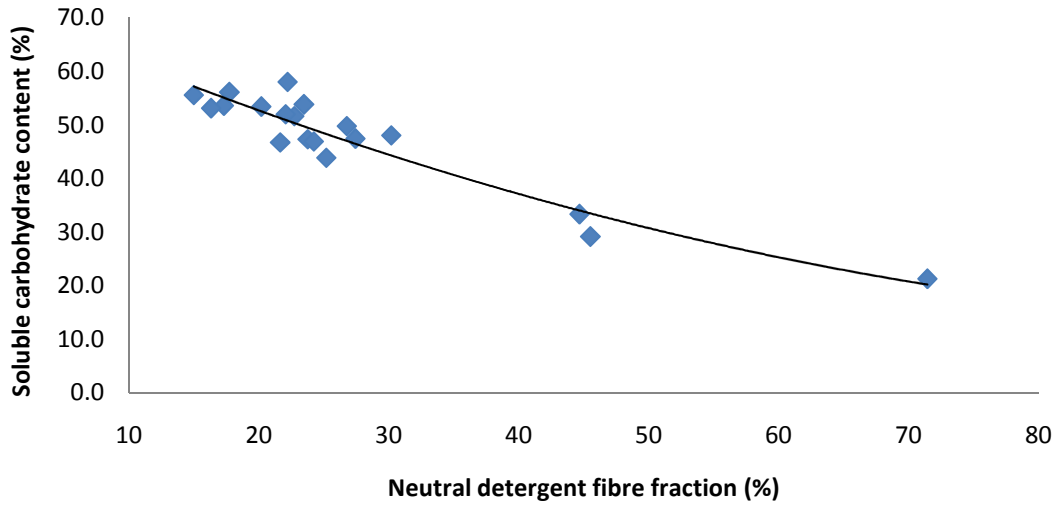

Figure 3.1 Relationship between the neutral detergent fibre fraction of common South African horse feeds, and the soluble carbohydrate content of these feeds, where CHO_{sol} (%) = $0.004x^2 - 1.059x + 71.99$ and $R^2 = 0.892$.

There are positive correlations (84%) between the soluble carbohydrate contents of the horse feeds and the apparent degradability (Figure 3.2) with the limit to this in the *in vitro* situation being the capacity of the inoculum to digest the substrate.

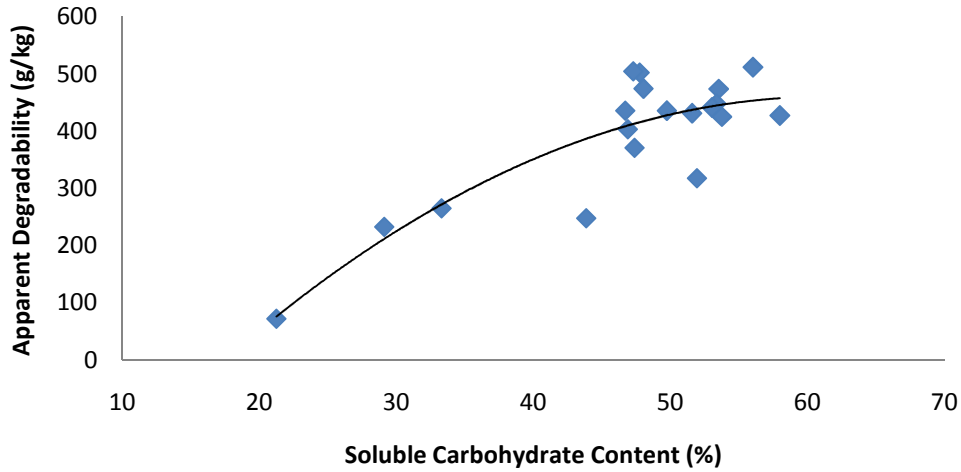

Figure 3.2 Response of Apparent Degradability to the Soluble Carbohydrate Content of several South African horse feeds, where $y = -0.237x^2 + 29.19x - 437.0$, and $R^2 = 0.738$

With an increase in the fibre content of the feeds, the apparent digestibility decreases.

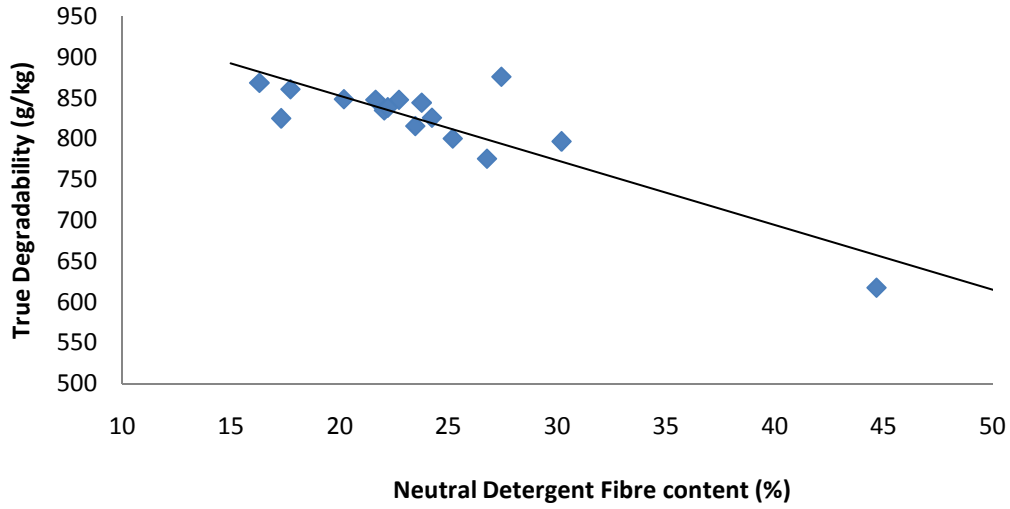

Figure 3.3 Inverse relationship between the True Degradability and the NDF content of several South African horse feeds, where True Degradability = -7.9209NDF + 1011.3 and $R^2 = 0.7569$.

Discussion

Poor correlations between the advertised CP% and the content in the feed (R^2=0.54), with there being more CP in the feed than that advertised, make this a poor indicator of the value of the feed, and undermines the value of marketing feeds on the basis of a CP percentage. A disparity in fat percentages in the feeds affords a means of segregating the feeds, but feed clusters based on these components of the feed (Table 3.1) don't necessarily eludicate fermentation characteristics of the feeds, which can provide a means of discerning between the fibre and fermentable portions of the feed.

pH of the inoculum was reduced from the control by the addition of the horse feeds (P<0.001). No reduction in pH is apparent in the control samples (Table 3.2) although differences in the reduction in pH were observed for other feeds. The pH values of the digesta were in the range mentioned by other authors (Hussein *et al*., 2004; Swyers *et al.*, 2008). The maintenance and Hi5 feeds (idle horse feeds) were significantly lower in pH, and the hay, lucerne, M14 and VVRed produced the highest pH. This would have been related to the products of digestion. Adjusted for a covariate of initial pH, the addition of concentrate lowered the pH of the digesta product. The most significant reduction in pH occurred with higher fibre feeds. Many authors have shown a reduction in pH of digesta with the addition of grains to the feed (Willard *et al.*, 1977;

Hussein *et al.,* 2004; Ellis and Hill, 2005; Swyers *et al*., 2008). This is due to the biochemistry of the breakdown of the grains, and the proportion of soluble carbohydrate reaching the caecum. No strong correlations between the reduction in pH following *in vitro* incubation and the proximate analyses of the tested horse feeds could be found. In feeds containing high NDF, there was concomitantly lower soluble carbohydrate content (Figure 3.1). This inverse relationship indicates the substitution of carbohydrate for fibre in the lower quality feeds. This is beneficial because the digestibility of fibre is usually reduced in the presence of non-degraded starch in the hindgut (Swyers *et al*., 2008). Hussein *et al.* (2004) found that the apparent digestibility of DM and OM could increase to a level commensurate with a high starch load (36% starch).

GP kinetics of the feeds produced significantly different parameters in respect of slow and rapidly fermentable components of the feed (Table 3.2). Adjusted with CP as a covariate, the amount of gas produced and fitted to the model of Campos *et al*. (2004) revealed that the feeds could be separated by the rapidly and slowly fermentable fractions of the feed. Low levels of rapidly fermentable substrates occurred in two feeds (SWperformance and MK14) and SWWB, SWSG and VSC10 contain rapidly fermentable components. This can only be attributed to the soluble carbohydrate content of the feed (38% correlation). The "A" value does indicate a high proportion of rapidly degradable substrate, and in this category were feeds that contained a high nutrient density and contained fermentable substrate**.**

The highest gas production identified by the fitting of the Campos *et al.* (2004) model to common SA horse feeds resulting from a high proportion of slowly degradable fractions was in the SWPerformance. This feed contained the lowest level of rapidly fermentable fractions, but contained a high percentage of fermentable carbohydrate. It is a feed intended to produce a lot of volatility in the behaviour of the horses, such as in polo ponies, or in horses that tend to be lazy and require an energy boost. Relating this characteristic of the feed to the glycaemic index may be valuable. The maize in this feed was extruded. Processing can alter the site of utilization of the maize (Julliand *et al*., 2006). The use of *in vitro* fermentation in testing horse feeds attempts to mimic the passage of digesta into the caecum. This assumes an overflow of digesta from the stomach, passing through the intestine, for utilization in the hindgut of the horse. This is commensurate with high levels of feeding in either one or two meals in a day (Clarke *et al.,* 1990; Rosenfeld and Austbø, 2009). This overflow leads to the site of digestion of feed being altered. It is interesting that the hindgut makes limited use of the rapidly fermentable portions, and that the slowly degradable fractions of the feed produce more gas. The aim of the extrusion

is the gelatinization of the starch, to increase foregut digestion (Ellis and Hill, 2005; Hill, 2007) and where hindgut fermentation is making full use of the fibre components as well, the percentage utilization of the feed as a whole must then be increased. That undigested starch in the hindgut might reduce fermentation is a subject addressed by nutrient synchrony.

Four of the low budget feeds provided the highest amounts of slow fermentable substrate. One of these contained many whole, unprocessed grains that are not digested by the horse, whereas the others contained no grain. The Campos *et al.* (2004) model elucidated no significant differences in the degradation rate of the substrates and no difference in lag or colonization time. Since the inoculum was sourced from horses that were receiving meals of concentrate daily, it is assumed that the populations of microbes were effectively sourced in the faeces (Harris, 1996) and the substrate could therefore be utilized. Least cost formulations frequently compromise on quality of raw materials. *In vitro* fermentation demonstrates this.

Apparent degradability is an indication of the breakdown of the feed material, and the feeds significantly improved the degradable portion in comparison to hay ($P<0.001$) and lucerne (Table 3.3). Apparent degradability was generally high, except for feeds with poor specifications like the MK 14 which is high in ADF.

The EFTrankilo is a feed that is purported to provide a maize-free feed to horses that will not cause bad behaviour, by providing energy through lipids. However, other feeds purporting to do the same thing have double the apparent degradability of the EFT. Many of the maize-free options include a lot of hominy chop (Lindsay, 2005) and in this way dilute the digestible energy by reducing the nutrient density of the feed.

There are positive correlations (84%) between the soluble carbohydrate contents of the horse feeds and the apparent degradability (Figure 3.2) with the limit to this in the *in vitro* situation being the capacity of the inoculum to digest the substrate.

With an increase in the fibre content of the feeds, the apparent digestibility decreases. This situation is mirrored in the true digestibility. The true digestibilities of the feeds depend on the soluble carbohydrate and fibre contents. The lowest degradabilities (Table 3.3) occur in the feeds with the lowest NDF content, which are sold as cubes. Finely ground feeds may present a large surface area for utilization by microbes (Ellis and Hill, 2005) which can result in high utilization of their substrate.

Conventional horse feed ingredients may be substituted for “cooler” feeds. This is to calm a “hot” horse, which by definition is one that is excitable and frenetic in behaviour under saddle and on the ground. It has long been supposed that this behaviour is related to the crude protein of the ration that the horse is eating. Lucerne has even been blamed for this behaviour. This is one reason why CP became a primary criterion of feed categories in this SA. This is inappropriate because crude protein is usually not invoked in different responses of horses to different feeds. Excitable behaviour may also be blamed on fermentable carbohydrate in the feed (Willard *et al.,* 1977; Johnson *et al.,* 1998; Hancock *et al*., 2004; Ellis and Hill, 2005). The passage of these substrates to the hindgut for fermentation releases a lot of energy in the form of volatile fatty acids (VFAs) but also produces a distention in the caecum, and the concomitant discomfort has been blamed for irritable behaviour. The feeds under study produced different amounts of gas under incubation (Table 3.3). Feeds with an average gas production have average nutrient densities, with a lot of lucerne in the feeds. The highest gas productions are observed in the feeds that have the most fermentable substrate (Tables 3.2 and 3.3). Maximum gas production is correlated to the A value (the rapidly degradable fraction) of the feed. Where the energy is supplied by whole oats or extruded maize, the maximum gas production is lower.

Partitioning factor (degraded substrate:gas volume; $mg.ml^{-1}$), as described by Blümmel *et al.* (1997), has been used to describe the efficiency of microbial protein synthesis in ruminants (Ouda, 2007; Dijkstra *et al*., 2005). In the horse, it reflects the relationship between substrate entering the hindgut and its fermentation. In this respect, most of the budget feeds, or feeds that employ some substitution of raw materials, have a lower partitioning factor (Table 3.3). Superior partitioning is evident in the oat feed and the feed with extruded maize. This is consistent with the work of Jackson *et al*. (2010) found an improvement in the PF with the processing of maize. Much of the value of the PF lies in its ability to predict microbial efficiency and in conjunction with issues like nutrient synchrony, in the horse, the PF can be a valuable indicator of the use the horse can make of concentrate feeds. Microbial activity is close to maximum at half maximum gas volume (France *et al*., 1993; Davies *et al*., 2000; Makkar, 2004; in Ouda, 2007), and $T_{½}$ can therefore be used to predict nutritive value (Murray *et al*., 2006). In the horse feeds, this provided a segregation of the lucerne based feeds from the rest. The correlation to crude fibre was higher, but the feeds that take longer to generate half of their maximum gas production were also the feeds that are formulated to contain either more lucerne or more raw materials in an unprocessed form. The DEF is correlated to the rapidly

fermentable portion of the feed and inversely proportional to the maximum GP, but not to any proximate characteristic of the feed.

Conclusions

Concentrate feeds offered to horses can demonstrate differences in gas production kinetics, and that these parameters and their inferences are more informative in the description of the feeds than proximate analyses. IVGPT can provide a more effective, rapid assay of horse feed characteristics. The addition of concentrates, even *in vitro*, decreases pH, and improves the degradable portion of feed over hay only. Gas production kinetics produced different parameters in respect of slow and rapidly fermentable components of the feed. Apparent and true digestibilities are negatively correlated to fibre content. Attributes of the feed from a feed analysis point of view can be inferred from gas production parameters, and IVGPT can be used to discern between feed groups more informatively than CP percentage.

CHAPTER FOUR: GLYCAEMIC RESPONSES TO SEVERAL SOUTH AFRICAN HORSE FEEDS AT TWO FEEDING LEVELS

Abstract

Common South African horse feeds were tested for glycaemic response in miniature horses using the hexokinase method with deproteinization using an auto analyser (Roche Diagnostics). Covariance Estimation for Cluster Analysis (SAS, 2005) produced four groups of feeds (R2=87%) from 18 common South African horse feeds, based on feed characteristics (neutral detergent fibre, crude protein, fat and non-structural carbohydrate). Blood glucose parameters of feeds (Mean, peak, slope and time to peak and area under the curve) in each group were compared by analysis of variance and regression with covariates. The addition of concentrate to the diets of the horses at 20% and 40% of the diet produced responses over the blood glucose of 4mmol/L of horses fed hay only. Peak and slope to peak blood glucose in feeds high in fat were lower than feeds containing more carbohydrate. Maintenance feeds (higher in fibre and lower in nutrient density) produced a peak response to feeding level of up to 5.8mmol/L. A high starch riding feed produces a faster rise to a higher peak (8mmol/L) at higher feeding levels. Glycaemic responses to feeds can also indicate the extent of preceacal digestion, which is influenced by the volume of feed per meal. With inordinately inflated concentrate:roughage ratios for work and body condition scores provided to sport horses, the timing of feeding relative to competition or work is important in governing the constancy of performance and temperament.

Introduction

There are three routes of entry of glucose to intermediary metabolism: direct absorption of the monosaccharide, enzymatic digestion of the di- and oligo-saccharides to yield glucose and fermentation of dietary carbohydrate to produce volatile fatty acids which are converted to glucose in the liver (gluconeogenesis) (Simmons and Ford, 1991). The horse as a hindgut fermenter is suited to the trickle feeding of plant material (Hill, 2007), 70% of which is carbohydrate. This carbohydrate varies both in characteristic and solubility (Figure 4.1).

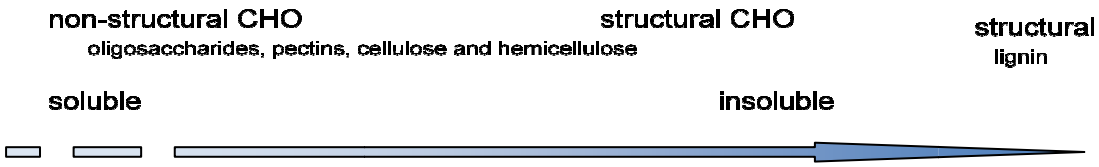

Figure 4.1 Characteristics and solubility of carbohydrates in animal feeds (after Hoffman, 2003)

The increase in nutrient requirement associated with sport horses (NRC, 2007) means that horses are provided nutrient dense feeds, with consequences for behaviour (Cooper *et al.,* 2005). There exists a disparity between the proximate analysis of feeds and response in the animal, similar to that outlined in Figure 4.2 by Hoffman (2003).

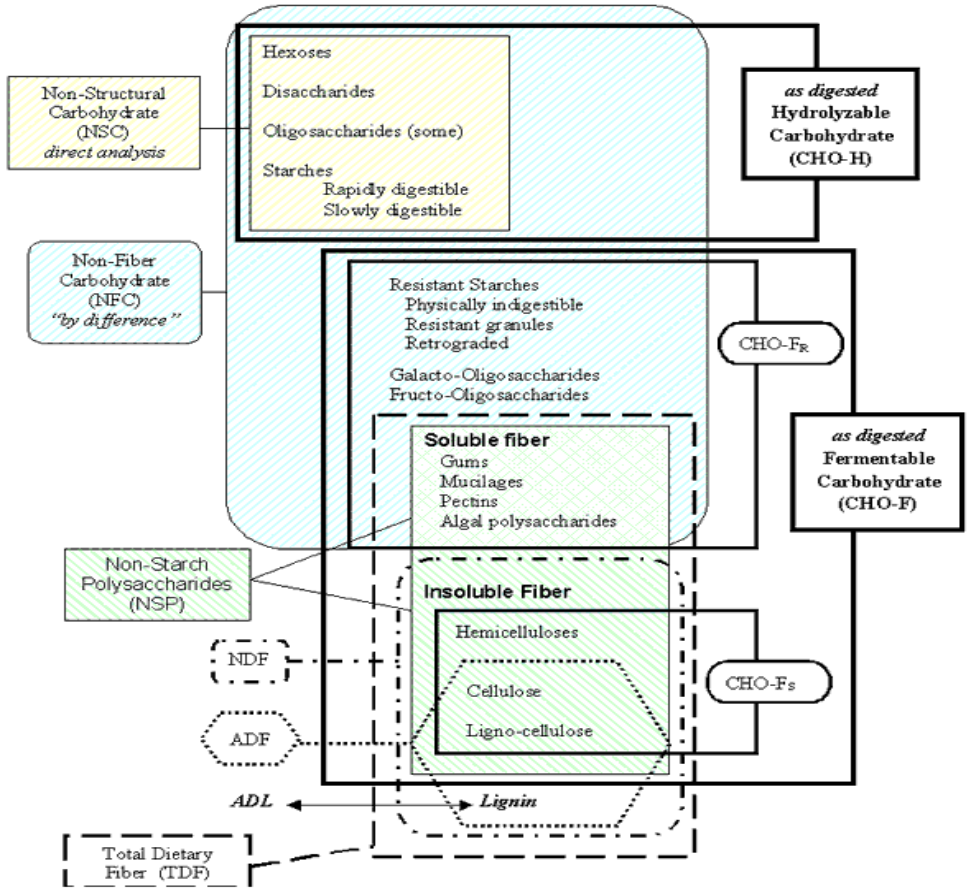

Figure 4.2 The difference between the proximate analysis of the feed (on the left hand side) and the animal's reception of the feed (on the right hand side of the diagram) after Hoffman (2003).

The order of enzymatic degradation and fermentation in the horse affects substrate choice (Dyer *et al.,* 2002) and the type (Staniar *et* al., 2007), amount and rate of provision of the rations (Medina *et al.*, 2002). The higher concentrate to roughage ratio has as a consequence: reduced saliva, increased lactobacilli, increased acidity in stomach, ulceration of gastric mucosa, lamellar ischemia

(Hill, 2007) and behavioural modification (Mills and Nankervis, 1991), as well as altering digestibility of starch in the small intestine (Meyer *et al.*, 1993).

Glycaemic potency (Munro, 2005) is a term that was first introduced as a means of determining the pre-caecal digestion of feed. Feeding large meals containing soluble carbohydrate necessarily precluded much pre-caecal digestion (Mētayer *et al.*, 2004), consequently providing substrate for fermentation in the hindgut (Medina *et al.*, 2002). Wolever *et al.*, (1991) calculated the glycaemic index of carbohydrates as

$$GI_{carb} = \frac{\text{IAUC 50g avail CHO}}{\text{IAUC 50g glucose}} \qquad \text{Equation 4.1}$$

The concept of glycaemic index was extended to accurately account for the blood glucose response in meal feeding by the glycaemic load (GL). A glycaemic gram equivalent (GGE) followed which provided the weight of glucose that would induce the same glycaemic response as a given weight of food (Monro and Williams, 2000). Wolever (1997) summarises some of the reservations of the glycaemic response in his review. Glycaemic index in its relativity has made many of the papers in the equine literature difficult to compare, as sometime oats and sometimes maize are used as a baseline (Ralston, 1984). Glycaemic responses do provide a useful tool in equine ration formulation (Harris and Geor, 2009).

Many horse feeds available in South Africa (AFMA, 2006) use muesli type feeds, molasses and other high GI raw materials. The literature does consider the glycaemic response of composite feeds in humans (Wolever, 1997) and in horses (Harbour *et al.*, 2003), and also considers the effect of processing of raw materials on this parameter (Hill, 2007). In horse feeds, the timing of feeding relative to exercise is important in terms of the glycaemic response (Lawrence *et al.*, 1993; Vervuert, 2009c). Together with the *in vitro* gas production of these feeds, glycaemic response in the feeds may provide the means of discerning between feeds of different quality that are intended for different purposes. Eighteen horse feeds were tested at two feeding levels in miniature horses. Feeds were selected from four categories as determined in Chapter 2. The glycaemic response was related to the starch and carbohydrate contents of the rations, and meal size.

Materials and Methods

Ten miniature horses (mean age in years seven) in good health and with up to date vaccinations and deworming treatment were kept in individual stables. Miniature horses were available for use at the Ukulinga Research Farm of the University and had been successfully protocolled for use as representatives of the larger Thoroughbred horses (Lindsay, 2005). Water was available *ad libitum*, and horses were walked in hand and groomed daily. Horses were subject to standard animal ethics guidelines for their care. An acclimation period of ten days for new feeds was stipulated (Lindsay, 2005). Horses received their daily ration at 2.5% of body weight. Concentrate feed and *Eragrostis curvula* hay were provided in a ratio of 20%:80% and 40%:60% of the daily intake as two meals. Horses were randomly allocated to feeding treatments of 18 feeds over two time periods during which blood glucose responses were obtained twice on each horse. Hay was available *ad* libitum and blood glucose was obtained from capillary blood (ear) before feeding and every 30 minutes after feeding the concentrate breakfast meal for four hours by the hexokinase method with deproteinization using an auto analyser (Roche Diagnostics®) immediately after sampling. Time to peak (hours), peak glucose (mg/dL), mean glucose concentration (mg/dL), slope to peak (mmol/L/h) and area under the curve (AUC) were determined using line-exp regression analysis (Genstat, 2007), Analysis of Variance (with initial blood glucose concentration as covariate), and Approximate Covariance Estimation for Cluster Analysis (SAS, 2005).

Results

Ration composition

Table 4.1 shows the nutrient specifications on an as is basis of several South African horse feeds fed to miniature horses at two feeding levels to determine glucose response parameters.

Cluster analysis

A correlation analysis was done on the analysed compositions of the feeds. A multivariate, hierarchical cluster analysis technique (SAS, 2005) was used in Chapter 2 to segregate feeds into homogeneous groups (clusters) (Figure 2.1) on the basis of classifying variables (CP%, NDF%, NFC% and fat %) derived from proximate analysis. The variable reduction ACECLUS procedure summarized the multivariate set of data into orthogonal canonical variates. Ward's method (William, 1994) was used to ensure that within-cluster differences and between-cluster linkages were minimized. Feeds (Table 4.1) were then offered to horses as described above to determine the glycemic responses on

the feeds. The cluster of each feed was used as a treatment factor in analyzing the significance of the response of the horse to each feed.

Table 4.1 Nutrient specifications (dry matter basis) of several common South African horse feeds that were fed to miniature horses to determine blood glucose response parameters.

Feed	Advert CP %	CP %	Ca %	P %	Moist %	fat %	starch %	NDF %	ADF %	CHO sol %
SPURWING HI5	11	10.97	1.32	0.51	11.90	3.28	25.00	27.60	12.66	34.39
SPURWING TRANKILO	14	15.07	1.09	0.49	13.4	4.31	12.99	23.77	12.39	48.6
SPURWING BROODMARE	14	16.25	1.27	0.57	11.4	4.13	18.75	17.3	9.6	53.1
SPURWING PERFORMANCE	13	13.51	1.11	0.42	11.2	4.03	24.72	17.73	10.33	57.0
SPURWING MAINTENANCE	11	11.71	1.01	0.46	15.1	4.26	17.01	22.72	9.09	53.0
SPURWING SUPA START	14	15.78	1.27	0.42	12.2	3.91	25.06	14.98	8.66	56.7
SPURWING WARMBLOOD	12	13.55	1.04	0.58	10.4	4.28	23.73	20.19	10.47	54.3
SPURWING HACK	12	13.72	1.01	0.52	13.4	3.49	18.3	22.05	9.37	53.1
SPURWING SHOW HORSE	14	15.17	1.11	0.5	13.9	4.76	11.55	24.23	11.2	46.7
SPURWING SUPA GROWTH	14	15.85	1.2	0.52	12	3.87	20.52	16.31	8.56	54.4
EQUIFEEDS ENDURO	12	13.53	1.58	0.47	10.8	8.23	16.9	21.65	11.14	48.1
EQUIFEEDS WARMBLOOD	12	10.15	1.18	0.4	11	4.11	23.76	23.47	10.34	53.8
EQUIFEEDS TRANQUILO	14	13.17	1.03	0.5	11.4	9.29	13.21	25.19	12.59	43.9
ROMIX PONY	10	10.09	0.93	0.35	12.8	2.49	23.54	22.2	8.86	56.7
ROMIX HACKING CUBE	12	11.1	0.92	0.52	10.8	3.9	22.92	26.76	9.1	49.7
VUMA VALU RED	12	14.11	0.24	0.56	11.5	5.43	19.74	27.43	10.53	44.5
VUMA SUPA COOL	10	13.18	0.27	0.55	11.6	2.96	19.71	30.19	11.68	45.2
MKONDENI HI ENERGY CUBES	14	11.77	1.14	0.58	11	2.89	11.7	44.67	21.13	32.2

Where: Advert CP = advertised feed bag label CP%; CP = crude protein, Ca=calcium, P=phosphorous, moist=moisture, NDF=neutral detergent fibre, ADF=acid detergent fibre, CHO_{sol}=soluble carbohydrates

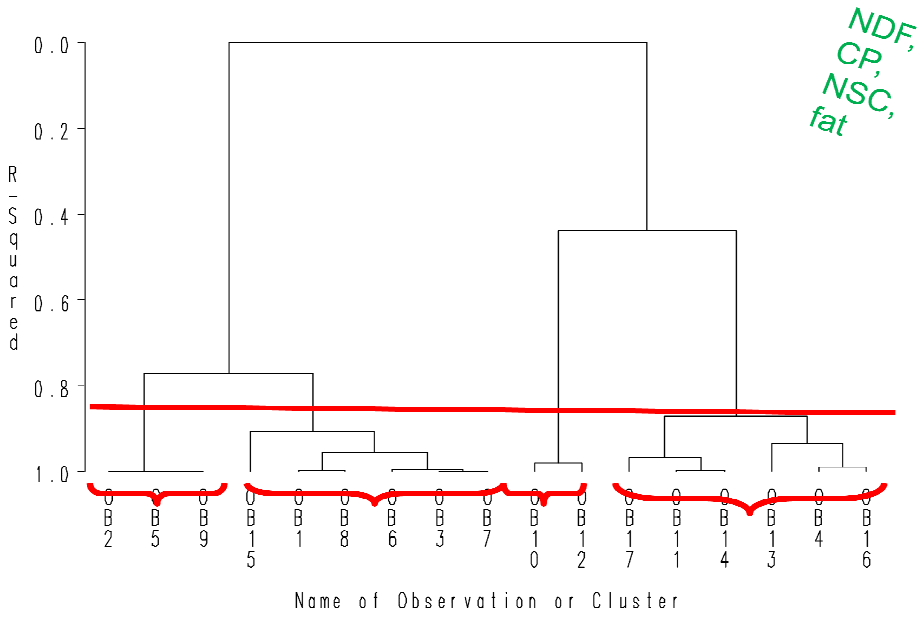

Figure 4.3 Dendrogram indicating that four groups of feeds can be derived from an hierarchical cluster analysis of classifying variables (NDF, CP, fat and NSC) which account for 87% of the variation observed between the compositions of the feeds.

Glycaemic parameters

Feeds representative of each group produce different glycaemic parameters in respect of the average blood glucose concentration, time and slope to peak, peak and area under the curve (Table 4.2), where the fifth cluster in the analysis is a hay diet.

Table 4.2 Postprandial glucose responses of miniature horses fed 20% and 40% of daily intake as concentrate feeds. Four feed clusters were produced from a hierarchical cluster analysis of horse feeds classified by NDF, CP, fat and NSC, with *Eragrostis curvula* hay being used as the fifth basal cluster.

Feeding level		Average BG	Time to peak	Peak BG	Slope to peak	Area under curve
% of daily feed	Cluster	$mMOL.ml^{-1}$	Minutes	$mMol.ml^{-1}$	$Mmol.ml^{-1}.h^{-1}$	Units
20	1	4.284	68.51	5.836	0.03331	1095
	2	4.459	80.48	5.998	0.03309	1142
	3	4.252	75.81	5.458	0.02651	1068
	4	4.211	69.59	5.280	0.02789	1057
	5	3.733	51.73	4.922	0.03155	849
Mean		4.19	69.22[a]	5.50	0.0305[a]	1042
40	1	4.499	84.48	4.651	0.01382	1003
	2	4.301	97.16	5.199	0.01769	1101
	3	4.862	86.36	6.269	0.03524	1200
	4	4.491	90.11	5.467	0.02351	1102
	5	4.602	106.87	5.678	0.02216	1187
Mean		4.55	93.00[b]	5.45	0.0225[b]	1119
SED		0.778	29.84	1.0315	0.0120	183.3
$LSD_{5\%}$		1.560	59.92	2.063	0.0239	366.6
CV%		17.74	36.31	18.76	46.29	16.74
Cluster Group		Ns	Ns	Ns	Ns	Ns
Feed Level		Ns	**	Ns	**	Ns
Cluster/Feed		Ns	Ns	0.093	0.073	Ns

Where BG = blood glucose, SED = standard error of difference, LSD = least significant difference 5%, Ns = not significant (P>0.05).

The addition of concentrate produced a response in blood glucose over a hay only diet, while the blood glucose response is ameliorated by the addition of fat (Figure 4.4). This is a common tool in sport horse diets, as it produces conditioning of the horse and reduces volatility of temperament in sport horses (Pagan *et al.,* 2000).

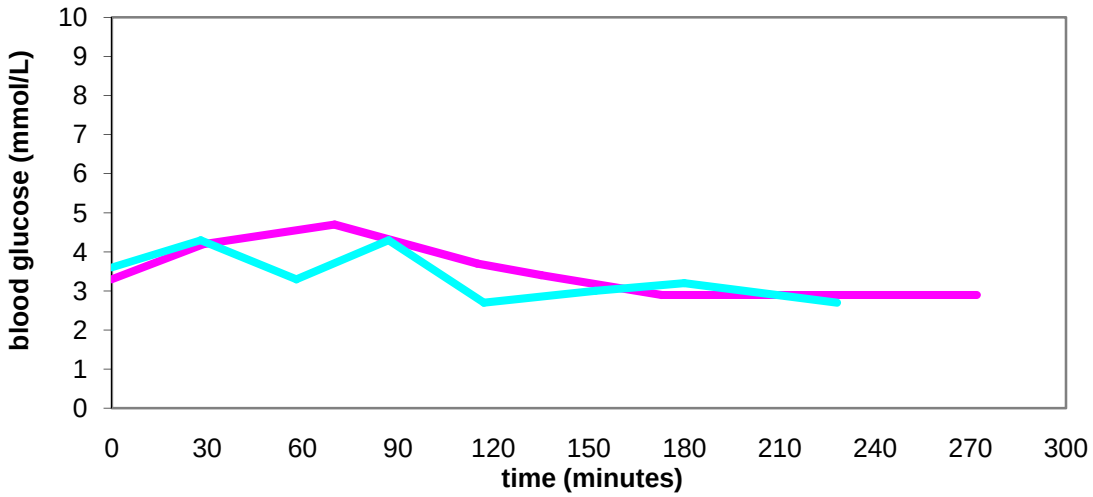

Figure 4.4 Mean blood glucose response in miniature horses over four hours to feeds that are high in fat at low (---) and high (---) feeding levels.

Feeds in the third category (maintenance feeds) reflect the soluble carbohydrate content of the feeds in that as feeding level is increased, the glucose peak is increased and shifted (Figure 4.5).

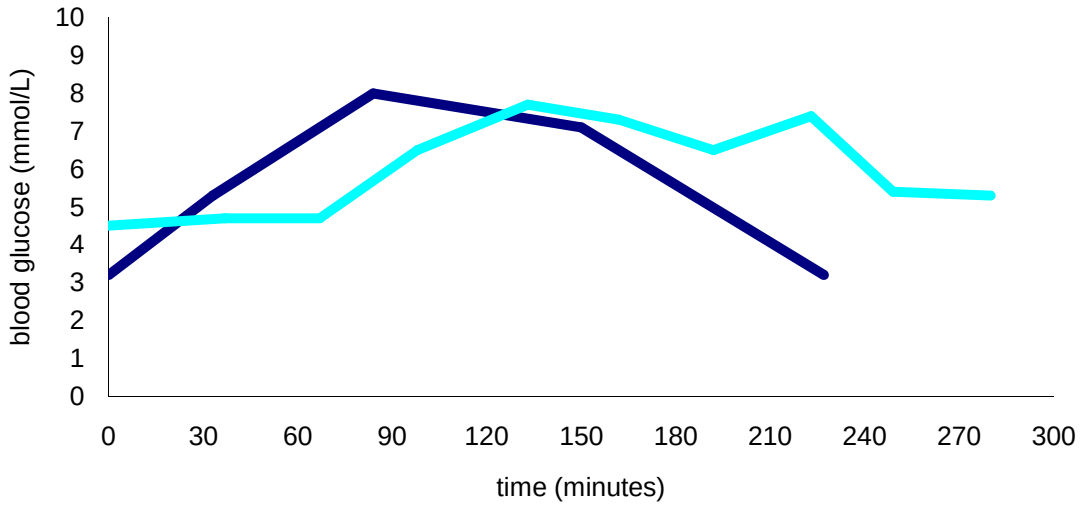

Figure 4.5 Mean blood glucose response of miniature horses over four hours to riding feeds that are high in soluble carbohydrates fat at low (----) and high (----)feeding levels.

Table 4.3 Intakes of starch in grams per kilogram body weight (BW) and non structural carbohydrate (NSC) intake of several horse feeds offered to miniature horses to test glycaemic response.

Feed	Cluster	Starch intake g starch/kg BW/meal		NSC intake g/kg BW $g.kg^{-1}$ BW		CHO_H $g.kg^{-1}$	CHO_{FR} $g.kg^{-1}$
Feeding level		20%	40%	20%	40%		
SPURWING TRANKILO	1	0.32	0.65	1.77	3.55	5.46	29.99
SPURWING BROODMARE	1	0.46	0.94	2.15	4.29	6.61	36.31
SPURWING PERFORMANCE	1	0.62	1.24	2.28	4.55	7.02	38.52
SPURWING MAINTENANCE	1	0.43	0.85	1.91	3.82	5.89	32.32
SPURWING SUPA START	1	0.63	1.25	2.26	4.51	6.95	38.18
SPURWING WARMBLOOD	1	0.59	1.19	2.18	4.36	6.72	36.87
SPURWING HACK	1	0.46	0.92	1.97	3.93	6.06	33.28
SPURWING SHOW HORSE	1	0.29	0.58	1.70	3.39	5.23	28.71
SPURWING SUPA GROWTH	1	0.51	1.03	2.20	4.40	6.78	37.20
EQUIFEEDS ENDURO	2	0.42	0.85	1.89	3.78	5.82	31.97
EQUIFEEDS WARMBLOOD	1	0.59	1.19	2.16	4.33	6.67	36.60
EQUIFEEDS TRANQUILO	1	0.33	0.66	1.65	3.30	5.08	27.87
ROMIX PONY	1	0.59	1.18	2.22	4.44	6.84	37.58
ROMIX HACKING CUBE	1	0.57	1.15	1.97	3.94	6.08	33.36
VUMA VALU RED	2	0.49	0.99	1.68	3.35	5.17	28.36
VUMA SUPA COOL	3	0.49	0.99	1.70	3.41	5.25	28.82
MKONDENI HIGH ENERGY CUBES	4	0.29	0.59	1.08	2.17	3.34	18.33
Mean		0.47	0.95	1.93	3.85	5.94	32.60

Where CHO-H = hydrolysable carbohydrate; CHO-FR = rapidly fermentable carbohydrate

Discussion

Much work on human glycaemic responses probably prompted the interest in the equine's response to carbohydrates in the feed. This current investigation produced glycaemic parameters that were similar to other researchers. Stull and Rodiek (2007) use individual raw materials in their investigations, while composite feeds can behave differently (Kronfeld *et al.,* 2004). Harbour (2003) used complete feeds which produces parameters in agreement with this study. Rodiek and Stull (2007) used ten feeds from a high and low glycaemic "square" from which to test glycaemic responses in horses. They concluded that from a practical standpoint, categories of GI values may be useful for formulating feeds with different glycaemic objectives. Rodiek and Stull (2007) said that NSC is a good predictor of GI. Although AUC was weakly positively correlated to the NSC intake, a stronger positive correlation exists between starch intake and AUC in this study.

Glycaemic load incorporates feeding level into the glycaemic response. In this study, feeding level affected the time to peak, in that double the feed allocation produced a peak blood glucose concentration 20 minutes later. The Peak BG tended to an interaction between the feeding level and the feed group cluster, in that the response across the five group clusters didn't appear to be constant across both feeding levels. Feeding level affected the slope to peak in that the 20% feeding increased the blood glucose slightly quicker. There was an indication (P=0.073) that this increase wouldn't be consistent over the cluster groups though.

No differences in the area under the curves were evident across feeding levels and clusters. A very good reason why we are not getting the greatest disparity between the feeds is that they are all in the higher zones of Hoffman *et al.* (2009) recommendations, greater than their point of inflexion at 0.296g NSC intake/kg BW. They showed the glycaemic response to be smaller in this zone. Regressions of NSC intake against AUC show very little correlation at the 20% and 40% feeding levels, although there is a slight positive trend in the data. There is a stronger correlation between the GR and the starch intake at the 40% feeding level, although the starch intake is not shown to be greater than the 1.1g.kg-*1* BW/meal recommended by Vervuert *et al.* (2009c) (Table 4.3).

NSC includes hydrolysable CHO (CHO_H) and non-hydrolysable, but rapidly fermentable CHO (CHO_{FR}) (Figure 4.2). In the horse, CHO_H is digested in the small intestine and is fermented in the hindgut when starch intake exceeds 0.4% BW/meal (Hoffman *et al.*, 2001). CHO_H can be calculated as $0.154xNSC + 0.00136NSC^2$ ($R^2 = 0.978$. $P<0.001$) Equation 4.2

CHO_{FR} is calculated as (NSC - CHO_H) and is more or less equivalent to the NDF. CHO_H accounts for all NSC in a typical sweet feed grain mix for horses (Hoffman *et al.,* 2001). For the SA feeds, these values are calculated in Table 4.3.

If starch intake is recommended as 1.1g starch/kg BW/meal (Vervuert, 2009c) and the horses in the current investigation are eating less than 1g starch/kg BW/meal at the 40% feeding treatment, it means that the CHO_H is within limits in these feeds (Table 4.3). Hoffman *et al.* (2009) identifies a point of inflexion of 0.296g NSC intake/kg BW above which dietary changes have less of an influence on blood glucose responses. In the current investigation, NSC intakes/kg BW are more than six times larger than this. This leads us to conclude that the SA feeds predispose to more hindgut fermentation and must have higher NDF's. Indeed, in comparison to the standard grain mix of Hoffman *et al*. (2001), where NDF's are about 15%, SA feeds contain at least 30% NDF.

As a result, we have an amelioration of glycaemic response due to the inadvertent replacement of hydrolysable with non-hydrolysable but rapidly fermentable CHO, which favours hindgut fermentation. As a result there are thermal issues in energetic efficiency, which is greater for hydrolysis than for fermentation and greater for glucose metabolism than for acetate metabolism (Kronfeld, 1996). This thermic effect in humans (Belko *et al.*, 1986) is also experienced in equine athletes (Kronfeld, 1996) and the heat increment of feeding forms an integral concept in the effective energy system of Emmans (1994). Thermic effect has important implications for performance horses in hot climates.

Intakes of starch greater than 0.4% BW accelerate to the hindgut for fermentation. The starch intakes of SA feeds are between 0.05 and 0.01% BW per meal at 40% concentrate proportions, but the CHO_{FS} content of the feeds presents a thermal load due to excessive hindgut fermentation. Feed intakes are often far in excess of this in sport horse stables. Hackland (2006) reported up to 90% concentrate proportions in racehorse diets, while most sport horse owners feed in excess of 60% of the daily feed intake of the horse as a concentrate mix in two meals.

From a nutritional technology perspective, if horses are fed according to their glycaemic response post exercise to maximise glucose availability for muscle glycogen synthesis (Kronfeld *et al.*, 2005), the method of processing and type of cereal starch are important (Hill, 2007). Two to four hours pre-exercise, providing carbohydrates can affect racing performance by increasing reliance on carbohydrate stores as the insulin inhibits lipolysis and fatty acid oxidation in skeletal muscle (Jose-Cunilleras *et al.*, 2002). Far from flogging a dead horse (Wolever, 1997), Rodiek and Stull (2007)

agree that it is a tool in producing useful feed categories, and this study has successfully discriminated between feed categories using basic glycaemic parameters.

Conclusions

The addition of concentrates to the diet of the horse is reflected in the blood glucose response of the horses. Current methods of feed characterisation do not help in feed selection, but this study has successfully discriminated between feed categories using basic glycaemic parameters. Four clusters of feeds have some dissimilar glucose parameters. Cluster analysis can elucidate more appropriate categories of feeds to assist consumers in their purchasing of feeds. Level of feeding in current production systems is high and given the glucose response at high concentrate to roughage ratios, timing of feeding relative to competition is important.

Glycaemic response can be correlated to starch intake and to the components of the feed that have an impact of equine performance, like thermic load, effective energy and hindgut fermentation. This information can be used to challenge the present system of ration presentation, and to define new groups that are more akin to the response of the horse relative to performance.

CHAPTER FIVE: RATE OF PASSAGE, DIGESTIBILITY AND IN VIVO PARAMETERS OF SEVERAL LOCAL HORSE FEEDS USING MINIATURE HORSES

Abstract

Digestibility is the product of the retention time and the degradation characteristics of a feedstuff. Passage rates and mean retention times were calculated using acid insoluble ash as a marker in miniature horses as a means of comparing *in vivo* parameters between 18 horse feeds offered at two feeding levels The concentrate horse feeds differed in respect of the forgut and hindgut retention times (calculated after the method of Thelemans *et al.* (1978)), as well as total mean retention times. Differences in retention time are correlated more strongly to the hindgut residence time than the foregut. The capacity of the feed to reside in the caecum, with the comcomitant characterisation of feeds in terms of their fermentative and glycaemic capacity (Chapters 3 and 4), means that compartmental flow parameters may well offer an explanation for the behaviour of feed ingredients in sections of the tract.

Introduction

In addition to proximate and Van Soest analysis, *in vivo/in situ* (including *in sacco* and fistulated and cannulated) techniques are widely used to study gastrointestinal processes, providing information about their nature and sensitivity to various changes (France *et al.,* 2000). These overcome many of the limitations in the routine use of *in vivo* trials (Broderick and Cochran, 2000). However, *In vivo* trials remain the gold standard, particularly in digestibility experiments.

The unrecovered fraction of the feed intake, expressed as a percentage of feed intake, is called the coefficient of digestion. Where nutrients of endogenous or metabolic origin are considered, the apparent digestibility can be adjusted to calculate a "true" digestibility. Factors which affect digestibility of feeds and nutrients are linked to animal factors (species, breed or subspecies differences, variation between animals, age, exercise and health status) and feed factors (chemical composition, ration composition (associative effect), effect of feed processing, level of feed intake). Digestibility is the product of the retention time and the degradation characteristics of a foodstuff (Forbes, 1996). Rates of passage are a measure of how long digesta is retained in the gut for

mechanical mixing, digestion, microbial fermentation and absorption (Warner, 1981; Mesochina *et al*., 1998; Sponheimer *et al*., 2003). Along with rate of fermentation, the mean retention time (MRT) of feeds within the gastrointestinal tract (GIT) is important in determining the extent of feed digestion and efficiency of microbial synthesis in herbivores.

Indigestible markers recovered from faeces provide a non-invasive method of calculating passage rate and mean retention time (MRT). Acid insoluble ash (AIA) has been the most frequently used marker in equine studies (Sutton *et al*., 1977; Cuddeford and Hughes, 1990; McMeniman *et al*., 1990; Cuddeford *et al*., 1992; Barbisan *et al*., 1993; Miraglia *et al.*, 1999), and is superior to chromic oxide (McCarthy *et al*., 1974; Orton *et al*., 1985). The cost of using N-alkanes (Ordakowski *et al*., 2001) or Ytterbium (Pagan *et al*., 1998; Moore-Colyer *et al*., 2003) as markers is preclusive in many instances.

Moore-Colyer *et al.* (2003) concluded that the pattern of faecal marker excretion could be accurately described by time dependent models, which use non-exponential residence time distributions to describe the time-dependent passage of digesta through different segments of the GIT. Time-dependent compartmental models fit equine faecal excretion data much better than time-independent models (Dhanoa *et al*., 1985; Pond *et al*., 1988; Moore-Colyer *et al*., 2003) as digesta passes through the caecum and large colon through narrow flexures between the right and left ventral, and left and right dorsal chambers in the horse. Furthermore, polynomial models of post-prandial percentages of marker (as a percentage of the total marker) excreted from the faeces against time (Thielemans, 1976) yield foregut (5% excretion) and hindgut (95%) transit times. In the absence of needing to compartmentalise digesta flow in faecal marker excretion data, this is more appropriate than time-independent representations of marker kinetics such as those developed by Grovum and Williams (1973) (Lindsay, 2005).

Because of the glycaemic and *in vitro* gas production properties that have been shown in previous studies (by this author) to be significant in the description of horse feeds on the South African sport horse market, *in vivo* parameters were sought at two feeding levels using 18 horse feeds. The glycaemic parameters should demonstrate a correlation with the foregut transit times. In this study, *in vitro* parameters such as mean retention and hindgut transit times were determined at two feeding levels in 18 feeds, using an acid insoluble ash marker technique to determine total tract transit time, and marker excretion. Residence times in the tract will be related to glycaemic and fermentation characteristics determined in other Chapters.

Materials and Methods

Animals and Housing

Eight mature miniature horses (males and females) of 132.91±12.89 kg (CV%=9.7) and less than 1000mm at the wither were used in this digestibility rate of passage trial at the Equine Section of the Ukulinga Research Farm of the University of KwaZulu-Natal (UKZN), Pietermaritzburg. The horses were brought in off kikuyu pasture for an acclimatization period. The animals were in excellent health and body condition, with an average body condition score of 6 (Henneke *et al.*, 1983) and were easy to handle, both in and out of the digestion stall, and were up to date with vaccination and antihelmintic programmes, as prescribed by an attending veterinarian. Animal Ethics protocols of UKZN were adhered to in their care.

Each horse was housed in an individual stall (1.5m x 1.7m) inside a roofed building. The metabolism stalls consisted of a cement floor with three cement walls and a wire gate in the front. The floor was covered with wood shavings during the acclimatization period, and was changed to an inflexible woven rubber matting during the collection period to prevent contamination of the faeces with urine and minerals in the cement floor, as well as to provide a comfortable surface for the animals to stand on. Hay was available *ad libitum* in a hayrack and feed was offered in black plastic feed troughs. Each stall contained a water bucket that was filled once a day or as often as needed. Water was available to the horses *ad libitum.* Hay and water intake were measured.

Animal comfort was ensured at all times. Horses could move around in their stalls. Horses were groomed daily, and hand-walked for 1km per day on a flat tar surface, as well as being allowed to stand in the sun for a period while the stalls were cleaned daily. Walking is indicated to prevent them going off their feed, to prevent oedema of the legs and sheath, and to alleviate boredom and digestive disorders. No access to feed was allowed on the walks and faeces were collected if produced.

Dietary composition and feeding

Horse feeds that represented a range of nutrient contents (Table 5.1) were randomly allocated to nine miniature horses in this digestibility and rate of passage study over two replicated 120 hour collection periods at two feeding levels, with the same horse receiving the diet at the two feeding levels. Celite®, a diatomaceous earth derivative, was used as an acid insoluble ash marker. It was administered at three percent of the total diet (hay and concentrate) once at the 8am feeding on the first day of the 120 hour collection period. The Celite® was mixed thoroughly by hand into the

concentrate portion of the diet. The marker was completely consumed by all horses. Concentrate was predetermined as a 20% and a 40% portion of 2% of body weight. The voluntary intake of horses has been anticipated as 2% of BW in previous work (Kohnke, 1998; Lindsay, 2005) and the meal sizes were therefore predetermined. While *Eragrostis curvula* and *w*ater were available *ad libitum*, voluntary intake was measured in actuality as 2.42% of BW, which slightly altered the proportions of hay and concentrate, but nevertheless provided two significantly different feeding levels. Concentrate feed was offered in two meals at 8am and 4pm, although the Celite® was administered only at the 8am feed.

Faecal output, collection and preparation

Faecal collection commenced on average two hours after the administration of the Celite® and continued for 120 hours post marker administration. Faeces were collected as they were voided and the time was recorded. In each collection period, a grab sample was taken from each dropping, placed in a glass jar and clearly marked for date, time and animal. Samples were dried in a force-draught oven at 90°C immediately for 48 hours and then milled through a 0.5mm screen, before being analysed using the acid-insoluble ash method in the laboratory (Schneider and Flatt, 1975). At the end of the collection period, faeces were also pooled for each horse and mixed to ensure a homogenous sample, and a glass jar was labelled with date, diet and animal for nutrient digestibility proximate analysis in the laboratory.

Analytical laboratory procedures

The concentrations of acid-insoluble ash in the feed and faecal samples were determined using a slightly modified method of the A101 Method from the Nutrition Research Laboratory, Adelaide University, Roseworthy, Australia (1998). Five grams of dried, ground sample was weighed into numbered crucibles and dried overnight in a drying oven. After been cooled in a desiccator for 45 minutes, each crucible was weighed (crucible + dry sample). The crucibles were transferred to a muffle furnace where they were ashed overnight at 480°C. After allowing the crucibles to cool in a dessicator for 90 minutes, each crucible was weighed (crucible + ash). Each crucible was then placed in a tall 250ml beaker. 4M HCl was slowly poured into the beaker until the sample was wet from underneath, and the crucibles were three-quarters filled with 4M HCl. The beakers with the crucibles inside were then boiled for 15 minutes on a hotplate, ensuring that the crucibles did not boil dry. After boiling, the crucibles were placed in a Buchner vacuum flask and the HCl was removed under suction. The sample was rinsed with 4M HCl, followed by distilled-deionised water. The crucibles

were transferred to the muffled furnace and ashed overnight at 480°C. After cooling once more in a dessicator for 90 minutes, each crucible was weighed and recorded (crucible + acid-insoluble ash).

Calculations

MSExcel (2007) was used to calculate the acid-insoluble ash percentage for each faecal deposit over the 96 hour collection period, using Equations 5.1 and 5.2.

$$\text{Dry Matter}\% = \frac{(\text{Crucible mass} + \text{Dry Sample mass}) - \text{Crucible mass}}{\text{Sample Mass}} \text{ x}100 \qquad \textbf{Equation 5.1}$$

$$AIA\% = \frac{(Crucible\,mass + Acid\ InsolAsh\,mass) - Crucible\,mass}{(Crucible\,mass + Dry\ Sample\ mass) - Crucible\,mass} x100 \qquad \textbf{Equation 5.2}$$

The percentage of AIA excreted in 120 hours was plotted against time to give excretion curves. Total tract mean retention time was estimated algebraically (TMRTalg – Equation 5.3), according to the equation described by Thielemans *et al*. (1978) in Goachet *et al.* (2009):

$$\mathbf{TMRT_{alg}} = \frac{\sum_{i=1}^{n} c_i \Delta t_i T_i}{\sum_{i=1}^{n} c_i \Delta t_i} \qquad \textbf{Equation 5.3}$$

where t_i is the time (h) from the dosage of markers to the midpoint of the *i*th collection interval, C_i is the concentration of the marker in the *i*th sample, Δt_i is the interval (h) between the two samplings, and n is the number of samplings. This method is considered the most accurate in achieving the total tract residence time (Moore-Colyer *et al*., 2003). Digesta flow in ponies fed fibrous diets is a time-dependent process and is therefore best described using time-dependent models, with which the model of MRT of Thielemans (1978) agrees.

Apparent digestibility (AppDig) was calculated from total dry matter intakes (DMI) and faecal dry matter outputs (DMO) over the five-day collection periods. Coefficients of digestion were calculated for the nutrients as ([intake – faecal output]/intake x100).

Statistical analysis

Feed and hay intake, water intake, frequency of production of faeces and faecal quantity data were obtained in the *in vivo* digestibility and rate of passage trial using miniature horses. Nutrient digestion coefficients were obtained by calculation, with digestibilities of the entire ration being obtained. Polynomial regressions were fitted in Genstat (2007) to the cumulative AIA excretion curves, as per the method of Castle *et al.* (1956), from which the mean retention times, 5% and 95%

marker excretion values were obtained. Response variates were analysed as a randomized blocks design in Genstat (2007), using Body Weight as a covariate, and with Feeding Level as a treatment structure, with comparisons of treatment means being made by least significant differences, which were considered significant at the 5% level.

Results

The proximate and detergent analysis of several riding, breeding, maintenance and grain-free feeds is shown in Table 5.1.

Table 5.1 Proximate and detergent analyses on a dry matter basis of common South African riding, breeding, maintenance and grain-free feeds, showing advertised and actual protein percentages (CP%), % fat, % neutral and acid detergent fibres (NDF and ADF, respectively) and soluble carbohydrate contents.

CODE	Feed Type	Advert CP %	CP (%)	Fat (%)	NDF (%)	ADF (%)	CHO_{sol} ($g.kg^{-1}$)
67swbm	Breeding	14	18.34	4.66	19.53	10.84	535
69swm	Maint	11	13.79	5.02	26.76	10.71	516
73swsh	Grain free	14	17.62	5.53	28.14	13.01	469
75efe	Riding	12	15.17	9.23	24.27	12.49	467
77eft	Grain free	14	17.40	4.98	27.45	14.31	439
68swp	Riding	13	15.21	4.54	19.94	11.63	560
70swss	Breeding	14	17.97	4.45	17.06	9.86	555
71swwb	Riding	12	15.12	4.78	22.53	11.61	534
72swh	Riding	12	15.84	4.03	25.46	10.82	519
74swsg	Breeding	14	18.01	4.40	18.53	9.73	531
76efwb	Riding	12	11.40	4.62	26.37	11.62	538
77eft	Grain free	14	14.86	10.49	28.43	14.21	439
78rxp	Maint	10	11.57	2.86	25.46	10.16	580
79rxhc	Riding	12	12.44	4.37	30.00	10.20	519
80vv12	Riding	12	15.94	6.14	30.99	11.90	474
81vv10	Grain free	10	14.91	3.35	34.15	13.21	480
82mk	Riding	14	13.22	3.25	50.19	23.74	333

Where: Advert CP = advertised feed bag label CP%; CP = crude protein, Ca=calcium, P=phosphorous, moist=moisture, NDF=neutral detergent fibre, ADF=acid detergent fibre, CHO_{sol}=soluble carbohydrates

Over the four periods (two feeding levels and two periods), BW varied very little, with a coefficient of variation of 9.7%. The horses had a body condition score of 6 (Henneke *et al.*, 1983) throughout the trial. Concentrate was provided at 20% of the total intake, which worked out at 2.42% of BW. Horses drank on average 5.9% of BW as water. Each dropping weighed about 400g for each miniature horse, which made about ten droppings per day (Table 5.2).

Table 5.2 Daily feed (wet basis) and water intakes and faecal output (wet basis) of miniature horses fed a ration at a 20:80 concentrate:roughage ratio over consecutive four day collection periods in a digestibility trial

Feed	body weight (kg)	conc intake (kg)	hay intake (kg)	intake as %BW	water intake (L)	avg weight per dropping (g)	No. of droppings (per day)
Efe	135.75	0.82	2.22	2.24	7.19	363.07	11.13
Eft	158.00	0.97	1.96	1.86	7.38	462.40	8.63
Efwb	142.00	0.86	2.37	2.29	7.54	579.58	9.00
Hay	124.50	0.00	3.10	2.50	7.05	323.85	13.63
Mk	133.50	0.81	2.53	2.50	7.40	426.84	14.63
Rxhc	116.50	0.69	2.54	2.78	8.83	384.90	10.88
Rxp	120.75	0.71	2.70	2.82	7.99	408.65	11.88
Swbm	135.25	0.81	2.53	2.47	8.17	318.87	11.13
Swh	130.25	0.81	2.74	2.73	10.13	353.69	12.25
Swm	134.25	0.81	2.01	2.11	6.89	415.84	8.00
Swp	113.50	0.66	2.56	2.84	7.96	313.56	9.13
Swsg	120.25	0.72	2.46	2.64	8.00	395.96	13.00
Swsh	142.25	0.85	2.80	2.56	7.89	558.11	8.00
Swss	118.25	0.70	2.58	2.78	7.68	294.96	11.13
Swt	134.50	0.82	2.13	2.20	7.75	430.79	10.75
Swwb	157.00	0.98	2.35	2.12	8.13	525.39	8.63
v12	134.75	0.81	2.01	2.09	7.90	372.02	12.63
vv10	134.25	0.81	1.91	2.02	6.64	429.82	8.75
MEAN	132.53	0.76	2.42	2.42	7.81	408.79	10.73
SED			2.48	1.269	1.269	59.272	1.993
LSD			0.981	2.665	2.665	124.526	4.186
CV%			18.8	16.3	16.3	14.5	18.6
Feed			ns	ns	ns	P<0.01	ns

Where SED = standard error of difference, LSD = least significant difference 5%, CV% = coefficient of variation, Ns = not significant (P>0.05).

While the concentrate intakes were calculated and provided to the horses, there was no difference in the hay intake between the rations, and all horses ate what was anticipated in terms of the percentage of dry feed intake (Kohnke, 1998; Lacasha *et al.*, 1999). Rations did not influence the water intake of the number of droppings per day. The average weight of the droppings was lower in the higher nutrient density diets.

For levels of feeding up to 40% of intake as concentrate in the diet, no interactions between feed type and feeding level are apparent (Table 5.3). In the absence of feeding level as a main effect, the feeds produce significant differences in terms of the foregut (5%) and hindgut (95%) and mean retention parameters (Table 5.4)

Table 5.3 Foregut (5%) and hindgut (95%) transit times, including the total tract mean retention time, after the method of Thielemans *et al.* (1978), for concentrate rations offered at 20 and 40 percent of the daily dry matter intake of miniature horses, using Celite ® as an acid insoluble ash marker.

Feed level	Foregut (5%) retention time (h)	Hindgut (95%) retention time (h)	Total mean retention time (h)
20%	2.69	64.00	33.3
40%	3.29	74.00	36.1
Mean	2.99	69.00	34.7
SED	0.33	7.43	4.5
LSD	0.74	16.55	10.04
F Prob	0.10^{ns}	0.21^{ns}	0.54^{ns}

Table 5.4 Cumulative excretion curves of an acid insoluble ash (Celite®) marker for several concentrate horse feeds at 20% of the total as fed intake of miniature horses per day, fed in combination with hay, showing the calculated 5% and 95% excretion times and the calculated total mean retention time, using the method of Goachet *et al.* (2009).

FEED	5% excretion (h)	95% excretion (h)	Mean retention (h)
Efe	2.26	43.0	22.63
Eft	2.90	75.2	35.10
Efwb	2.63	62.7	29.77
Mk	2.24	42.6	22.44
RxHc	2.16	41.0	21.59
Rxp	2.58	61.1	29.23
Swbm	2.82	65.8	31.70
Swh	2.97	56.5	29.73
Swm	3.23	70.6	35.10
Swp	3.11	69.2	42.85
Swsg	2.26	64.8	28.77
Swsh	2.29	62.5	28.37
Swss	2.54	58.8	28.92
Swt	2.49	81.9	34.46
Swwb	2.54	69.0	31.44
v12	3.61	89.4	45.59
vv10	2.29	43.5	22.90
MEAN	2.641	62.2	30.62
SED	0.266	12.06	4.561
$LSD_{5\%}$	0.581	26.28	9.937
Significance of effect	0.005**	0.047**	0.007**

Discussion

The use of a stronger acid (for example 4M HCl) and the inclusion of more acid insoluble ash (3% in the current protocol), underscore the success of AIA as a marker technique in digestibility and rate of passage studies in horses (Goachet *et al.,* 2009).

Digesta flow in ponies fed fibrous diets is a time-dependent process and is therefore best described using time-dependent models. The model of MRT of Thielemans (1978) is considered accurate in achieving the total tract residence time (Moore-Colyer *et al.*, 2003). Compartmental retention times can be obtained from time-dependent two compartment models.

Total tract residence time, the mean retention times (the pooling of ingesta in the digestive tract; MRT) will be different depending on feeding level, dietary composition and physical form, animal body size and exercise, and markers and passage models (Austbø and Volden, 2006). For passage kinetics, it is assumed that the hindgut (the caecum and colon) are mixing segments with the possibility of selective retention of particulate material, with little mixing and backflow in the small intestinal flow (Austbø and Volden, 2006). Algebraic algorithms (such as Thielemans, 1978) successfully produce mean retention times for feeds in the gastrointestinal tract of horses from marker retrieval kinetics.

As explained by Austo and Volden (2006), digesta kinetics can be represented as a two-compartment model with a gamma dependency in the first compartment and age independency in the second compartment (GnG1, n=2–4). Moore-Colyer *et al.* (2003) fitted time dependent parameters to curve fitting data to reveal more sensitively the activities of the sections of the GIT than the algebraic algorithm, producing mean values for the time dependent compartment (λ), a time-independent factor (k_2), and a time delay factor (TD). The arrangement and anatomy of the caecum in the horse suggests that it could be a fast, time-independent compartment, with the large colon being time-dependent. The rate constant for the time-dependent section (λ) would then correspond to the colon, with time delay referring to the small colon. The time that digesta remains in the stomach and small intestine is included in the λ and k_2 compartment retention times. While retention times were successfully measured, Moore-Colyer *et al*. (2003) concluded that, “conclusive biological interpretation on the compartmentalization of the different segments of the equid digestive tract is hampered by variability in the data”, which means that the model parameters remain at best an indication of what is actually happening to feed in the digestive tract.

In the model calculations it is assumed that a transit time (TT) represents the time delay from marker dosing to the first appearance of marker in the faeces (Pond *et al*., 1988), which is represented by the 5% retention time in the method of Thielemans, 1978).

Mean retention times have been calculated as 25.8 hours and 25.9 hours using the Best Fit Models (Moore-Colyer *et al.*, 2003). These are similar to the values calculated algebraically in this chapter. Rosenfeld *et al.* (2006) calculated a 26 hour retention time with his models, which are simpler to interpret biologically. They assume a time spent outside the mixing compartments (transit time-stomach and small intestine and caudal part of the large intestine), a mean retention time in the time-dependent compartment (CMRT1-colon) and a mean retention time in the time-independent compartment (CMRT2-caecum). Foregut transit time (5% excretion data) calculated in this study at 2.26 hours are within the time delay of approximately nine hours of Moore-Colyer (2003), which includes the caudal part of the latter GIT as well. The current algebraic data are therefore similar to biological interpretations of transit times of the compartmental models, and provide a simpler explanation.

Goachet *et al.* (2009) feeding at 40%:60% concentrate to roughage, at 3.1% of BW intake per day, achieved MRT of 23 hours at two meals a day as well. Similarly, at 2% of BW feed intake, using a 30:70 C:R ratio of a high nutrient density diet, Austbø and Volden (2006) achieved 23.4hour retention times, and the time equivalent to the 5% excretion of Thielemans (1978) calculated in the present study, was calculated by Austbø and Volden (2006) to be 8 hours. This corresponds to the TT (transit time) in the compartmental model (Pond *et al.,* 1988).

Proportions of 20% and 40% of the daily ration given as concentrate do not produce different retention times (Table 5.3). While it is anticipated that larger feed intakes of concentrate feeds in horses should spend less time in the stomach and proximal GIT (Varloud *et al*., 2004), feeding concentrate feed at a 40% proportion of the ration was not sufficient to affect the passage of the feed through the GIT. Given that hay intakes were not significantly different in the 20% and 40% feeding levels, it is assumed that residence time in the latter part of the GIT made up for small increments in transit in the foregut. Strong positive correlations exist between the mean retention time and both 5 and 95% transit times, with the hindgut transit forming a greater proportion of the total mean retention (Figure 5.1). This illustrates also that differences in retention time are probably correlated more strongly to the hindgut, rather than the foregut, transit times. Ellis and Hill (2005) tabulate MRT's for different feedstuffs in horses, and the longer MRT's are usually associated with more fibre in the diets. There appears to be a need for more research on the relationship between

the MRT and the roughage: concentrate ratio and the correlation to digestion coefficients should be determined.

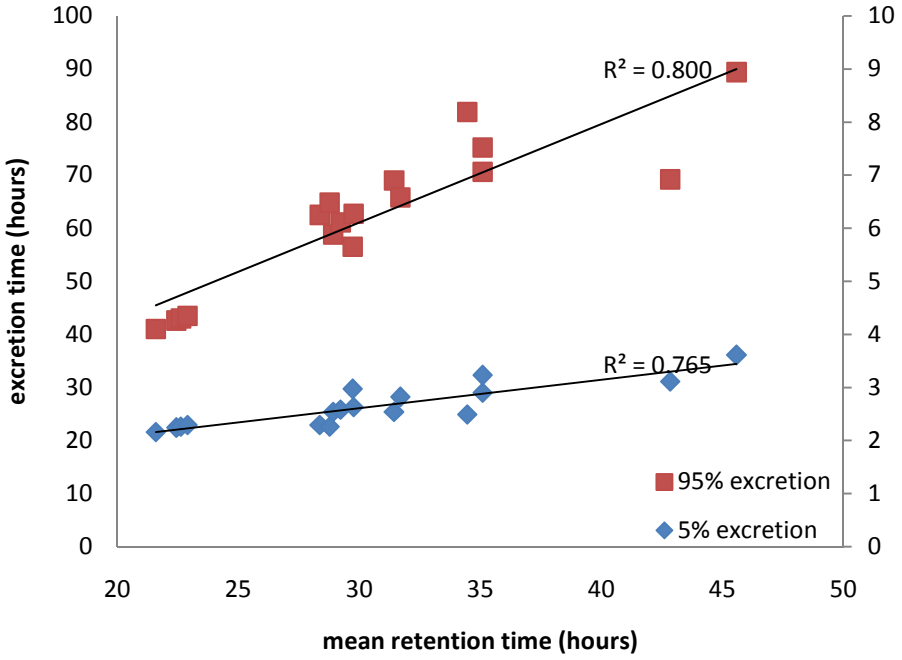

Figure 5.1 Correlation between the mean retention time and the relative contributions of the 5% and 95% transit times as determined algebraically by the Thielemans *et al*. (1978*),* (In Goachet *et al*.,2009).

Calculations from this work indicate some weak positive correlations between mean retention time and soluble CHO (27%) and crude protein (21%), while negative correlations to CF (33%) and NDF (31%) exist.

Conclusion

While the topic of compartmental modelling in the equine digestive tract has received recent attention, the method of Thielemans *et* al. (1978) in Goachet *et al.* (2009) provides an effective means of discriminating between a character of the rations that sees the initial excretion of the marker (foregut transit time) being different. In addition the time taken for the total marker to be excreted differs between feeds, but the residence time in the tract becomes a function of the ration as well (Varloud *et al.*, 2004). Weak correlations exist between the retention times and the chemical components of the concentrate feed. The literature reports scarcely on the relationship between

retention time and digestion coefficient. This work will still be done and should be extended by the consideration of processing effects on the time spent in the GIT.

What is significant about these conclusions is that a relatively simple five day digestion trial using an effective marker (AIA) can produce digestibility data. It can also produce a crude estimate of residence times in the tract, and also a measure of the times approximating those spent in the time independent portions. The capacity therefore of the feed to reside in the caecum, with the comcomitant characterisation of feeds in terms of their fermentative and glycaemic capacity (Chapters 3 and 4), means that compartmental flow parameters may well offer an explanation for the behaviour of feed ingredients in sections of the tract that that may offer means to corroborate any relationship to behavioural manifestations in the horse (Ellis and Hill, 2005).

CHAPTER SIX: CARBON : NITROGEN RATIOS IN HORSE FEEDS FOR OPTIMAL HINDGUT FERMENTATION

"the 'nutritive ratio' of a food is the term used to express the proportion which exists between the nitrogenous matter of the food, and the other energy producing constituents, namely, starch, sugar and fat. It is generally advisable to try to obtain a mixture which has a nutritive ratio somewhat similar to that of oats"

Captain M.H.Hayes (1900)

Abstract

The need to balance nitrogen levels with a proportional supply of fermentable carbohydrate is in contrast with the excess provision of protein to horses, and provides the opportunity to explore nutrient synchrony and its implications for nutrient utilization in the hindgut of the horse. There is an optimal carbon nitrogen ratio for microbial fermentation, which is often violated in the composition of the rations offered to the horse. Several horse feeds were evaluated by means of *in vitro* gas production on the basis of their ability to support microbial action as a firm indicator of the value of the feed to the horse as a hindgut fermenter. Feeds differed in respect of the calculated carbon and carbohydrate to nitrogen ratios. CHO_{sol}:N and C_{biodeg}:N ratios more accurately inform the changes to degradation efficiency and partitioning factors calculated from *in vitro* gas production. Microbial efficiency in particular had a strong negative correlation to C_{biodeg}:N. Optimal nutrition for the horse relies on the accurate representation of feeding value using the concept of nutrient synchrony.

Introduction

Some indication of the validity of linking caecal microbes and the ratio of nutrients available to them can be found in relation to soil (Titshall, 2008) and to ruminants (Reynolds and Kristensen, 2008). Both environments favour the propagation of microorganisms that break down substrate to utilizable fractions. Interest in eutrophication (Wilson *et al.,* 2006) and increased global pressure to reduce phosphorous sinks, predominantly in Europe (Lawrence *et al.*, 2003), has prompted research in this sphere. The concept of nutrient synchrony in respect of the supply of carbon and nitrogen and their influence on microbial systems, has been evaluated most recently by a few authors (Cole and Todd, 2008; Hall and Huntingdon, 2008; Reynolds and Kristensen, 2008). The need to balance

nitrogen (N) levels with a proportional supply of fermentable carbohydrate (CHO) contradicts widely used protein intakes in the horse, which indicates the opportunity to explore nutrient synchrony and its implications for nutrient utilization in the hindgut of the horse. Wastage in Thoroughbred (TB) race horses owing to inappetence (2.2%) or a myriad of "unknown" causes (9.7%) represents a loss to the trainer and the owner, and the industry as a whole (Olivier *et al.,* 1997). Identifying reasons to keep horses in work is important as they are expensive ornaments, and the consequences of current feeding practice to their health in the long run should be evaluated.

Protein has been identified as a major reason that people purchase a horse feed (Hiney and Potter, 2002). While digestion of protein occurs in the small intestine (Slade *et al*., 1971; Wootton and Argenzio, 1975), deamination of excess amino acids can take place in the hindgut, where exogenous (bacterial) D-amino acid oxidases convert amino acids to keto acids, H_20_2 and NH_3. The H_20_2 is immediately converted to water and oxygen by catalase and three major pathways of amino acid metabolism all lead to the release of ammonia which must be cleared from metabolism rapidly, usually via the formation of urea in the liver (Figure 6.1) (Ellis and Hill, 2005). N supplied to the large intestine will be utilized by the microflora or be utilized in the synthesis of non-essential amino acids from ammonia (Potter, 2002; in Ellis and Hill, 2005). However, protein supplied in excess of requirement (as frequently happens) exceeds the ability of the horse to synthesize urea efficiently, being beyond the capacity of the urea cycle (Miller-Graber et al. 1991), and may be detrimental. No ergogenic potential can in fact be identified in the excess provision of protein (Harris and Harris, 2005). Excessive N intake increases water intake and NH3 excretion (Hintz, 1994), which in the stabled horse could induce respiratory problems. The small intestine contains little ammonia, but in a slaughter experiment, Hecker (1971) confirmed more ammonia in the large intestine, particularly in the rectum, as did Mackie and Wilkins (1988). Excess protein intakes increase the burden of unusable nitrogen (Frape, 1994). N retention also plateaus after 0.202g N/kg BW or at 1.26g CP/BW for horses at maintenance (NRC, 2007). Excess protein also slows times in race horses (Glade, 1983). No consequence of this appears to have been considered, nor are recommendations made for the equine nutritionist to optimize provision of protein in the horse, although the incidence of metabolic disorders and gastrointestinal disturbances is increasing (Kalck, 2009).

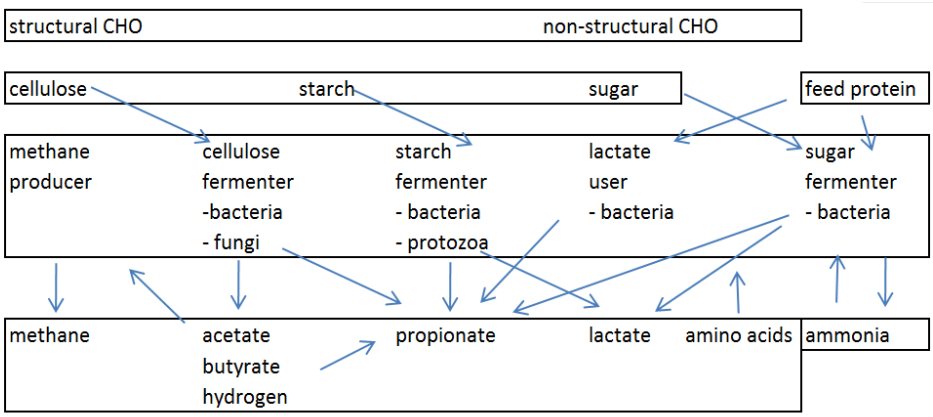

Figure 6.1 Microbial populations and their end products in the equine large intestine (adapted from Spring, 2000; in Ellis and Hill, 2005)

Along with stereotypic behaviours as a result of grain overload (Freire *et al*., 2009), a reduction in pH is a consequence of highly fermentable carbohydrate diets (Ellis and Hill, 2005) and is a common problem in the sport horse industry (Williamson *et al.,* 2007). Medina *et al.* (2002) found increased gut ammonia to be a consequence of high starch diets. Hay prevents hindgut acidification (Zeyner *et al.,* 2004), the proportion of which is reduced in sport horse diets as they seek to increase energy density for work (Lawrence, 1990). N forms other than ammonia are required for optimal microbial growth and fibre digestion by caecal bacteria (Carro and Miller, 1999; Maczulak *et al*., 1985). Ruminal and caecal bacteria ferment structural and non-structural carbohydrates, affecting the extent of NH_3 absorption and urea N recycling and excretion (Reynolds and Kristensen, 2008). Bacteria utilizing non-structural carbohydrate (NSC) produce less ammonia when carbohydrate fermentation is rapid. Ammonia production is moderated by amino acid and peptide uptake, but 34% of ammonia production is insensitive to rate of carbohydrate fermentation (Russel *et al*., 1992). A balanced dietary NDF/starch ratio and probiotic support (*S. cerevisiae)* can limit the extent of undesirable changes in the intestinal ecosystem of the horse (Medina *et al.,* 2002).

The fate of N and the production of ammonia is potentially damaging to microorganisms, given an adequate supply of energy for the microbes (Medina *et al*., 2002; Mair *et al.,* 2002). The inability of 79.5% of caecal isolates to grow with ammonia as the sole nitrogen source, indicates that caecal bacteria need N other than ammonia or urea for growth (Medina *et al.,* 2002). Thoroughbred (TB) race horses consume in excess of 80:20 concentrate to roughage ratios (Hackland, 2007). Once retired from racing, adaptation to work rate and to concentrate: roughage ratios and to handling should take two months to achieve, following realimentation. Kriebiel *et al*. (1995) speak of short

term severe insults in the small ruminant having implications for long term organic acid absorption. Hall and Huntingdon (2008) refer to anecdotal observations in dairy cows. The off-the-track TB's often take up to a year to regain body condition and vitality (Author's own observations). The association between primary hyperammonemia and antecedent or concurrent signs of gastrointestinal disease, raises suspicion that excessive ammonia production in the large intestine is a possible etiology (Mair *et al.,* 2002).

Prompted by the observation of this retarded alimentary adjustment and the consequence that the protein content of horse diets can have on microbial health in hindgut fermentation systems, the current work investigated the nutrient synchrony in terms of C:N provided in a range of horse feeds and the consequences for hindgut fermentation *in vitro*. It is alleged that C:N ratios in horse feeds are inflated over that required for optimal microbial health, leading to undesirable levels of volatile nitrogenous compounds that can damage the lining of the GIT, sometimes with long term consequence. More importantly, key substrates may become unavailable to gut microbes for absorption in solution. For readily degradable substrates, a ratio in the order of 20-25:1 is acceptable. A high C:N ratio causes an increase in acid formation which retards methanogenesis activity, while with a low C:N ratio, nitrogen is converted to ammonium-N at a faster rate than it can be assimilated by the methanogens (Ghasimi *et al.*, 2009). Horses have a higher capacity for acetogenic over methanogenic activity (Ellis and Hill, 2005). This endorses further the need to refine the profiling of horse rations such that optimal nutrient supply for microbial health, and consequently for horse health is achieved.

In vitro gas production techniques generate kinetic data and measure the appearance of fermentation gases (Schofield and Pell, 1993; Adesogan, 2002). Artificial rumen fermentation techniques can be utilized to study the digestibility of forages and feedstuffs by caecal microflora (Vallance, 1966; Applegate and Hershberger, 1969; Ouda, 2007). The donor animal has little effect on *in vitro* digestibility determinations of horse feedstuffs (Murray *et al.,* 2003). Hindgut fermentation can be modelled using the method of Pienaar (1994), substituting equine faecal inoculum to determine rapidly the digestibilities of nutrients, following hindgut fermentation (Hackland, 2007). The following figure (Figure 6.2) demonstrates the response in *in vitro* gas production to the incubation of maize silage, using the model of Campos *et al*., (2004).

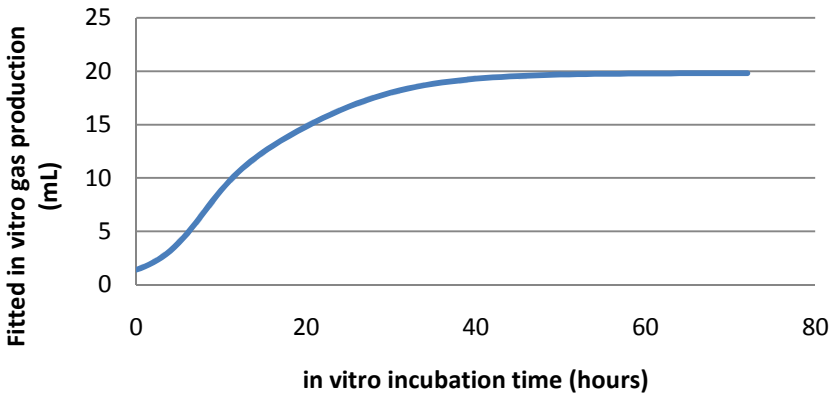

Figure 6.2 Fitted *in vitro* gas production (ml) from the model of Campos et al. (2004) fitted to data of corn silage, with R^2=0.998

Drogoul *et al.* (2000) gave caecal retention times of 8 and 4 hours for long stemmed hay and ground pelleted hay, respectively. Horses retain fibrous particles for only ten hours and liquid for 9 hours in the caecum (van Soest, 1994; in Ellis and Hill, 2005). Hyslop (2006) indicated that current mean retention times for total tract work may overestimate the usefulness of the nutrient to the horse, and that shorter caecal retention times may be more realistic.

Rapid transit times can explain the position of the fermentation centre in the context of the GIT. Ruminal fermentation products (CO_2, H_2 and CH_4) are lost through eructation, but in the horses, limited fermentation must occur or microbial adaptations must occur to produce low levels of methane and hydrosome metabolism (Russel and Gahr, 2000). It is particularly the amount of starch that reaches the hindgut that depresses pH and can lead to microbial dysfunction (Bailey *et al.,* 2002; Ellis and Hill, 2005), with consequent intestinal damage (Ellis and Hill, 2005), in addition to nutrients being less available pre-caecally (Hoffman, 2003). Any concept of microbially available carbon has to be considered in light of substrate flow, composition of digesta post ileum and spill over of nutrients not utilized in the ante-caecal section of the GIT (Ellis and Hill, 2005). Source of substrates, their efficiencies of use and yields of products are affected by inherent properties, interactions, transformations, and passage (Hall and Huntingdon, 2008), and nutrient synchrony needs to balance the animal and these transformations in the GIT.

Gas production as a measurement of hindgut fermentation is important, because there is limited capacity for precaecal digestion of CHO when so much is fed. As a result, GP and proportional changes in VFA's in the hindgut occur. There will be a similar degradation between feeds precaecally which still allows one to use the GP as a measure of the difference between feeds in terms of their

ability to provide substrate for hind gut fermentation if provided in excess. This effect is greater in some feeds than others.

There is a consequence to the feeding of excess protein in the horse. Precaecal protein digestion is often overwhelmed by volume intake, which does make protein of major importance in the hindgut. The evaluation of several feeds on the basis of their ability to support microbial action should be considered as a firm indicator of the value of the feed to the horse as a hindgut fermenter. Correlations between the actual and perceived benefits of feeds to horses will remain anecdotal in nature, until the value of the feed to the GIT microflora is quantified. The degradability efficiency factor and other anomalies of the IVGP technique (Ouda, 2007) in selected feeds can be used in the current context as a means of evaluating the value of the feed to microflora, and the products of fermentation can be evaluated in terms of their use or harm to the horse.

Materials and methods

Feeds and chemical composition analysis

The trial compared the gas production characteristics of seven horse feeds of differing C:N ratios, using faecal inoculum from race horses (20:80) and from idle horses fed 70:30 roughage: concentrate ratios (Table 6.1). A control treatment of inoculum in the absence of additional substrate and seven feeds were incubated in faecal inoculum for 24 hours using two sources of inoculum (from race and maintenance-kept horses), in a completely randomized arrangement using the IVGP incubator method of Pienaar (1994). There were therefore four replicates per feed and the runs were replicated three times. The feeds were dried at 50˚C overnight and were milled through a 1mm screen. Total dry matter (DM) was determined by drying the samples in a fanned oven at 100˚C overnight. Total ash was determined by igniting samples in a muffle furnace at 500˚C overnight, and was used to calculate total organic carbon (Moral *et al.,* 2005). Nitrogen was measured by the micro-Kjeldahl technique. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) determinations were described by van Soest *et al.* (1991). Soluble CHO were calculated by the method of Harris (1970), where CHO_{sol} ($g.kg^{-1}$) = 1000 – (NDF + crude fat + CP +soluble ash) = NFC (non- fibre CHO (Hoffman, 2003)).

Gas production analysis

The *in vitro* gas production technique (IVGPT) described by Pell and Schofield (1993) was used. A total of 1.0 ± 0.0010g DM of each feed was weighed into 250ml Duran glass bottles for

incubation, with the control having no additional substrate. The incubation was replicated three times for 24 hours. Buffer solution was prepared using the method of McDougall (1948) whereby 4ml of Solution B (5.3g $CaCl_2.2H_20$ in 100ml distilled water) was added drop wise to 4L Solution A ($NaHCO_3$ + diNaH_3PO_4(anhydr) + KCl), to make a complete buffer solution, which was warmed to 37˚C. To the one gram of sample (DM), 67ml of buffer was added, which was then left to equilibrate at 37˚C in the incubator.

Equine faecal inoculum was prepared from freshly voided faeces from Thoroughbred horses maintained on pasture and fed 0.6% of body weight of concentrate feed in two meals. Every effort was made to maintain anaerobic conditions. The faeces were collected as they were produced, into a sealed packet, and sealed in the flask that had been infused with CO_2. Faecal samples were collected before the morning feed into a flask maintained at 37˚C and were transported within 15 minutes to the laboratory. Faeces were also collected from TB race horses in training. Faecal samples were collected into a 37˚C flask in the morning before the morning feed and were transported promptly to the laboratory. 500ml of faeces was mixed with 500ml buffer solution and stirred with a glass rod and squeezed through four layers of cheese cloth into a glass beaker flushed with CO_2. Initial pH was measured. Inoculation was done by adding 33ml of faecal fluid to the sample plus buffer under a stream of CO_2. The bottle lids were tightened and pressure sensors fitted. Samples were allowed to settle for 20 minutes before pressure logging started at 20 minute intervals for 24 hours. The justification for the 24 hour period was to accommodate the shorter caecal residence time in contrast to the rumen retention times of 72 hours on which the procedure was based. Previous work also indicated that no further peak in maximum GP was achieved after this time, making it a suitable time frame for comparative purposes.

The pressure data was converted to gas volumes (in ml) using a predetermined calibration equation where 1kPa=2.2489ml gas (Nsahlai and Umunna, 1996).

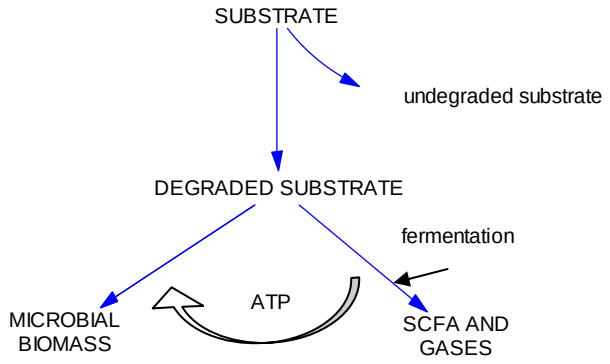

Figure 6.3 The partitioning of degraded substrate between different products (after Ouda, 2007).

Gas production arises from the fermentation of carbohydrate, and can be either direct (CO_2, CH_4) or indirect (CO_2 from the bicarbonate buffering of short chain fatty acid (SCFA) production, which will be predominantly the acetate and butyrate fractions) (Blümmel and Orskov, 1993) (Figure 6.3). Gas profiles, corrected for control fermentation profiles in the absence of substrate, were fitted to the model described by Campos *et al*. (2004) to derive GP kinetics as follows.

$$Y= A/[1+\exp(2+4a_1(C-t))] + B/[1+\exp(2+4b_1(C-t))] \quad \text{Equation 1}$$

Where y is the total gas volume (ml) at time t, A and B are the gas volumes (ml) from the fast and the slow degradable fractions, a_1 and b_1 are the degradation rates (h^{-1}) for the fast and the slowly degradable fractions and C is the colonization or lag time (h).

In vitro gas production and *in vitro* apparent and true degradability are highly correlated, with an inverse relationship between *in vitro* gas production and microbial biomass yield, so selecting roughages by *in vitro* gas production only, may well be a selection against maximum microbial yield. Hence, a combination of *in vitro* gas volume measurements with a complementary determination of the substrate truly degraded was proposed in the partitioning factor (PF), which reflects the variation of short-chain fatty acid production per unit substrate degraded (Blümmel *et al.,* 1997). The substrate degraded: gas volume produced (termed the PF) depicts the efficiency of rumen microbial production *in vitro* (Blümmel *et al*., 2003). For concentrate feeds in ruminants, there is a strong non-linear relationship between PF at peak microbial production (T½) and the efficiency of microbial production (EMP) *in vivo*. Low gas productions per unit substrate (high PF) were correlated to high EMP determined *in vivo* (Blümmel *et al.*, 2003). Microbial activity is close to maximum at half maximum gas volume (France *et al*., 1993; Davies *et al*., 2000; Makkar, 2004; in Ouda, 2007). This is when the relationship between EMP *in vivo* and the PF is strongest for concentrate feeding in ruminants (Blümmel *et al.,* 2003). $T_{½}$ could be used to predict the nutritive value of horse feeds (Murray *et al*., 2006), and the calculation of a degradation efficiency factor (DEF), incorporating both these concepts, is investigated to evaluate the gas production technique in assessing the nutritive value of feeds for the horse.

The degradation efficiency factor (DEF) is calculated from the partitioning factor as follows:

$$PF = \frac{deg}{V} \qquad \text{Equation 2}$$

and

$$DEF = \frac{Deg}{T_{½} \times V_{½}} = \frac{2PF}{T_{½}} \qquad \text{Equation 3}$$

Where deg = true degradability (mg), V = total gas volume (ml), $T_{½}$ = time taken to produce $V_{½}$, and $V_{½}$ = half of V (ml).

Determination of degradability and carbon and nitrogen contents

After incubation, terminal pH was determined, and the samples were centrifuged in a Beckman Centrifuge at 18 600 G for 15 minutes at 4˚C, with rotor JA14. The supernatant was processed for ammonia and VFA analysis. Total dry matter (DM) of the pellet residue (R) was determined by drying the samples in a fanned oven at 100˚C overnight for 24 hours. The difference in weight between R and 1gDM was regarded as the apparently degraded fraction (AppDeg). R was refluxed with neutral detergent solution (NDS) and the residue (NDF) was dried. The weight of the NDF was subtracted from the 1g DM to yield the truly degraded fraction (degradability). The difference between the true and the apparent degradability is the microbial yield (Blümmel *et al.,* 1997). The remaining fraction was ashed in a muffle furnace at 500˚C overnight, and was used to calculate total organic carbon. The cellulose and hemicelluloses were calculated as the difference between the ADL and ADF, and the NDF and ADF, respectively (Richards *et al*., 2003). The non-lignin C:N ratio (Ghasim, 2009) was calculated from the digestible fraction of the cell wall calculated by Richard (1996), where the digestible fraction of cell wall = $100-5.41(\text{lignin\%}_{\text{cell wall}})^{0.76}$, and $\text{lignin\%}_{\text{cell wall}} = \text{lignin\%/NDF\%*100}$.

Ammonia and volatile fatty acid determination

After incubation, terminal pH was determined, and the samples were centrifuged in a Beckman Centrifuge at 18 600 G for 15 minutes at 4˚C, with rotor JA14. 4ml of 25% H_3PO_4 was added to 20ml of supernatant to produce a pH of below 2. 5ml of the supernatant solution was then stored in 5ml aliquots and was frozen, pending Ammonia analysis. The method of Maynard and Kalra (1993) was used to determine nitrate and ammonium nitrogen in the samples, and N was measured using the micro-Kjeldahl technique, to determine the C:N ratio in the fermentation product after 24 hours. 5ml of the treated supernatant was centrifuged at 18 600 G for 10 minutes at 4˚C, with rotor JA25.50. The supernatant was filtered through Cameo 20 (0.45µm) filters, of which 1µl sample was used in the Gas Chromatograph (HT300A Liquid Gas

Chromatograph Autosampler). Concentrations and relative percentage yields of volatile fatty acids were obtained from GC software (YLClarity®), which was calibrated for acetic, proprionic, iso-butyric, N-butyric and iso-valeric acid.

Statistical analysis

Feeds were randomly allocated to 24 pressure transducer channels in the incubator (Pienaar, 1994) to generate gas production data by *in vitro* gas production (Pell and Schofield, 1993), following a modified Tilley and Terry (1963) method. SASv9.1 (2005) software was used to fit the gas production responses over the control *in vitro* treatment to the model by Campos *et al*. (2004). ANCOV (Genstat, 2007) was performed, for predetermined comparisons amongst feed type across the three replications. Treatment means were compared by Fisher's least significant difference (LSD) test and were considered different at $P < 0.05$ for the ammonia and volatile fatty acid and gas production results.

Results

Chemical analysis

Proximate and van Soest analysis yielded the following results for the feeds used in this trial (Table 6.1). Calculations from these are produced in Table 6.2 and further calculations are detailed in Table 6.3.

Table 6.1 Nutrient specifications of five South African horse feeds (dry matter basis) subjected to *in vitro* gas production technique to determine the implications of various carbon and nitrogen scenarios on microbial health.

	CP	Ca	P	Ca:P	Moist	Fat	NDF	ADF	ADL	Starch	Ash
FEED NAME	**%**	**%**	**%**		**%**	**%**	**%**	**%**	**%**	**%**	**%**
LUCERNE	16.35	1.33	0.26	5.1	11.75	1.27	45.52	37.59	10.72	2.0	7.71
EQUUS ALL TIME BALANCER	25.05	0.35	0.20	1.8	12.00	2.5	27.0	10.61	2.76	1.8	8.00
SPURWING BROOD MARE	16.25	1.27	0.57	2.2	11.4	4.13	17.3	8.38	1.81	18.8	9.22
ROMIX PONY	10.09	0.93	0.35	2.7	12.8	2.49	22.2	13.04	2.80	23.5	8.52
EQUIFEEDS RACE MEAL	15.53	1.14	0.70	1.6	6.06	6.97	26.0	7.52	1.70	31.8	8.63

Where: CP = crude protein, Ca=calcium, P=phosphorous, moist=moisture, NDF=neutral detergent fibre, ADF=acid detergent fibre, ADL = acid detergent lignin

Calculations from Table 6.1 produce several parameters to describe the ratio of carbon and nitrogen in the selected feeds (Table 6.2). A 15% difference in crude protein percentage together in the All Time Balancer and the Romix Pony, for example, with a considerable soluble carbohydrate difference leads to a variation in the CHO_{sol}:N ratio.

Table 6.2 Soluble carbohydrate and nitrogen specifications calculated on a dry matter basis of five South African horse feeds subjected to *in vitro* gas production technique to determine the implications of various carbon and nitrogen scenarios on microbial health.

FEED NAME	CHO_{sol} %	C_{OM} %	N %	C_{OM}:N	CHO_{sol}:N	Starch:N	Cellulose:N	NDF:Starch
LUCERNE	28.28	91.42	2.62	34.9	10.8	0.76	10.26	22.8
EQUUS ALL TIME BALANCER	37.50	92.00	4.00	23.0	9.4	0.45	1.96	15.0
SPURWING BROOD MARE	53.54	91.22	2.60	35.1	20.6	7.23	2.53	0.9
ROMIX PONY	58.00	92.78	1.61	57.5	35.9	14.6	6.36	0.9
EQUIFEEDS RACE MEAL	42.87	91.37	2.48	36.8	17.3	12.8	2.35	0.8

Where: CHO_{sol} (%) = soluble carbohydrates (NFC) =100-(NDF%+crude fat%+crude protein%+ash%), C_{OM}=organic matter carbon =100-ash, N%=CP/6.25, NDF=neutral detergent fibre

The use of cellulose and hemicelluloses in the (cellulose+ hemicellulose) to lignin ratio provides an indication of the degradation potential of the feeds. Together with the amount of protein in the feed, the suitability of substrate for anaerobic degradation can be scrutinized in the C_{biodeg}:N (Table 6.3).

Table 6.3 Further nutrient calculations of the carbohydrate fractions of five horse feeds with various C:N ratios.

FEED NAME	(C+H)/L	Cellulose = ADF-ADL	Hemicell= NDF-ADF	C_{biodeg}	C_{biodeg}:N
LUCERNE	3.25	26.87	7.93	90.67	34.61
EQUUS ALL TIME BALANCER	8.78	7.85	16.39	91.34	22.83
SPURWING BROOD MARE	8.56	6.57	8.92	91.23	35.09
ROMIX PONY	6.93	10.24	9.16	91.49	56.83
EQUIFEEDS RACE MEAL	14.29	5.82	18.48	91.25	36.79

Where: (C+H)/L = (cellulose+hemicelluloses)/lignin; ADF = acid detergent fibre; ADL = acid detergent lignin; NDF = neutral detergent fibre; C_{biodeg} = biodegradable carbon; N = nitrogen.

These horse feeds were fermented *in vitro*, and the Campos *et al.* (2004) model was fitted to derive gas production parameters (Table 6.4).

Gas Production Parameters

The lack of an effective digestive enzyme in mammals that will cleave the linkages in carbohydrate polymers (except lactose) has led to the mutualistic association between the animal and microorganisms (van Soest, 1994). Following *in vitro* fermentation, the following degradation parameters were determined for the five feeds (Table 6.4 and Table 6.5).

The idle inoculum produced a slightly lower true degradability (P=0.053). Microbial biomass between treatments was not different. Normal terminal pH values were obtained. A reduction in pH is expected following the *in vitro* procedure, with the type of substrate indicating the extent of that reduction (Table 6.4).

Table 6.4 pH, apparent and true degradabilities (g.kg^{-1}DM), final 24 hour pH and the change in pH in five complete horse feeds differing in C:N ratio, fermented *in vitro* using faecal inoculum from racing and idle horses.

Faecal inoculum	Feed	App Deg g.kg^{-1}DM	True Deg g.kg^{-1}DM	Microbial Mass g.kg^{-1}DM	pH	Change in pH
IDLE MEAN		382.9	651.2	279.2	6.743	0.47
	EQUUS ALL TIME BALANCER	361.4	694.0	350.0	6.735	0.45
	SPURWING BROOD MARE	493.4	971.5	238.1	6.708	0.53
	LUCERNE	203.1	463.0	278.4	6.877	0.32
	ROMIX PONY	421.3	692.9	271.6	6.725	0.51
	EQUIFEEDS RACE MEAL	435.3	433.6	257.6	6.706	0.51
RACE MEAN		379.3	706	329.5	6.657	0.47
	EQUUS ALL TIME BALANCER	406.6	760.1	353.5	6.717	0.41
	SPURWING BROOD MARE	394.2	734.9	340.7	6.607	0.52
	LUCERNE	121.7	511.6	389.9	6.800	0.34
	ROMIX PONY	422.5	709.8	387.3	6.607	0.52
	EQUIFEEDS RACE MEAL	465.6	762	296.4	6.593	0.54
FEED MEANS	EQUUS ALL TIME BALANCER	382.7^{ab}	724.7a	351.7	6.727^{b}	0.43^{ab}
	SPURWING BROOD MARE	414.9^{a}	701^{a}	286.4	6.661^{c}	0.52^{a}
	LUCERNE	164.7^{c}	485.6^{b}	331	6.841^{a}	0.32^{b}
	ROMIX PONY	421.9^{a}	700.8^{a}	279	6.670^{b}	0.52^{a}
	EQUIFEEDS RACE MEAL	449.6^{a}	720.6^{a}	275.9	6.654^{c}	0.52^{a}
SED		33.18	62.48	65.75	0.03	0.06
LSD		66.4	124.9	131.5	0.06	0.12
CV		16.1	17.2	40.5	0.9	24.5
Variation	Faecal inoculum	Ns	0.053	0.088	**	ns
	Feed	**	**	ns	**	**
	Faecal x feed	0.079	ns	ns	ns	Ns

Where: App Deg = apparent degradability; True Deg = true degradability; means with different superscripts in the same column differ significantly (P <0.05). Ns= non-significant.

The time taken to half maximum gas production was not different among the treatment groups Table 6.5). More gas is produced on the race horse inoculum, but this is not consistent over the feeds. The lowest CHO_{sol}:N feed on the inoculum least suited to the high protein, had the lowest gas production. The higher CHO_{sol} regardless of inoculum produced more gas. The higher roughage feeds on the race horse inoculum did not ferment, and the higher protein feeds on the idle horse inoculum did not ferment well, as indicated by the max gas volume. The gas volume per unit substrate for the idle inoculum is less. Degradation efficiency is a function of both PF and $T_{½}$, and was higher on the race inoculum.

Table 6.5 Mean gas production kinetics of five complete horse feeds differing in C:N ratio fermented for 24 hours *in vitro*, using race and idle equine faecal inoculum, where $T_{½}$ is the time (hours) to half maximum gas production, max GP = maximum gas production (ml/g DM), gas volumes (ml) of the rapidly and slowly degradable fractions, degradation rate (h^{-1}), and the lag/colonization time (h) as determined by the Campos *et al.* (2004) model, PF = partitioning factor (degraded substrate:gas volume mg/ml) and DEF = degradation efficiency factor, for five complete horse feeds differing in C:N ratio, fermented *in vitro* using faecal inoculum from racing and idle horses.

Faecal Inoculum	Feed	$T_{½}$ (h)	Max GP ml.gDM^{-1}	gas vol (ml) for rapidly degradable	gas vol (ml) for slowly degradable	degradation rate of rapidly degradable fraction (h^{-1})	lag/ colonization time (h)	PF	DEF
IDLE		9.399	122.5	49.9	74.8	0.130	2.153	6.398	1.185
	EQUUS BALANCER	9.38	106.1	40.1	66	0.084	1.73	9.135	1.744
	SPURWING BROOD	9.50	138.6	57.9	78.1	0.070	1.6	8.077	1.013
	LUCERNE	11.13	90.3	38.2	52.1	0.197	3.01	5.517	1.004
	ROMIX PONY	9.50	136.6	42.8	933.8	0.199	2.61	4.803	1.010
	EQUIFEEDS RACE MEAL	8.28	140.8	70.6	84.0	0.098	1.81	4.713	1.146
RACE		9.428	123.1	91.2	44.8	0.113	2.35	5.941	1.324
	EQUUS BALANCER	8.33	122.1	57.4	64.8	0.214	2.61	7.422	1.815
	SPURWING BROOD	9.00	140.7	150	1.77	0.091	0.93	5.386	1.234
	LUCERNE	11.78	75.1	67.9	7..2	0.085	2.00	6.423	1.092
	ROMIX PONY	9.61	131.3	27.3	160.1	0.116	3.45	5.529	1.197
	EQUIFEEDS RACE MEAL	9.29	146.3	153.3	6.2	0.060	2.75	5.468	1.261
FEED	EQUUS BALANCER	8.90	114.1	48.7	65.4	0.149	2.17	8.335	1.779
	SPURWING BROOD	9.27	139.7	104.0	38.2	0.081	1.27	6.821	1.122
	LUCERNE	11.43	82.7	53.1	29.7	0.141	2.51	5.940	1.048
	ROMIX PONY	9.55	134.0	35.0	126.9	0.158	3.03	5.142	1.102
	EQUIFEEDS RACE MEAL	8.72	143.5	11.9	38.9	0.079	2.28	5.065	1.203
SED		2.131	5.27	14.73	16.99	0.0514	0.427	1.266	0.1588
LSD		4.277	10.79	30.63	35.15	0.1062	0.881	5.542	0.3195
CV%		38.43	6.1	29.5	40.2	59.9	26.8	34.60	20.68
Variation	Faecal inoculum	Ns	Ns	<0.001	<0.001	Ns	Ns	0.002	**
	Feed	Ns	<0.001	<0.001	<0.001	Ns	<0.001	Ns	0.052
	FxR	Ns	0.004	<0.001	<0.001	0.023	0.005	Ns	Ns

The ammonia and volatile fatty acid contents of the fermentation products are detailed in Tables 6.6 and 6.7. The fermentation product in the race horse inoculum produced more ammonia than the idle horse faeces, while the low CHO, N and CP treatment had the lowest ammonia values (Table 6.6).

Ammonia Results

Table 6.6 Ammonia (mg/L) in the fermentation product of five complete horse feeds differing in C:N ratio, fermented *in vitro* using faecal inoculum from racing and idle horses.

Faecal inoculums	FEED	NH_3 ($mg.l^{-1}$)	
IDLE			275.9
	EQUUS ALL TIME BALANCER	322.3	
	SPURWING BROOD MARE	243.2	
	LUCERNE	309.3	
	ROMIX PONY	248	
	EQUIFEEDS RACE MEAL	264.9	
RACE			310.9
	EQUUS ALL TIME BALANCER	344.9	
	SPURWING BROOD MARE	248.5	
	LUCERNE	394.4	
	ROMIX PONY	265.6	
	EQUIFEEDS RACE MEAL	324.8	
FEED MEANS	EQUUS ALL TIME BALANCER	332.6	
	SPURWING BROOD MARE	245.6	
	CONTROL	334	
	LUCERNE	348	
	ROMIX PONY	256	
	EQUIFEEDS RACE MEAL	292.2	
OVERALL MEAN		292.1	
SED		34.44	
LSD		23.98	
CV%		20.7	
Variation	Faecal inoculum	0.018	
	Feed	<0.001	
	Faecal x feed	0.431	

An increase in propionate occurred at the expense of acetate as the grain ratio in the feed increased. More butyrate was produced from idle horse inoculums fermentation, with more soluble CHO increasing the butyrate (Table 6.7).

VFA Results

Table 6.7 Molar proportions of short chain fatty acids determined by gas chromatography following *in vitro* gas production for 24 hours in two set of faecal inoculum, using five feeds differing in C:N ratio.

Faecal Inoculum	Feed	Acetate	Propionate	N-Butyrate	C2:C3	TOTAL VFA(mg.ml^{-1})
IDLE	EQUUS ALL TIME BALANCER	44.23	39.08	14.00	1.29	0.15
	SPURWING BROOD MARE	49.43	35.08	15.33	1.42	0.08
	CONTROL	54.75	34.15	10.80	1.62	0.04
	LUCERNE	58.87	29.10	9.43	2.03	0.16
	ROMIX PONY	53.35	28.70	16.55	1.88	0.8
	EQUIFEEDS RACE MEAL	51.35	31.43	17.23	1.65	0.4
	IDLE mean	51.05	32.86	14.77	1.63	0.10
RACE	EQUUS ALL TIME BALANCER	50.33	44.40	5.15	1.13	0.15
	SPURWING BROOD MARE	46.68	45.88	7.45	1.02	0.14
	CONTROL	69.65	27.10	2.80	2.59	0.02
	LUCERNE	63.53	31.93	4.53	2.01	0.12
	ROMIX PONY	52.00	41.18	6.75	1.26	0.13
	EQUIFEEDS RACE MEAL	50.03	41.85	8.13	1.24	0.12
	RACE Mean	51.93	41.53	6.50	1.30	0.13
FEED MEANS	EQUUS ALL TIME BALANCER	47.28	41.74	9.58	1.21	0.15
	SPURWING BROOD MARE	48.06	40.48	11.39	1.22	0.11
	CONTROL	62.20	30.63	6.80	2.11	0.03
	LUCERNE	61.20	30.52	6.98	2.02	0.14
	ROMIX PONY	52.68	34.94	11.65	1.57	0.47
	EQUIFEEDS RACE MEAL	50.69	36.64	12.68	1.45	0.26
Overall Mean		51.49	37.19	10.64	1.47	0.12
SED		4.26	3.92	1.29	0.22	0.033
LSD		6.17	5.67	1.87	0.32	0.047
CV%		10.8	13.8	15.9	19.7	36.9
Fprob:Faecal Inoculum		0.631	<0.001	<0.001	0.001	0.042
Fprob:Feed		<0.001	0.003	<0.001	<0.001	0.016
Fprob: Faeces x Feed		0.435	0.367	0.105	0.332	0.082

Discussion

The literature would suggest a ratio of C:N of 20-25:1 for optimal microbial health (Ghasimi *et al.*, 2009). Horses are eating rations with parameters exceeding that (Figure 6.4), even if one considers the CHO_{sol}:N ratio (Richard, 1996).

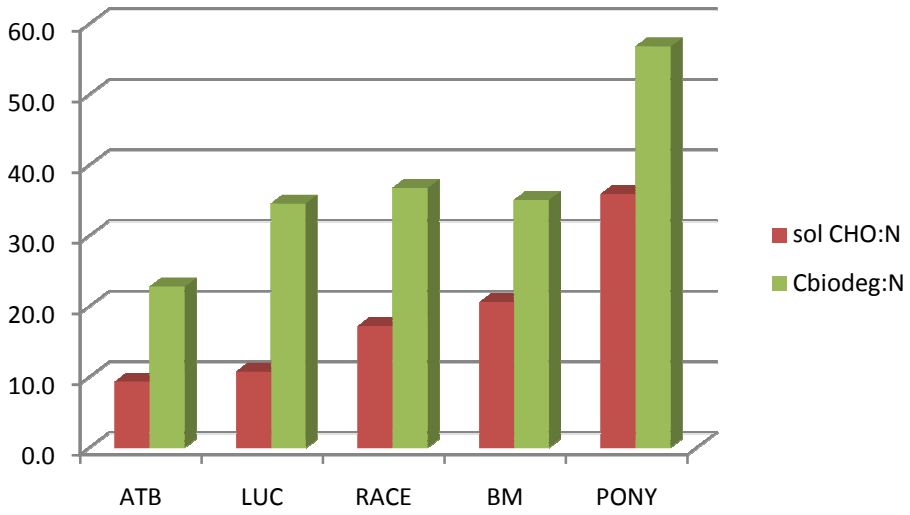

Figure 6.4 The range of Cbiodeg:N ratios and CHOsol:N ratios in five horse feeds, illustrating the greater disparity observed in the latter parameter.

What the microbes have available to them depends on the extent of precaecal digestion of feed components (Figure 6.5). *In vitro* fermentation evaluates the consequence of the arrival of undigested protein, CHO and lipid in the caecum, and the production of fermentation products. With it are recommendations for ration optimization. Foregut digestion is rapidly overcome by a combination of feeding frequency, meal size and ration composition, and measures of hindgut efficiency should be obtained as a result. What Figure 6.5 also intends to demonstrate is that there is no guarantee of the similar precaecal degradation of feeds, and that the assumption that precaecal digestion is additive to fermentation in the utilization of nutrients in the horse may not be valid. While one can ascribe the site of degradation to certain sections of the GIT, more research should be governed by the effect of nutrient synchrony, particularly in respect of the forage:concentrate ratios, and the concomitant delivery and interaction of nutrients at the site of digestion.

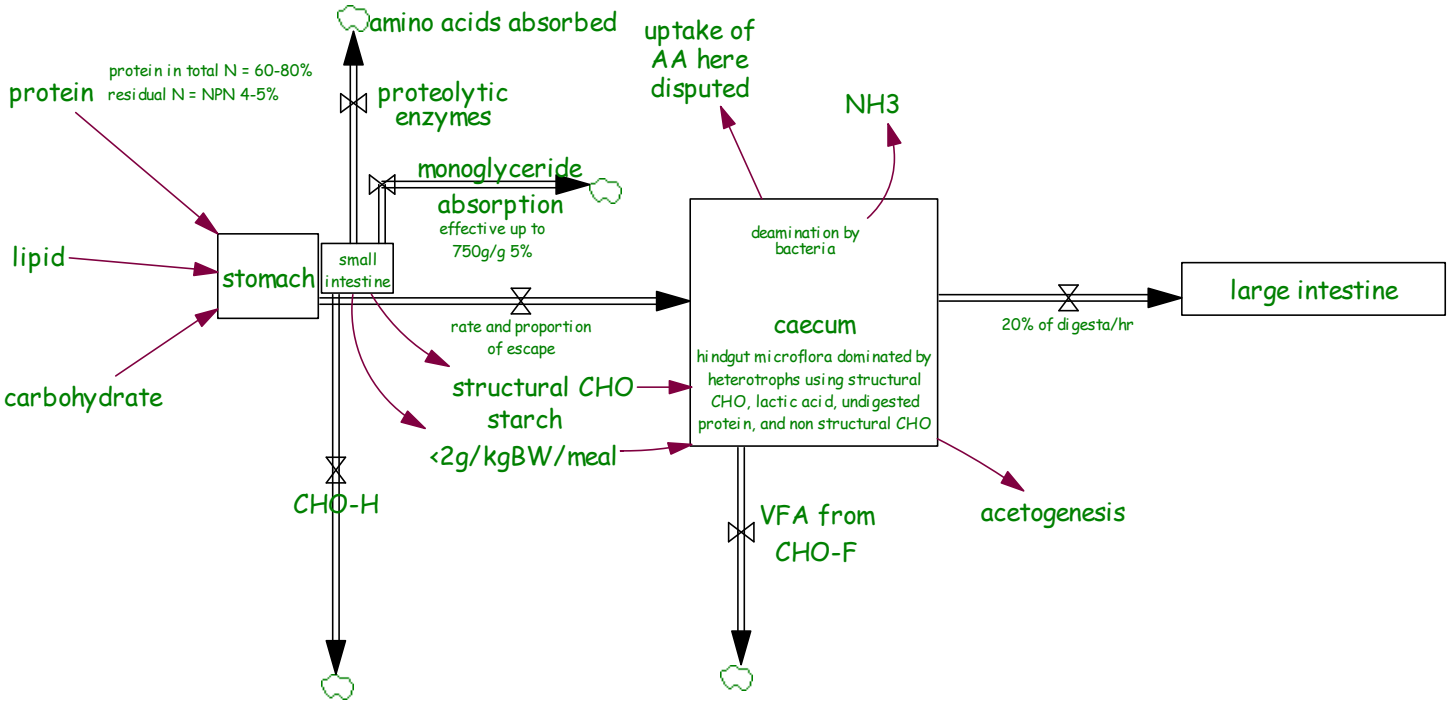

Figure 6.5 Heuristic model of nutrient flow in the horse (author's own).

The response in apparent degradability of the feeds was almost not consistent (P=0.079) across the faecal treatments for the feeds, but only the lucerne demonstrated the lowest apparent and true degradability, the values of which were slightly lower than the rumen fluid IVGPT results of Ouda (2007) (Table 6.3). The idle inoculum produced a slightly lower true degradability (P=0.053). Low degradability in the forage only treatment can be explained by the higher ADF% and the lowest (C+H)/L ratio (Figure 6.6). Microbial biomass between treatments is not different. Normal terminal pH values were obtained (Wiliamson *et al.*, 2007; dos Santos *et al.*, 2009). The change in pH with the forage is least, containing the lowest CHO_{sol}. Final pH following fermentation using the race horse faeces was lower and this difference was consistent across feeds, reflecting the differences in microbial ecology of the horses adapted to different nutrient density diets. Microorganisms using high fermentable substrates proliferate in this inoculum and the race feed produces the lowest pH following fermentation on this inoculum. The protozoal depolymerization of the cellulose and hemicelluloses by glycosidases is highest at pH 6 for the protozoal and bacterial fractions of the caecal contents (Bonhomme-Florentin, 1988). The $CP:CHO_{sol}$ parameter appears to explain the final pH best.

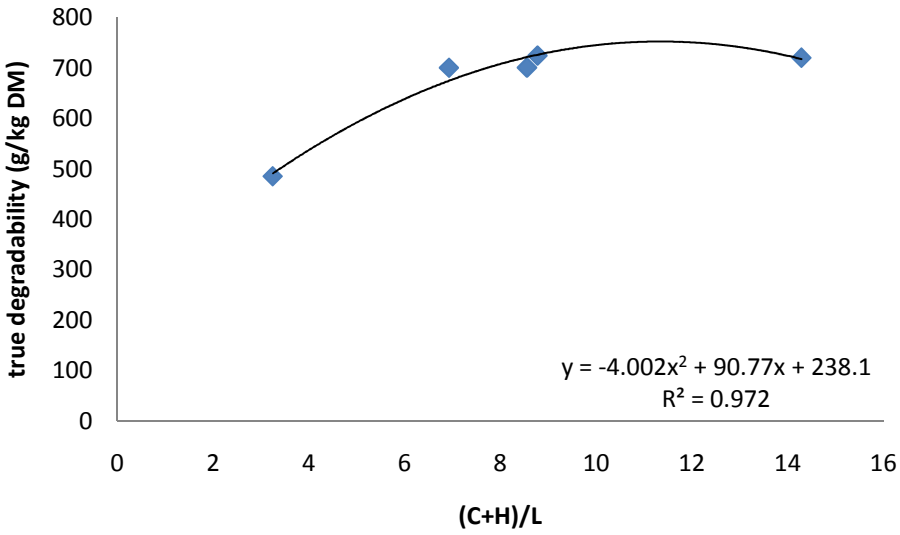

Figure 6.6 Response in true degradability to the cellulose and hemi-cellulose to lignin ratio ((C+H)/L) in feeds following *in vitro* fermentation in equine faecal inoculum.

The time taken to half maximum gas production was not different among the treatment groups Table 6.5). This indication of how rapidly fermentation begins tends to be lower in the forage treatment. The interaction between treatments is significant. More gas is produced on the race horse inoculum, but this is not consistent over the feeds. The lowest CHO_{sol}:N feed on the inoculum least suited to the high protein, had the lowest gas production. The microbial ecosystem would have been suited to more cellulose in the feed. The higher CHO_{sol} regardless of inoculums then produced more gas. The higher roughage feeds on the race horse inoculum did not ferment, and the higher protein feeds on the idle horse inoculum did not ferment well, as indicated by the max gas volume. This is a function of the microbial adaptation to the substrate. The lower CHO diets do demonstrate a shorter lag time. This is due to the higher NDF feeds needing time before gas production starts.

PF is an authentic means of contrasting treatments over short incubation times (Blümmel *et al.,* 2005). The gas volume per unit substrate for the idle inoculums is less. The PF is a measure of the efficiency of rumen microbial production, and in the horse, where the total mg VFA was less and the ammonia lower in the idle inoculums, where the volumes of GP were lower, but the PF is higher, indicates that the volume alone is not necessarily a degradability issue. The true degradability (P=0.03) was slightly lower in the idle inoculum, indicating a higher efficiency in the idle inoculums.

PF is defined as degradation per unit volume, and with the volume being lower, the PF should have been higher if the degradabilities were the same. But the true degradability was lower as well. This implies that PF is a good indicator of microbial efficiency in the horse as well. The feeds were

not different, but the difference in faecal inoculums was picked up, making it a characteristic of the inoculum and therefore the fermentation process.

Degradation efficiency is a function of both PF and $T_{½}$, and was higher on the race inoculum. The highest DEF was on the lowest C_{biodeg}:N and CHO_{sol}:N and lowest C:N, with a feed having the lower GP characteristics (Figure 6.7). From the PF, the efficiency of microbial production can be inferred, so the DEF can say which microbes have to work harder for their gain (race>idle) and produce the least gas doing it when the microbes are not acclimatized to the substrates. The higher C_{biodeg}:N provided to microbes that are used to it, are able to metabolize the substrate, which means that the DEF and PF indicate an opportunity to select feeds for enhancing the rate of substrate degradation (Figure 6.8).

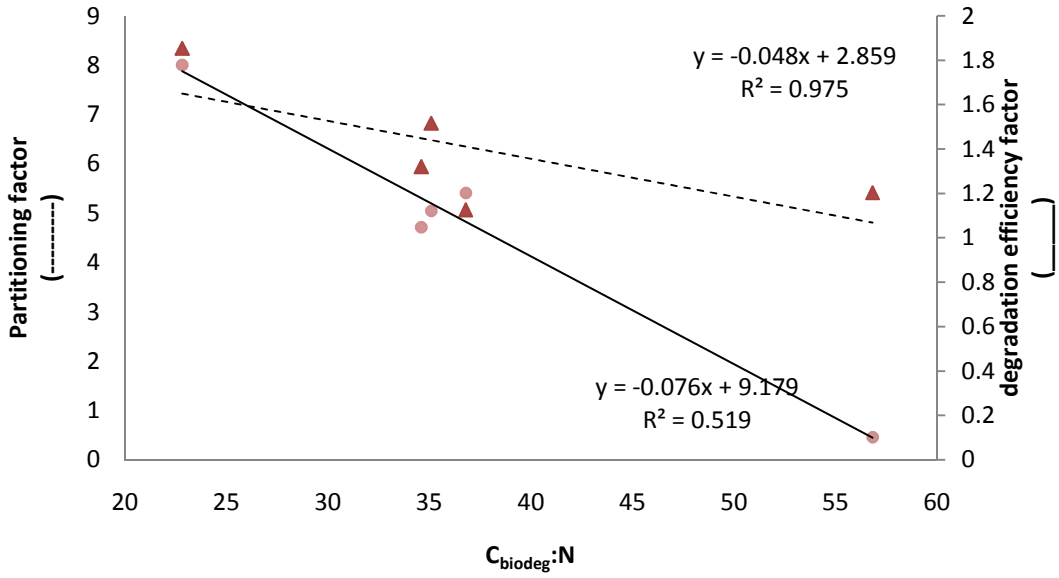

Figure 6.7 The ratio of biodegradable carbon to nitrogen (C_{biodeg}:N) strongly influences both the partitioning factor and the degradation efficiency factor calculated following *in vitro* fermentation of horse feeds. Biodegradable carbon is calculated as = C_{total}(NDF%/100)(1-0.054(lignin%/(NDFx0.01))$^{0.76}$) + C_{total} (1-NDF/100 (after Richard, 1996).

The importance of carbohydrate fermentation to bacterial N metabolism in the ruminant is well known (Glade, 1984). Reduced NDF fermentability decreases the energy available to caecal and colonic microbes, slowing microbial capture and incorporation of the ammonia resulting from urea hydrolysis and increasing faecal water soluble N. A similar effect is observed in ruminants fed poorly fermentable substrate (Van Soest, 1982). In addition, decreased microbial capture may have resulted in increased ammonia damage to large intestinal mucosal cells (Anderson *et al*., 1964; Glade, 1984), increasing the secretion of intestinal cell-bound N.

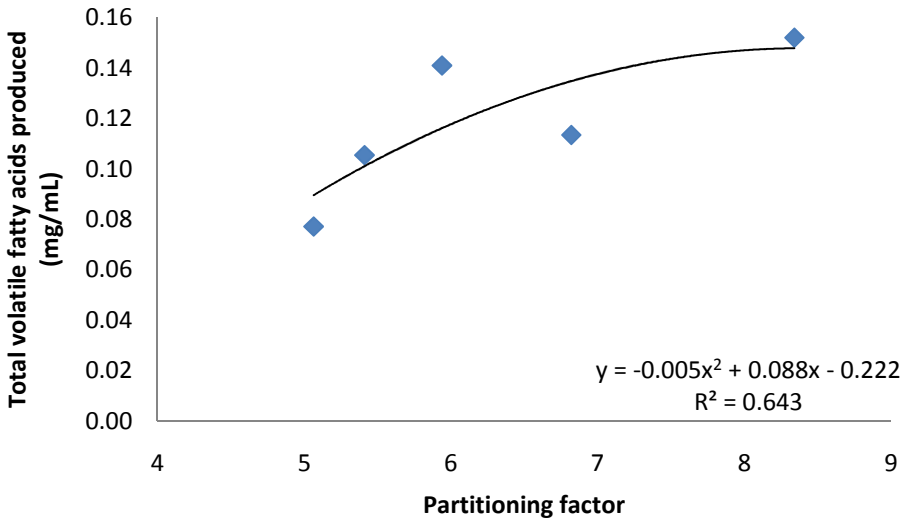

Figure 6.8 The Partitioning factor is correlated to the total production of volatile fatty acids in horse feeds subjected to *in vitro* fermentation.

The race horse inoculum had a lower partitioning factor, so less microbial efficiency, because of the higher gas productions, although the DEF on the race horse inoculum was ultimately higher. This incorporates the time, the gas production and the degradability, and it can therefore be concluded that the slightly higher microbial yield in the race horse inoculums must have enhanced this parameter. The lowest CHO_{sol}:N ratio showed the highest DEF, which is interesting in that the fermentation appeared to succeed for more conventional feeds.

The fermentation product in the race horse inoculums produced more ammonia than the idle horse faeces, while the low CHO, N and CP treatment had the lowest ammonia values. There is a negative correlation (63%) between the CHO_{sol} :N and the ammonia produced, with a positive correlation between the N% of the diet and the ammonia it produced. If the C:N ratio is too low, nitrogen is converted to ammonium-N at a faster rate (Ghasimi *et al.*, 2009). Glade and Bieseik (1986) found that when the amount of N in the diet is increased to, for example, a 38% increment over the NRC specification for N intake in yearlings (NRC, 2007) there is more ammonification, leading to double the % total faecal N in the high N diets. High starch diets increase the blood urea levels (Zeyner *et al*., 2002). Nitrogen associated with cellulose or cellulose-like substances promoted earlier microbial degradation (Fioretto *et al.*, 2005). With ammonium nitrate supplementation, both cellulose and lignin degradation was reduced in soils, with the higher N soils also having lower active bacterial biomasses (Entry, 2000). Microbial extracellular enzymes are regulated by soil N

availability (Waldrop *et al*., 2004), and it appears to be the case that in the horse microbial ecosystem, that N availability is certainly implicated in the efficacy of degradation.

The faecal inoculum reflects what is occurring in the hindgut (Müller *et al.*, 2008). The lucerne feed produced more acetate (Table 6.7), which occurs in roughage diets (Ellis and Hill, 2005; Frape, 1989; Meyer, 1987). An excessively high C:N ratio causes an increase in acid formation (Ghasimi *et al.*, 2009). An increase in propionate occurred at the expense of acetate as the grain ratio in the feed increased, similar to results obtained by Hoffman (2003). Higher ADF feeds have lower propionate (Ellis and Hill, 2005). More butyrate was produced from idle horse inoculums fermentation, with more soluble CHO increasing the butyrate. Ruminal fermentation characteristics were largely unaffected by the source of dietary carbohydrate, with similar ruminal pH and volatile fatty acid and ammonia concentrations for the first 6 h after the morning feeding (Gozho and Mutsvangwa, 2008). Dry matter digestibility of wheat straw was increased when glucose was available as an energy source, so that the degradation of lignin was enhanced to release the hemi/cellulose for conversion to VFAs by microbes. The addition of ammonium sulphate unfortunately ruined this improvement in DMD (Abdullah *et al*., 2004). The ratio of C2:C3 is reduced when concentrate is added to a feed. The adaptation of the race inoculums to the higher concentrate diets produces a lower ratio. The absence of lactate in the fermentation profiles (Müller *et al*., 2008), can be explained by the absence of a positive control, but the race faeces should theoretically have produced more. The value in the VFA analysis from GPT is realized in mechanistic modelling including digesta passage rate (Dijkstra *et al.*, 2005).

The nutritive value of the feed depends on the proportions of soluble, insoluble but degradable, and undegradable fractions of the feed (Getachew *et al*., 2005). CF, ADF and NDF measure fibre in horse diets, with the under-estimation of NSP by the NDF (Ellis and Hill, 2005). An effective scheme for the representation of the usefulness of CHO to horses would include three main fractions (Figure 6.9): 1. A hydrolysable group (yields mainly glucose), 2. A rapidly fermentable group (lactate and propionate, metabolised as 3C and 6C units mainly via glucose, and 3. A slowly fermented group (yields primarily acetate and butyrate, metabolised as 2C and 4C units through acetyl coA (Hoffman, 2003).

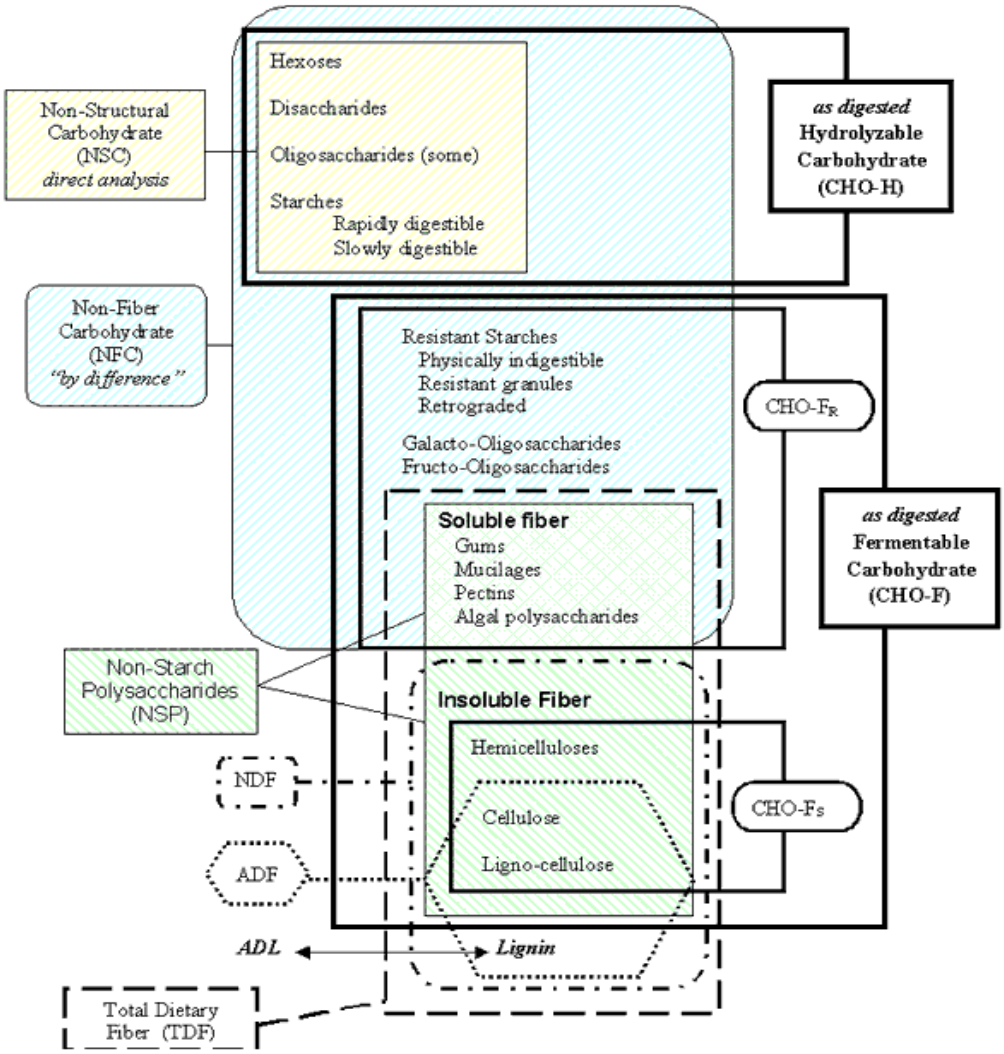

Figure 6.9 The scheme of carbohydrate fractions for the horse, as a comparison of proximate analysis fractions (left) with fractions as digested (right). Abbreviations include ADL, acid detergent lignin; ADF, acid detergent fiber; NDF, neutral detergent fiber; CHO-FS, slowly fermentable carbohydrate; CHO-FR, rapidly fermentable carbohydrate. (Adapted from Hoffman *et al.*, 2001).

In the horse, hydrolysis to simple sugars occurs in the small intestine and involves the enzymatic hydrolysis of α-1,4 linked molecules. Disaccharides (sucrose, maltose and lactose), some oligosaccharides, and starch are hydrolused by small intestine enzymes to get free sugars, glucose, galactose, and fructose. CHO escaping enzymatic hydrolyis in the SI, is subject to bacterial fermentation in the caercum where the horse obtains energy from the production of VFA's. The β-1,4 links are fermented. Hemicellulose, cellulose lignocellulose, soluble fibes and oligosacharides (galactans and fructans implicated in laminitis) and starch by the action of the microbial ecosystem, produce acetate, propionate, butyrate and latate and valerate to a lesser extent. The transmucosal pH gradient in the large intestine facilitates the passive diffusion and absorption of acetate, over

proprionic, butyric and lactate (based on MW). VFA's also function as nutrient stimuli in the control of meal frequency (Ralston and Baile, 1983b).

Cellulose is a β-1,4 linked, unbranched, D-glucose units. Cellulases cleave the glycosidic linkages. Hemicellulose is made of D-pentose sugars, especially xylose. It can be estimated from the difference between the NDF and ADF fractions in the feed. The increase in the grain ratio in the feed will increase the proportion of prop:lactate, at the expense of acetate, suggesting the support of rapid over slow fermentation. This favours the proliferation of lactobacilli, which decrease the pH. A pH of 6 indicates subclinical acidosis, with less than 6 indicating clinical acidosis. In excess of 0.4% of BW per meal (or as low as 0.2% of BW) of CHO-H overload will lead to this occurring (Hoffman, 2003).

There are two classes of glucose carrier proteins. SGLT1 carrier proteins have a high affinity and low capacity for absorption of D-glucose/galactose absorption across the intestinal lumen membrane and in the kidney. There is a lag time in SGLT1 production in mice, and the hypothesis is that if in the horse the activity is similar, then abrupt changes to CHO exacerbate CHO overload because of the 12-24 hour lag time in the production of SGLT1 production for its absorption in the small intestine. The GLUT proteins are facultative, and three classes exist. Fructose is absorbed by GLUT5 (of GLUT 5-12) and represents a small fraction of the absorption of simple sugars, which are mainly as glucose (Hoffman, 2003). Fructose substitution for glucose does not improve glucose metabolism in animal studies (Gerrits and Tsalikian, 1993). Of the pentose sugars, xylose is the most abundant, and while it has been demonstrated to be largely non-nutritive in the small intestine, overflow to the hindgut will also yield VFA's by its microbial degradation (Ralston and Baile, 1983b). Starch should be kept to a <1.1g starch /kg BW /meal (Vervuert *et al.*, 2009c). NSC can be 32-36% in the high performance horse while idle horses need little NSC in their diet (Coleman, 1999).

Maximum performance occurs when the non-lignin-carbon to nitrogen ratio of feed mixtures is between 25 and 32, utilizing anaerobic digestion of dairy manure and field crop residues (Hills and Roberts, 1981). One can also use the C_{biodeg} : N ratio (Richard, 1996, cited in Richards, 2003). One should then reduce the C:N goal. Richards *et al.* (2005) made a successful point on the calculation of the (C+H)/L ratio as a means of evaluating degradation potential, with good correlations being achieved between this ratio and the gas production of anaerobic waste degradation.

Cellulase has been shown to reduce digestibility of fibre components in a hay-based diet (O'Connor-Robison *et al.*, 2007). Supplemental yeast enhances growth and reduces ammonia (Glade

and Sist, 1990), but to influence the degradability of hemicelluloses and lignin, the amount you feed to mature horse is important (Hall *et al*., 1990). Significant improvements in cellulose digestibility with the addition of yeast cultures are observed, chiefly through the activity of the microflora involved in the digestion of the ADF (Jouany *et al*., 2008).

The pregastric fermentation of protein in the ruminant may make them more adaptable to asynchronous nutrient provision (Reynolds and Kristensen, 2008). The horse however does demonstrate shifts in hindgut fermentation parameters when provided with varying carbon:nitrogen ratios. Synchrony between other nutrients is important as well, especially fat (Geelen *et al.*, 1999). The replacement of fermentable carbohydrates with lipid in the diets of sport horses has been indicated for glycogen sparing (Geelen *et al*., 2001), energy provision without heat increment and conditioning (Ellis and Hill, 2005). Positive effects of adaptation to fat as a fuel in athletic training in horses have been identified (Pagan *et al*., 1995; Kronfeld *et al*., 1994), and is not supposed to have any long term effects (Zeyner *et al*., 2002), although fermentation effects were not considered, but should be (Figure 6.10). However, the depression of the activity of especially cellulolytic bacteria (Jenkins, 1993; Jansen *et al*., 2000; Jansen *et al*., 2007a) in the hindgut cannot be disregarded if optimal use of cellulose is to be made (Figure 6.9). Reduced fibre digestibility also occurs at levels of 25% and 35% NDF (Bragal *et al.*, 2008).

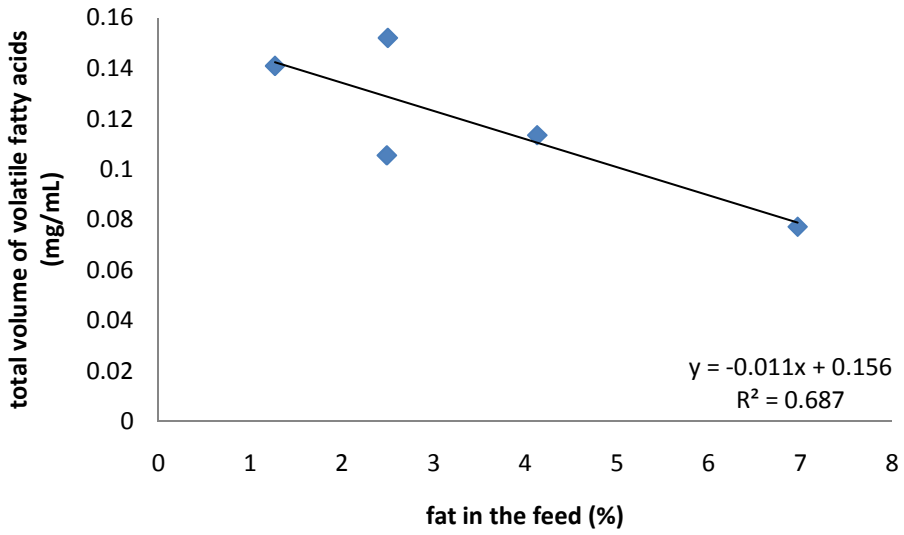

Figure 6.10 Indication of the antimicrobial properties of lipid on the production of volatile fatty acids in the diet of the horse.

There are some indications that the amount or pattern of supply of fermentable carbohydrate has a greater impact on microbial production and efficiency than does the pattern of protein supply

(Hall and Huntingdon, 2008). While practical ration formulation and theoretical modelling of nutrient synchrony are sometimes difficult to reconcile *in vivo* and *in silico,* there is sufficient evidence to suggest that such a reconciliation should be pursued. As a result, the partitioning between foregut and hindgut digestion as well as the nutrient synchrony in feeds supplied to horses should be considered by nutritionists for optimal health of the microbial system and long term health and productivity of the horse.

Conclusions

Horse owners currently buy horse feed for protein, and feed at levels far in excess of requirement. No consequence of this appears to have been considered, nor are recommendations made for the equine nutritionist to optimize provision of protein in the horse. The frequencies of feeding as well as the meal size influence the pre-caecal digestion of feed, with concomitant changes to the substrate available to microbes for fermentation in the hindgut. This emphasizes the role that nutrient synchrony has to play in the optimal formulation of rations for sport horses, and the prediction of response of horses on those feeds. Biodegradable carbon to nitrogen ratios in horse feeds are inflated over that required for optimal microbial health, leading to undesirable levels of volatile nitrogenous compounds that can damage the lining of the GIT, sometimes with long term consequence. CHO_{sol}:N and C_{biodeg}:N ratios more accurately inform the changes to degradation efficiency and partitioning factor in the hindgut, Microbial efficiency in particular has a strong negative correlation to C_{biodeg}:N. It is no longer sufficient to consider the provision of nutrients to the horse simply as reflected by primary analysis of nutrient contents of the feeds. Nutrient synchrony and the fate of ingested ingredients, ameliorated by feeding management, have to be considered.

CHAPTER SEVEN: REPRESENTATION OF SA HORSE FEEDS FOLLOWING IN VITRO, IN VIVO AND IN SILICO CHARACTERISATION

Abstract

In vitro and *in vivo* parameters for common South African horse feeds were compiled. Chemical analysis, *in vitro* gas production, *in vivo* retention times, glycaemic responses and various carbon: nitrogen ratios were used to describe several horse feeds. Statistically significant parameters and correlations were used to produce nine classifying variables: crude protein, fat, starch, soluble carbohydrate, (cellulose + Hemicellulose): lignin (CHL) ratio, the degradation efficiency factor, true degradability and the biodegradable carbon: nitrogen ratio and maximum gas production. A multivariate, hierarchical cluster analysis technique was used to segregate feeds into homogenous groups. Radar charts were produced to gauge the effectiveness of the feeds in meeting key parameters. Significantly the CHL, soluble carbohydrate and starch levels in the feeds were important in defining feed clusters, but equine nutritionists in this country need to quantify objectives in ration formulation to define the suitability of rations for groups of horses to allow the horse owner to provide appropriate, evidence-based nutrition for their horse.

Introduction

Nutritive values of horse feed depend on understanding chemical composition, intake level, digestibility and the efficiency with which the nutrients are released. The ratio of fore- and hindgut utilization as influenced by feeding management is crucial. Because of a poor understanding of feeding management in equines, there is an expectation of the hindgut to process raw materials, which the caecal microbes may not be able to fulfill. Nutrient synchrony is as yet unapplied in the field of equine nutrition and yet it is critical to the optimal utilization of feed ingredients, so that the performance of the horse on the various rations can be explained. Several methods have been developed to determine the nutritive value of horse feeds. Biologically-based methods are recommended as providing a more meaningful representation of equine nutrition than are chemical methods (van Soest, 1994). IVGPT should be coupled with residue determination (Blümmel *et al*., 1997), which together with gas fermentation kinetics (Campos *et al.,* 2004), can generate diverse information regarding the fate of feed undergoing hindgut fermentation in the horse.

Complemented by *in vivo*, glycaemic and chemical analyses, the fate of ingested ingredients can be used to model the extent to which feeds actually meet the requirements of the horse in various activity scenarios and to what extent nutrient synchrony is adhered to in the optimization of microbial and fermentative capacity. Characteristics of horse feeds should be used to consistently and meaningfully classify feeds into homogenous nutritional groups.

Materials and methods

Results from the preceding six chapters of experimentation were compiled in an Excel spreadsheet (MSOffice, 2007) to produce nutrient profiles for each of 18 feeds, which included chemical analysis, *in vitro* gas production, *in vivo* retention times, glycaemic responses, and various carbon:nitrogen descriptions of the feeds. Parameters which produced statistically significant differences in ANOVA between feed treatments in previous experiments were identified, and correlations in excess of 60% were identified in a correlation matrix containing all feeds and parameters (Genstat, 2007) to reduce multicollinearity. Nine classifying variables were identified: crude protein, fat, starch, soluble carbohydrate, the (cellulose+hemicellulose):lignin ratio, the degradation efficiency factor, the true degradability, the biodegradable carbon: nitrogen ratio and the maximum gas production.

A multivariate, hierarchical cluster analysis technique (SAS, 2005) was used to segregate feeds into homogeneous groups (clusters) on the basis of these classifying variables, derived from the previous chapters.

1. The variable reduction ACECLUS procedure summarized the multivariate set of data into orthogonal canonical variates.
2. Canonical variables were used in the hierarchical cluster analysis using Ward's Method to ensure that within-cluster differences and between-cluster linkages were minimized.
3. Cubic clustering criterion, pseudo-F and pseudo-T^2 analyses were unanimous for the number of clusters chosen to represent the solution, with a corresponding percentage variation accounted for.
4. The diets were then assigned into clusters by the TREE procedure, and adjusted using FASTCLUS.

To check the validity of the clusters, general linear model analysis (GLM) was performed. The classifying variables used in the creation of the clusters were included in the GLM procedure. Cluster

analysis was used iteratively to fathom the relationships between feeds in the clusters. For this reason, cluster analysis is repeated on feeds excluding the aberrant clusters in the previous analysis.

For each classifying variable, substantive proof in the literature was used to describe ideal parameters for the working horse, and the idle horse. Radar charts in Excel (MSOffice, 2007) were used to evaluate how effectively these parameters were met in horse feeds, on the hypothesis, that all horse rations should be adequate across these key classifying variables for successful ration formulation for the working and idle horse.

Results

SAS (2005) produced the following tree diagram to illustrate the allocation of clusters (Figure 7.1). Separate clusters of the 20 feeds are formed for a "high protein balancer" (OB17), and a "high energy riding feed" (OB 15) and lucerne (OB16). This can be explained by the low C_{biodeg}:N and high CP, low starch and low CHO_{sol} in the All Time Balancer and the fact that lucerne is a roughage only with low starch, CHO_{sol} and true degradability. Mkondeni Feeds is a rice based, very high ADF feed and would have been discriminated against on the basis of the low CHO_{sol} and true degradability (Table 7.3).

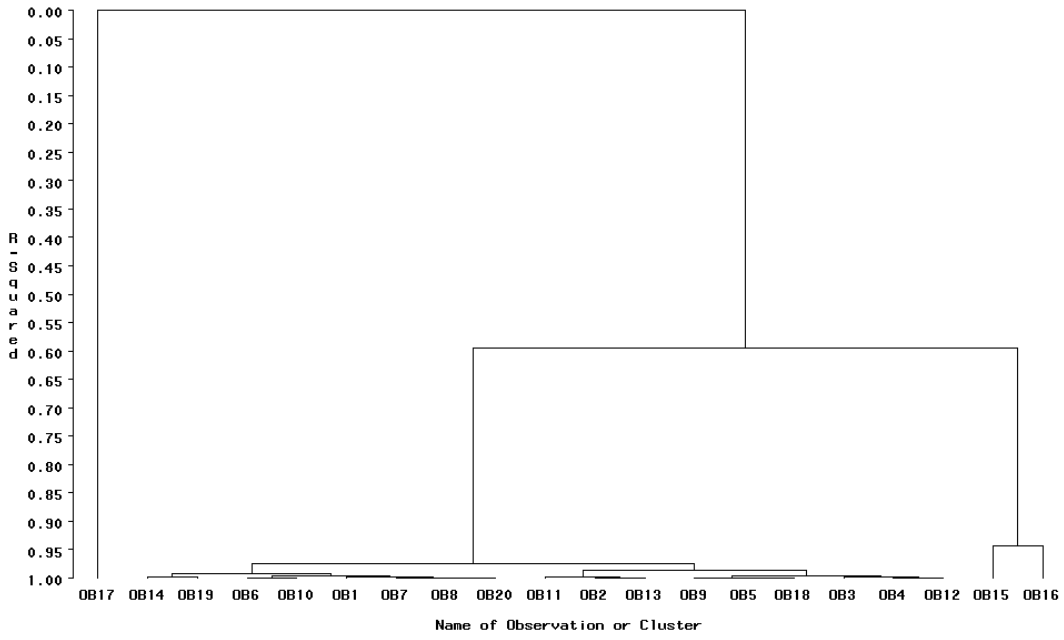

Figure 7.1 Hierarchical cluster analysis of feeds on the basis of nine classifying variables that separated feeds into 4 clusters, accounting for 97.5% of the variation between them

Excluding these obvious groups, four clusters were identified that accounted for 97.5% of the variation between horse feeds according to nine classifying variables (Table 7.1).

Table 7.1 Diet clusters produced from measurements derived fromlaboratory analysis on a dry matter basis, *in vitro* fermentation, glycaemic and C:N studies of horse feeds, using hierarchical cluster analysis (SAS, 2005).

FEED	CP (%)	FAT (%)	STARCH (%)	CHO_{SOL} (%)	(C+H)/L	DEF	TRUE DEG ($g.kg^{-1}$ DM)	C_{BIODEG}:N	MAX GP (ml)	CLUSTER
SPURWING TRANKILO	15.07	4.31	12.99	47.29	2.83	1.79	844.40	37.72	104.10	1
SPURWING BROODMARE	16.25	4.13	18.75	53.54	2.65	0.67	825.00	35.08	120.60	1
SPURWING PERFORMANCE	13.51	4.03	24.72	56.04	2.93	1.39	860.80	42.19	91.30	1
SPURWING MAINTENANCE	11.71	4.26	17.01	51.58	5.41	1.20	847.90	48.56	101.40	1
SPURWING WARMBLOOD	13.55	4.28	23.73	53.36	3.33	3.08	848.60	42.07	75.30	1
SPURWING HACK	13.72	3.49	18.30	51.94	4.27	3.55	834.90	41.54	45.80	1
SPURWING SHOW HORSE	15.17	4.76	11.55	46.89	3.32	0.76	825.90	37.54	122.70	1
SPURWING SUPA GROWTH	15.85	3.87	20.52	53.05	3.03	0.86	868.60	35.84	77.70	1
EQUIFEEDS ENDURO	13.53	8.23	16.90	46.70	3.30	0.64	847.90	42.00	154.80	1
EQUIFEEDS TRANQUILO	13.17	9.29	13.21	43.85	3.38	1.31	800.50	43.27	144.00	2
ROMIX PONY	10.09	2.49	23.54	58.00	6.36	2.40	838.30	56.71	90.60	1
ROMIX HACKING CUBE	11.10	3.90	22.92	49.74	6.81	1.12	775.70	51.38	135.20	1
VUMA VALU RED	14.11	5.43	19.74	47.39	4.47	0.86	876.10	40.75	124.10	1
VUMA SUPA COOL	13.18	2.96	19.71	48.03	4.60	0.97	796.70	43.65	110.90	1
SPURWING BROOD MARE	16.25	4.13	18.80	53.54	3.29	1.12	701.00	35.09	139.70	2
ROMIX PONY	10.09	2.49	23.50	58.00	4.29	1.10	700.80	56.67	134.00	3
EQUIFEEDS RACE MEAL	15.53	6.97	31.80	42.87	5.75	1.20	720.60	36.72	143.50	4

Where: CP = crude protein; CHO_{sol} = soluble carbohydrate; (C+H)/L= (cellulose + hemicellulose)/lignin; DEF = degradation efficiency factor; true DEG = true degradability; C_{biodeg}:N = biodegradable carbon to nitrogen ratio; max GP = maximum gas production.

Generalised linear models of the classifying variables produced the following mean values for members of each cluster, identifying which classifying variable was most responsible for the difference between the clusters (Table 7.2). In this analysis, the clustering of the Broodmare and Trankilo ration (Cluster 2), the pony meal (Cluster 3) and the race meal (Cluster 4), can be explained on the basis of the significant classifying variables. It is evident that Cluster 2 has the highest Crude Protein relative to the content of other nutrients. Cluster 3 has the lowest true degradability and soluble carbohydrate content. Cluster 1 has the lowest C_{biodeg}:N and a high CP content.

Table 7.2 Means of classifying variables produced by the generalized linear model (SAS, 2005) for clusters of horse feeds

Nutritive parameter	Clusters					
Classifying variable	1	2	3	4	SE	P
CP	13.64	11.77	16.35	15.53	1.98	0.003**
FAT	4.64	2.89	1.27	2.50	1.88	0.2385
STARCH	19.86	11.70	2.00	1.80	5.00	0.0016**
CHO_{SOL}	50.9	33.29	28.8	37.45	4.58	0.001**
CHL	1.29	4.12	3.64	1.24	1.29	0.1308
DEF	1.41	0.76	1.05	1.78	0.84	0.8112
TRUE DEG	812.6	617.7	485.6	724.7	56.43	0.001**
C_{BIODEG}:N	42.75	48.58	34.66	22.79	6.89	0.046*
MAX GP	112.69	156.9	82.7	114.1	29.51	0.3823

Where: CP = crude protein; CHO_{sol} = soluble carbohydrate; (C+H)/L= (cellulose + hemicellulose)/lignin; DEF = degradation efficiency factor; true DEG = true degradability; C_{biodeg}:N = biodegradable carbon to nitrogen ratio; max GP = maximum gas production.

In an effort to increase the displacement between the conventional feeds in Cluster 1 of the previous analysis, all the conventional horse feeds that were allocated to Cluster 1 (Figure 7.1) were reclustered. The other three clusters from the previous analysis were excluded in an effort to separate the normal horse feeds (Figure 7.2). Three clusters accounted for 88.3% of the variation observed between feeds. The reasons for the clustering are summarised in Table 7.3.

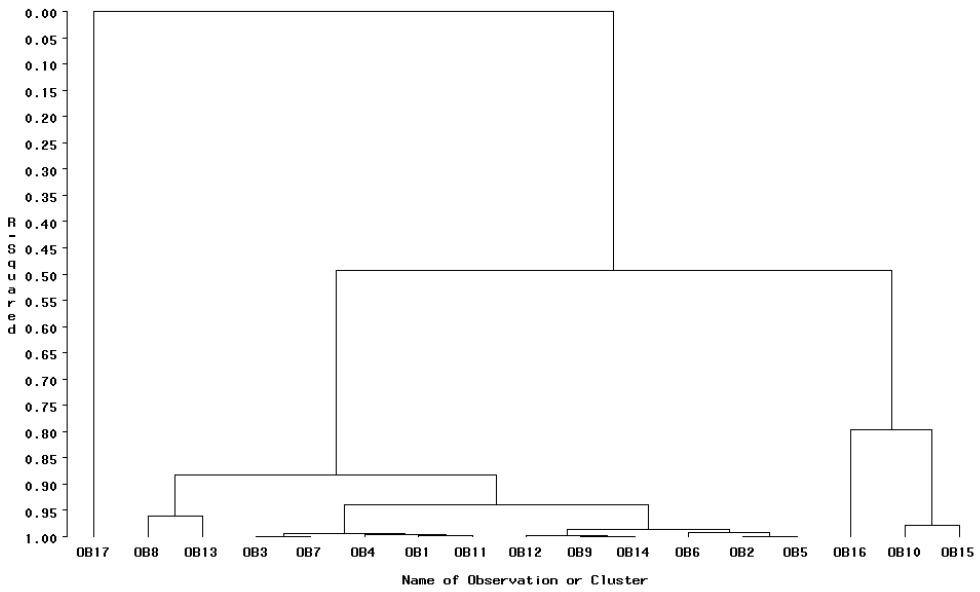

Figure 7.2 hierarchical cluster analysis of concentrate feeds, clustered on the basis of nine classifying variables, accounting for 79.7% of the variation by three clusters.

Table 7.3 Means of classifying variables for three clusters produced by Hierarchical cluster analysis. Three clusters account for 79.7% of the variation between horse feeds. A generalized linear model indicates the significance of each of the classifying variables responsible for the three clusters.

Nutritive parameter	Clusters				
Classifying variable	1	2	3	SE	P
CP	13.60	13.17	15.53	2.04	0.6109
FAT	4.32	5.30	6.97	1.86	0.3381
STARCH	19.26	18.50	31.80	4.0	0.034*
CHO_{SOL}	51.04	51.80	42.87	4.38	0.2134
CHL	4.10	3.65	5.75	1.290	0.394
DEF	1.48	1.18	1.20	0.88	0.839
TRUE DEG	837.75	734.1	720.6	3.57	0.003**
C_{BIODEG}:N	42.68	45.01	36.72	7.11	0.6101
MAX GP	104.19	139.23	143.5	26.63	0.0935*

Where: CP = crude protein; CHO_{sol} = soluble carbohydrate; (C+H)/L= (cellulose + hemicellulose)/lignin; DEF = degradation efficiency factor; true DEG = true degradability; C_{biodeg}:N = biodegradable carbon to nitrogen ratio; max GP = maximum gas production.

It is clear that true degradability, starch and maximum gas production are responsible for the differences between conventional horse rations. These variables are clearly not parameters used in feed formulations. What equine nutritionists are using to discriminate between rations are not apparent in differences between the rations at all.

Discussion

The intention was to be able to discriminate between horse feeds on the basis of their nutritive characteristics, as determined by IVGPT, *in vivo*, glycaemic and chemical determinations. Feeds were segregated into clusters and were verified by a generalised linear model of the prediction of response from the clusters. The nutrient that was driving the segregation into homogenous groups was identified in this way.

It is expected that in horse feeds, there are attributes that are more important in defining the quality of the feed than simply the chemical analysis thereof. The ability of biological assays like the IVGPT, and glycaemic and rate of passage assays, to reflect the fate of ingested feed ingredients is reported here. Two conclusions are important. One, feeds can be segregated into discrete groups, and two, the absence of discrete groups in horse feeds that are instead marketed as being homogenous begs interrogation. It is true that South African feeds are marketed on the basis of a CP

percentage. The irrelevance of this measure of "feed quality" is clear. Horse feeds are then broadly categorised as riding, or maintenance or breeding feeds. This fact was borne out by the simple segregation of feeds on the basis of the NDF, CP, Fat, and NSC (Chapter 2). The author annotated these as the fat, flat, idle and breeding feeds. Equine nutritionists, however, pride themselves on the cunning use of raw materials for some proposed benefit in temperament or performance in the horses (Kline, 2004). Some marketing ploys include: "low grain", "supa cool", "warmblood" and "performance" feeds. However, there is no discernable difference in the nutritional characteristics of these feeds, particularly if one considers only chemical analysis. There is little consideration of the consequence of the inflation of specifications to achieve these 'goals'. Equine nutritionists are quick to assert that feeds are not formulated at least cost, so that no expense is spared in the provision of optimal nutrition, but they fail to elucidate what those goals actually are, and how optimal that nutrition is, which therefore needs to be evaluated (Topliff, 2002)

A critical discussion to pursue is the consequence of the inadequacy in the feeds. Most often this is solved by a volume intake issue. Whatever is missing will be solved by feeding more. There are consequences however.

Table 7.4 summarises the nutritional goals that can be calculated from the literature for the classifying variables in the preceding tables. One could also consider the historic diet of the horse, and see what balance of nutrients should be available for it to eat. Should the ratio of nutrients in the historic program of the horse inform current feeding programs? Horses evolved over millions of years living on grassy plains, and prospered on a diet dominated by the intake of climax ecology grasses such as *Themeda triandra*. Modern diets should reflect the evolved requirements of horses that should be modified to adjust for increased work rates and the modified environmental architecture it finds itself in. It should however not compromise on digestive physiology or nutrient synchrony necessary for optimal physiological and psychological functioning.

Table 7.4 The recommended dietary allowance for classifying variables used to discern between groups of horse feeds, as obtained or calculated from sources in the literature, for a horse at maintenance.

classifying variable	reqt g/d	hay %	Nutrients From hay (g)	Nutrients from conc (g)	Balance of nutrients in conc (%)	Reference
CP	630	6.53	522.4	107.6	5.38	NRC, 2007
FAT	350	0.78	62.4	287.6	14.38	Duren, 2000; NRC, 2007
Starch	1000	2.05	164	836	41.8	Radicke *et al.* , 1991; NRC, 2007
CHOsol	930	12.12	969.6	0	0	Pagan *et al.*, 1998; Nicol *et al.*, 2005
chl		5.12			11.347	CH6; Varloud *et al.,* 2004
def					7.2574	CH6;
true deg					752.92	CH6; Silva and Pimentel, 2010
C_{biodeg}:N					25	CH6; Varloud *et al.,* 2004
max GP					150	CH6; Murray *et al.*, 2006; Abdouli and Attia, 2007

Where: CP = crude protein; CHO_{sol} = soluble carbohydrate; (C+H)/L= (cellulose + hemicellulose)/lignin; DEF = degradation efficiency factor; true DEG = true degradability; C_{biodeg}:N = biodegradable carbon to nitrogen ratio; max GP = maximum gas production.

Assumptions are that: a 500kg horse at maintenance (i.e. idle) is ingesting a roughage:concentrate ratio of 80:20 (Khonke, 1998) and consumes 2% of bodyweight (NRC, 2007). A standard hay diet is used to represent the roughage portion of the feeds, as would commonly occur in stable fed horses, which leaves a balance of the major nutrients to be supplied by the concentrate (on an as fed basis). The DEF, degradability, C_{biodeg}:N, and maxGP are calculated for the concentrate ration only.

The goal is approximating the 100% meeting of these recommendations (Figure 7.3), and relative to Table 7.4, the inadequacy of the outliers in Table 7.2 can be visualised in Figure 7.4. This is the equine equivalent to the RDA (Recommended daily allowance) in humans.

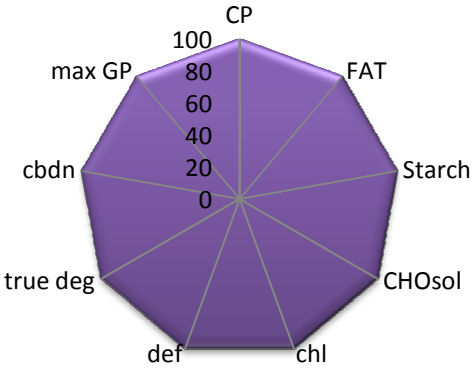

Figure 7.3 Radar chart illustrating the 100% matching of the dietary content of the feed and the dietary recommendation as calculated in Tables 7.4 and 7.5.

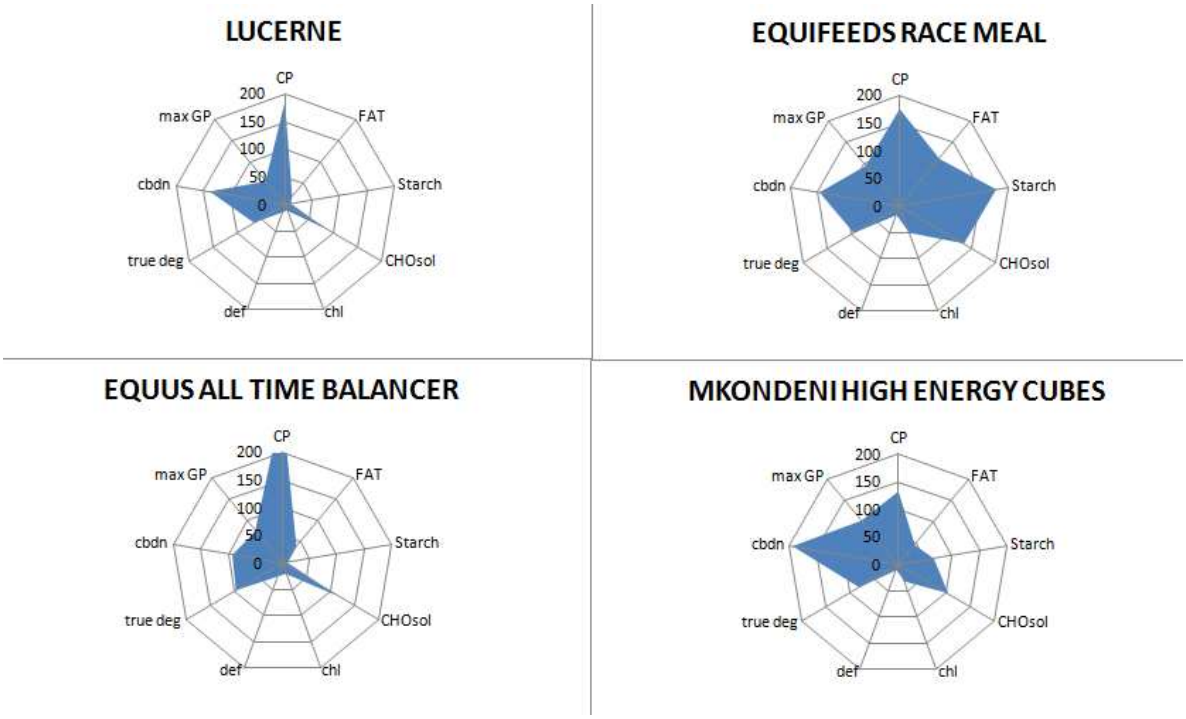

Figure 7.4 Radar chart of horse rations in Clusters2, 3 and 4 (Figure 7.1) and race meal, illustrating the inadequacy of the rations in meeting the dietary recommendation as calculated in Tables 7.4.

As indicated already, the failure of the rations to meet recommendations in terms of C_{biodeg}:N, CP, starch, CHO_{sol} and true degradability. The radar charts illustrate effectively that nutritional goals are not being met in a range of outliers, but are they being met in the conventional horse riding feeds?

If asked whether the classifying variables in the cluster analysis actually represent nutritional goals, we are reminded that they are identified in the literature as being important concentrations of nutrients, and as such constitute nutritional goals. One is hoping to be able to achieve consistent performance on a ration, and generate identifiers in the ration that provide evidence based nutrition (Ralston, 2007) principles for the behaviour of the horse on that ration. Can these identifiers be used to group feeds that the equine feed companies say are different? The answer is that *IF* feeds were indeed formulated to include some of these specifications, they would be able to group grain free vs. performance vs. idle feed, and be able to indicate how the horses would perform on these feeds.

It is necessary to recalculate Table 7.4 to reflect a change in the roughage:concentrate ratio for working horses, and the increment in requirement for work. Usually horses are fed in excess of 6kg of concentrate (i.e., an amount in excess of 50% concentrate), although the recommendations

(Khonke, 1998) state that this proportion should be lower. Moderate exercise in this example will incur a 50:50 roughage concentrate ratio, in a 500kg horse, with the assumptions as before. The recommendations to be provided by the ration then become 8.83%, 6.22%, 17.95% and 32% for CP, fat, starch and CHO_{sol}, respectively. The adequacy of current riding feeds of meeting these specifications in working horses can then be evaluated.

Consider the "grain free' feeds that are supposed to produce less volatile, "tranquil" behaviour in horses. Tryptophan is alleged to moderate behaviour but without scientific support (Grimmett and Sillence, 2005; Noble *et al*., 2008). There is no doubt that feeding affects temperament (Zietler-Feicht *et al.*, 2001; Hothersall and Nicol, 2009). Nutrition companies believe that it is the reduction in "grains" that will moderate temperament in the horse, but again, offer no substantive proof that their feeds address this claim. The test feeds in this report (Figure 7.5) vary in soluble CHO as well as starch.

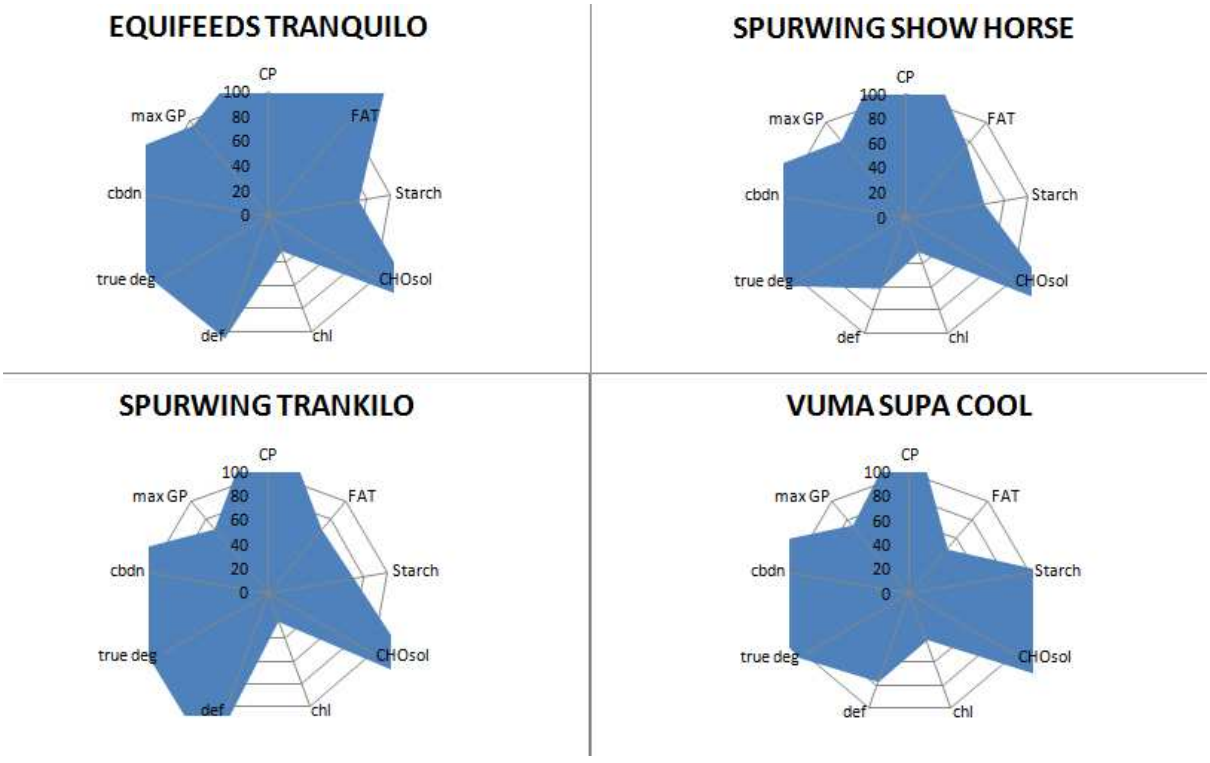

Figure 7.5 Horse feeds that are alleged to reduce excitable behaviour in horses by replacing grains with lipid sources, demonstrate an inconsistent change in CHO_{sol}, starch and fat.

Geor (2007) identifies that not all soluble CHO (NFC) is starch, and hence the use of both starch and CHO_{sol} as classifying variables in ration delineation. In addition, the CHO-F_s that includes the cellulose and hemicelluloses as proposed by Hoffman (2003), represents a source of energy from

fermentation of CHO. Temperament however, is related largely to carbohydrate fermentation in the hindgut. Starch is linked to acidosis and colic more than it is to temperament, as the precaecal digestion of it is supposed to be higher (Geor, 2007) than other CHO. Geor (2007) emphasises the need to identify the fermentable portion of the NSC, to get an idea of the CHO escaping enzymatic digestion. NSC is a poor predictor of glycaemic response, which is a good reason to have both starch and NSC in our classifying variables. Pagan (1997) correlated bad behaviour in the horse more to glycaemic response than to fermentation, with rhythmicity of glycaemia a function of feeding regimes (Piccione *et al*., 2008). Bad behaviour is linked to fermentation in the hindgut causing caecal distension (Cuddeford, 2000). Rowe *et al*. (1995) and Willard *et al.* (1977) relate behaviour to the reduction in hindgut pH or acidosis, as a result of the feeding of grains and pH is significantly correlated to starch in the hindgut (Medina *et al*., 2002). If it is necessary to advertise a feed as having an effect on temperament, therefore, one must include some measure of starch, as well as of fermentable fibre (CL) and CHO_{sol} in order to represent the relationship to behaviour. The distinction between the hydrolysable and the rapidly and slowly fermentable CHO fractions is available from GP data and can be interrogated more fully from these parameters. But doubtless, will be a more accurate predictor of behaviour than current ration parameters.

None of the rations actually fulfils the anticipated obligation for (cellulose+hemicellulose)/lignin. This calculation by Richard (1996) should elucidate the proportion of useable structural carbohydrate that the microbes have the capacity to release for utilization by the horse. The horse feeds presently are all oversubscribed in terms of the provision of soluble carbohydrate, some of which consequently ends up in the hindgut for fermentation. There is a lot of pentose sugar that is ignored as a substrate, but it is available through the action of the microbes, although longer lag times may be necessary (Sunvold *et al*., 1995). Using the CHL ratio as a parameter in feed formulation will mean that soluble CHO can be reduced while CHL is increased through formulation to provide cellulose for the horse to ferment for energy. Some of the hack feeds, which are quite popular in fact, have 40% of the RDA of CHL, and a concomitantly high gas production. Three of the four hack feeds below act as expected in terms of the max GP, as these feeds always have a high percentage of maize and fermentable substrate in them. Non-starch CHO sources minimise the risk of digestive disturbances, and cause beneficial alterations in energy and glucose metabolism in the exercising horse (Lindberg, 2004).

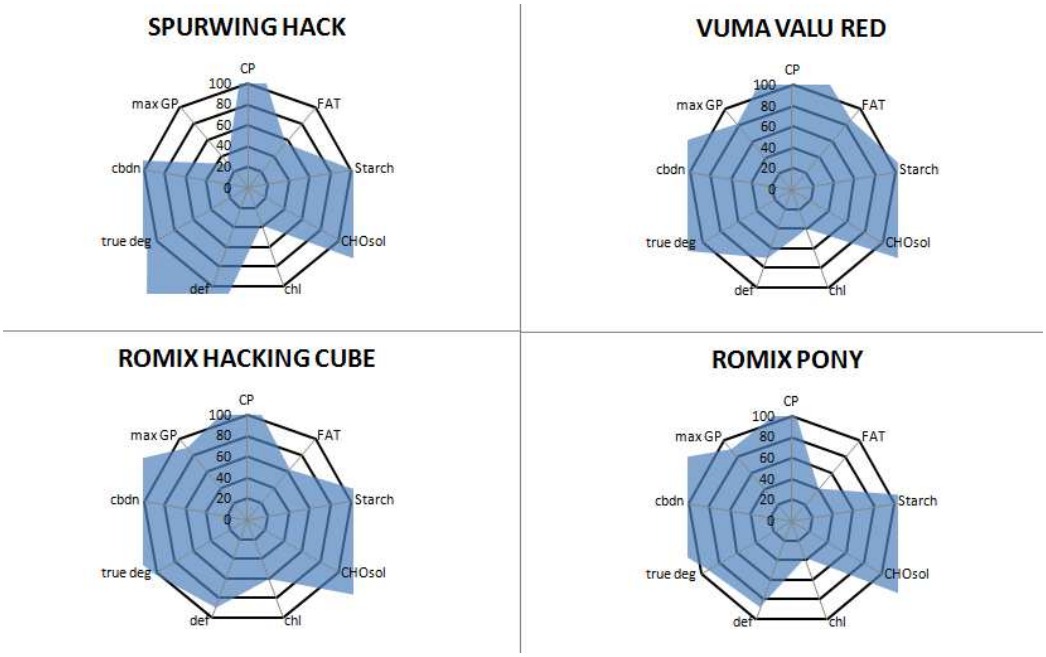

Figure 7.6 Hack feeds in general offer evidence of high gas production, as well as higher (cellulose+hemicellulose)/Lignin ratios.

The way horse owners present and inform their feed choices can be improved (Honore and Uhlinger, 1994). Deterministic models are preferred for the only branch of equine nutrition that has received any modelling attention, rates of passage (Cuddeford, 1999). Limited empirical models exist for growth rates (Staniar *et al*., 2004). The NRC (1989) provided requirements for nutrients that were related to energy requirement, which was empirically determined. Fortunately the new NRC requirements for horses addresses this, but only partially, by providing three energy levels to choose from, to which the requirement of other nutrients is related (NRC, 2007). It has been determined that protein requirements do not increase in the same way as do energy requirements in the working horse, but the NRC (2007) did it, rightly or wrongly (van Saun, 2006). But still, dietary composition will be dependent on the level of intake achievable under a given feeding management system (van Saun, 2006). Nutrition modelling in poultry has seen major advances in the last thirty years, but one of the most important of these was making feed intake an output from, as opposed to an input to, the growth model (Gous, 2007).

Van Saun (2006), himself a member of the committee responsible for new NRC (2007) for horses, acknowledges that there is a need to move to a level of predicting available nutrients from feeds, and data are needed to incorporate microbial fermentation into prediction of nutrient provision for consumed feeds in the horse. The problem with the prediction of response to horse rations is that the incorrect paradigm has persisted, even until now. Ration inadequacies are corrected by volume

intake, forcing the ingestion of high levels of concentrate feeds, with concomitant increase in metabolic disorders in the horse (Kalck, 2009). Feed intakes drive the provision of nutrients. Monogastric research notwithstanding, voluntary feed intake should be a function of the first limiting nutrient, and if it is not, in the horse, then this should be determined. Feed intake should be the output, and not the input of the system. The use of classifying variables such as those described above is a start in the mechanistic approach to the modelling of requirements in the horse. Nutritional principles should govern the provision of nutrients to the horses, and a paradigm shift towards that of requirement →predicting intake →predicting performance, is required. The strategic use of nutritional determinants that represent partitioning and fermentative capacity has been used in this study to demonstrate that horse feed formulators in South Africa are subscribing to the incorrect paradigm in their formulation. In fact, they are firing fifteen darts at a dart board and hoping one will hit the board, not the bullseye. Refining our characterisation of equine diets, with respect and cognisance of our colleagues in monogastric and ruminant nutrition, will allow us to advance equine nutrition science, towards the modelling of performance parameters that we can define in the equine (Bergero and Valle, 2007). Harris and Bishop (2007) identify that there has been very little change in the absolute requirements for horses, even in the new NRC, such that very little research has targeted core nutritional requirements. Where these nutritional constants (Vermorel *et al*., 1997b) can be incorporated into, for example, effective energy models (Emmans, 1994), a more precise effect of thermic load and utilization can be determined to improve specifications and raw material selections in feed formulation. Improved nutrient profiling of horse feeds will reposition our companion animals to the scope of the performance animal, where our expectation of their function in our lives will match their capability through optimum nutrition.

Conclusions

Hierarchical cluster analysis was used to produce clusters of horse feeds that could meaningfully and consistently classify feeds into homogenous groups of nutritive characteristics. Generalised linear models of the classifying variables identified which variables were responsible for the clusters, as a validation for the delineation into clusters. Radar charts were used to model the adequacy of the rations against a calculated goal, which identified that CHL, CHO_{sol} and starch as important in defining goals for horse feeds offered on the market, but which were undiscernable into separate clusters. Given that nutritional modelling in equines is in its comparative infancy, an evidence based, mechanistic approach was considered for a paradigm shift in the provision of effective nutrition for equines.

OVERVIEW AND FUTURE RESEARCH DIRECTIVES

"There is another group of signs and sounds which have been developed by horses through their contact with man. Many of these deal with feeding. The first and possibly oldest of these is easily understood:

1. **'Where is my bloody breakfast?'** ***This is shown in a multitude of ways, from the whicker of welcome to a bang on the food bin. Each horse owner will know how his horse does it. The sub-messages are several.***
2. **'I want water'** ***is often expressed by knocking the water bowl about and whinnying***
3. **'I want hay'** ***may be said by walking to the hay rack looking disgusted. When he has been fed***
4. ***The horse will say '*****thank you'*****, usually by a whicker of welcome or saying '*****I love you*** ***and showing affection'. He will of course indicate whether or not he likes his feed and will say***
5. **'This is nice'** ***by eating his food greedily with bits falling out of his mouth; or if you are feeding him tit-bits, he will say***
6. **'Give me some more'***, by whickering and nuzzling at your pockets and reminding you he is still there. If he does not like what you give him he will say*
7. **'That is horrible'** ***by spitting it out and wrinkling his lips and making ugly faces."***

(Henry Blake, 1975)

The goals of this thesis, as outlined in the Introduction, included the following:

1. An integrated approach to the effective representation of the nutrient quality in horse feeds;
2. The evaluation and combination of several effective means of nutrient evaluation;
3. The development of a platform for the scientific delineation of horse feeds in South Africa;
4. To create a scientific framework for the evaluation of horse feeds so that they can be accurately presented by equine nutritionists and feed representatives selling horse feed products;
5. To provide a scientific baseline from which horse feed companies can develop horse ration formulations that:
    a. Optimize nutrition for the health and performance of horses in different states of activity;
    b. Concurrently, reduce the costs of feed formulations, and reduce competition for feed resources with other agricultural activities. Horses have evolved to utilize primarily low value grassland plants, with a small contribution by broadleafed plants such as lucerne.

6. To investigate biological assays into the usefulness of common feed ingredients, and nutrient synchrony, based on the digestive capabilities and needs of both the horse, and the consortia of hindgut fermentative bacteria.
7. To develop an integration of the information captured in the above research, as an initial step towards the development of a more rational approach to the formulation of horse feeds.

The literature review dealt with digestive physiology in the horse, including nutrient supply and quality, and approaches to ration formulation. As a hindgut fermenter, many of the companion animal perspectives have been re-orientated to the horse as a production animal. Historically, a problem with much of the equine literature was that it was based upon data generated by ruminant studies. Together with advances in ruminant and monogastric research, equine research has progressed, although paradigms are difficult to shift. The new NRC (2007) recommendations made new recommendations in an attempt to do this. Formerly, all feed requirements of the horse were determined relative to its energy requirements (NRC, 1989). Prior to 2007, most of the literature on energy evaluation models used ruminant inputs in the calculation of horse requirements, meaning also that the derivatives of requirements in other nutrients, based on the energy requirements were therefore incorrect. The distinction of the new NRC (2007) document is that their recommendations on the requirements of horses are now moving towards parameters per kilogram body weight, and therefore report more accurately the requirements of the horse. The change in their recommendations is based on the body of research of the interim 17 years.

Performance in the horse is difficult to measure. In a broiler chicken, the responses in feed intake, body weight gain, feed conversion efficiency and days to 1.8kg slaughter weight are tangible, achievable goals, and define “performance”. Strictly by definition, it is the process or manner of functioning or operating. In a racehorse, the time taken to cover a certain distance would be the most useful parameter to measure. Similarly, the accuracy of performing set movements in a dressage test will earn a percentage score, or the number of faults in a show jumping round does produce a numerical output for “performance”. Endurance riders quantify performance effectively. Most of the time, there is a control treatment, and then some ergogenic aid is imposed to produce an increase or decrease in the parameter relative to the control. In the same way, output variables in the experimentation produce empirical data that suggest that one treatment was superior to another in respect of certain key response variables. Performance in the horse should not be

subjective, and for example, behaviour should be a subset of the performance parameters under evaluation in response to a particular feeding and management regime, as should body condition score. For horses in different disciplines, appropriate descriptions of performance should be defined. Nutrient levels for different disciplines of horse riding that correlate to measurable performance parameters should be enumerated.

Defining the level of fat intake relative to the activity of the enzymatic hydrolysis in the primary digestive zone in the small intestine, and quantifying the decrease in cellulolytic activity per unit increase in fat percentage is a step in the right direction for nutrition research. The elusive holy grail for nutrition research is the prediction of voluntary feed intake, and with simulation modelling this has proved successful for poultry and pigs. Because there are a myriad of factors affecting the microbial populations and hence the nutrition they contribute, that single factor that has remained elusive. It is the question of whether the horse eats to the level of the first limiting nutrient, independently of any social or psychological motivation for it to do such. The ingestive behaviour of feral horses would suggest that diets were complete before concentrates were added to their daily intakes. Horses can compete with grazing ruminants because the horse can use non-structural carbohydrates before fermentation and reduced the loss of carbon from acetogenesis. Incremental nutrition for exercise should not suppose that an increase in the level of all nutrients is necessary, as has been proposed in the NRC for many years. In fact, the contrary is probably true. And so the notion of nutrient synchrony is an essential field.

Research should continue with empirical and mechanistic modelling of responses to nutrients and their combinations in the rations, such that requirements can be determined, in order that consequence can be established for the overprovision of nutrients. Observations of horses and analysis of comments from horse owners over thirty years indicates that there is a fundamental paradigm that must shift in horse feeds (Vandergrift, 2002). In South Africa, untrained sales staff sell horse feed to horse owners that rely on the sales staff to inform their purchase.

It was the intention of this dissertation to take a sample of the over 100 horse feeds that are on the market and objectively evaluate their true nutritional value. In this country, companies have produced idle horse feeds (10% CP), riding feeds (12% CP), performance feeds (13% CP), breeding feeds (14% CP) and race feeds (16% CP), presupposing that these levels of protein are necessary in the feeds. The first observation is that there is great variability and a low correlation between the advertised and the actual analysed CP percentage. The nutrients that should be supplied by the feeds include protein, fat, fibre and carbohydrate. These nutrients were submitted to a hierarchical

cluster analysis technique in Chapter Two, and the feed clusters that were produced were titled fat, riding, idle and breeding feeds, accounting for 87% of the variation between the feeds. Cluster Four (fat) was characterized by higher fat %, Cluster Three by lower CP (idle), and Cluster One by the lower NDF (breeding) with the Cluster Two (riding) feeds slightly higher across the board. The fact that so many feeds can be reduced to these specifications reflects poorly on the smorgasbord of feeds that are available to the consumer to choose from.

IVGPT was used to gauge the fermentative capacity of the feeds. The four clusters did not explain the response of the feed in the horse, and the non-significant responses in gas production parameters means that the clusters fail to explain how the horse would react to those feeds. The final pH, apparent degradability and microbial mass were different between the clusters, so it was concluded that IVGPT produced useful results for evaluation of equine feeds using equine faecal inoculum. Using equine faecal inoculum is better than using rumen fluid, and the feeding regime of the inoulum donor should be strictly governed in this instance. Proximate analysis does not inform the best clustering of feeds with which to obtain parameters of cause and effect. Hence, IVGPT should be used to augment the techniques used for feed evaluation to permit more appropriate feed clustering techniques.

Because IVGPT was useful, the array of feeds was tested for differences between feeds using this technique. Much is made in South Africa of the formulation of “cool” feeds for “hot horses”. The effects of episodic feeding are absent or greatly attenuated when a normal foraging pattern is established in the horse. The addition of concentrates, even *in vitro*, decreases pH, and increases the degradable portion of feed over hay only. Gas production kinetics of the feeds produced different parameters in respect of slow and rapidly fermentable components, as determined by the fitting of the Campos *et al.* (2004) model to the responses of the feeds over the control. Apparent and true digestibilities were negatively correlated to fibre content. Many of the attributes of each feed could be inferred from gas production parameters, and IVGPT could therefore be used to discern between feed groups more informatively than CP percentage.

The appropriateness of the glycaemic response in revealing anything more than a post-prandial circadian rhythmicity to carbohydrate ingestion, induced by meal-feeding a trickle feeder, is still under debate. The glycaemic response in horses provides some value in informing the time of feeding relative to competition, and behaviour, and the effect of roughage: concentrate ratios. Many horse feeds use molasses as a binder, to reduce dustiness and to increase palatability, and

insulin resistance is just one of the metabolic disorders that have become a problem as a result of excessive sugar levels in many feeds (Frank, 2009).

In Chapter Four, blood glucose parameters of feeds (mean, peak, slope and time to peak and area under the curve) in each of the original chemical analysis clusters were compared by analysis of variance and regression with covariates. The addition of concentrate to the diets of the horses at 20% and 40% of the diet produced responses over the blood glucose of 4mmol.L^{-1} of horses fed hay only. Peak and slope to peak blood glucose in feeds high in fat were lower than feeds containing more carbohydrate. Maintenance feeds (higher in fibre and lower in nutrient density) produced a peak response to feeding level of up to 5.8mmol.L^{-1}. A high starch riding feed produces a faster rise to a higher peak (8mmol.L^{-1}) at higher feeding levels. The glycaemic response was usefully correlated to starch intake and to the components of the feed that have an impact of equine performance.

The glycaemic response was used initially to evaluate the extent of pre-caecal digestion. As a tool to differentiate between feeds, and in combination with fermentation and *in vivo* data, more complete feed characterisation can be achieved. Compartmentalising digesta flow in faecal marker excretion data is more appropriate than time-independent representations of marker kinetics such as those developed by Grovum and Williams (1973) (Lindsay, 2005). While the topic of compartmental modelling in the equine digestive tract has received recent attention, the method of Thielemans (1978) in Groachet *et al.* (2009) provides an effective means of discriminating between characteristics of feeds. Foregut transit time and the time taken for the total marker to be excreted differed between feeds, as did residence time in the GIT. The capacity of the feed to reside in the caecum, with the concomitant characterisation of feeds in terms of their fermentative capacity (Chapter Three), means that compartmental flow parameters may well offer an explanation for the behaviour of feed ingredients in sections of the GIT that can then be correlated to behavioural manifestations in the horse (Ellis and Hill, 2005).

Horse owners currently buy horse feed on the basis of crude protein content, and tend to feed protein to horses at levels far in excess of their requirements. No consequence of this appears to have been considered, nor are recommendations made for the equine nutritionist to optimize provision of protein to the horse.

The frequencies of feeding, as well as the meal size, influence the pre-caecal digestion of feed, with concomitant changes to the substrate available to microbes for fermentation in the hindgut.

This emphasizes the role of nutrient synchrony in the optimal formulation of rations for sport horses, and the prediction of the performance of horses on those rations. Biodegradable carbon to nitrogen ratios in horse feeds are inflated over that required for optimal microbial health, leading to undesirable levels of volatile nitrogenous compounds that can damage the lining of the GIT, sometimes with long term consequence. CHO_{sol}:N and C_{biodeg}:N ratios more accurately inform the changes to degradation efficiency and nutrient partitioning in the hindgut. Microbial efficiency, in particular, has a strong negative correlation to the ratio of C_{biodeg} to N. It is not sufficient to consider the provision of nutrients to the horse simply as reflected by primary analysis of nutrient contents of the feeds because this disregards the evolution of the horse as a hindgut fermenter of grass forages. In particular, the requirements of the caecal microbial population need to be considered as paramount to the health of the horse. Nutrient synchrony and the fate of ingested ingredients, ameliorated by feeding management, have to be considered. Nutrient synchrony is important in two respects: that of the ratio between nutrients offered to the horse (for example C:N), but also with respect to the degree of synchrony between what substrates are provided for the digestion that occurs in the fore-and hindguts (i.e., feeding the microbes in the hindgut; digestive synchrony).

One really wants to be able to profile one's horse, and select a suitable ration, then match the performance parameters of the horse to the nutrition that should be provided to achieve that. Nutritive value of horse feeds depends on the chemical composition, intake, digestibility and efficiency with which the nutrients are released, both in the foregut, and using hindgut fermentation. These factors can be incorporated into attributes of the feeds and the fate of ingested ingredients can be used to model the extent to which feeds actually meet requirements of the animal and to what extent nutrient synchrony is adhered to in the optimization of microbial and fermentative capacity.

Profiling the horse feeds in terms *of in vitro*, *in vivo*, glycaemic and chemical attributes elucidated several characteristics of the horse feeds. Chemical analysis will not reveal the horse's response to the feed. Sufficient other descriptors of the rations, particularly those that directly relate a capacity of the feed to induce, for example, post prandial hypoglycaemia or increase degradability post-fermentation, will differentiate one feed from another. For example, a "grain-free feed", should have a higher fat content, and a lower maximum gas production with lower NSC. For an idle horse feed, the CHL should be higher, and the fermentation produced should be higher. Feeds with incorrect C:N ratios should depress fermentation and produce more ammonia, while VFA ratios change with carbohydrates in the feed. All of these responses in the horse to the feed are what determine its nutritive value, and these are the basis of feed categories. The objective of

Chapter Seven was to integrate the most significant of these classifying variables across all of the feeds. Cluster analysis was not able to discern between any of the riding feeds on the market. This indicates that the approximately 80 feeds that are provided for riding horses, that are all marketed to be different, are not different in respect of their digestion in the horse.

An evidence-based, mechanistic approach is required as the basis for a paradigm shift in the provision of effective nutrition for equines. Parameters in ration formulation still need to be brought in line with mechanistic modelling processes such as have been developed for poultry. An obvious distinction is that monogastrics respond directly to what they eat, whereas the hindgut fermenter responds to the two-stage digestion of what it eats. The value of biological assays in the determination of nutritive quality remains important. But the approach to ration formulation needs to change. As shown in this thesis, ration characteristics should inform feed groups (clusters) appropriate for horses living at different activity levels. The nutritional paradigm which Gous (2007) developed for poultry production, where food levels are an output for models, and not an input, needs to be adopted in horse nutrition.

This work has shown that there are many feeds on the market that are not distinctive from each other, and for which there is no evidence that they result in changes in performance, temperament or body weight. In addition, these feeds do not relate to any defined nutritional requirements or goals. Several classifying variables have been proposed which can be used as linear constraints in feed formulation to prevent nutrient asynchrony, and to optimize both equine digestion and hindgut microbial fermentation, and to provide the horse with a ration that is specifically designed for a hindgut fermenter, which will enhance equine performance.

In addition to proposing a paradigm shift in our approach to ration formulation, the work highlights several areas of research that would need to be pursued in order to accurately model equine feeds. Emmans, Fisher and Gous (at the University of KwaZulu-Natal) developed EFG Software and Winfeed (2006). As a start, this feed formulation software could be used to develop a database for equine ration formulations, including the new variables. Test feeds could be formulated and evaluated *in vitro* and *in vivo*, using the new array of variables. Aspects of the requirements of the horse, such as the effect of fat in the diet on hindgut fermentation could be tested *in vitro*. A larger range of feeds could be evaluated in this respect so that the industry can be invited to review the research as a basis to inform horse nutritionists seeking to improve their prediction of response on their own feeds.

The ability of the horse to increase its capacity to use pentose sugars on reduced soluble carbohydrate diets must be evaluated, on the basis that more roughage and less “high quality” feeds such as grain and protein should actually cause a better response in the horse.

Riding and competition horses respond to the effects of each feed in their GIT. Equine nutritionists need to focus on the fate and proportion and synchrony between feed ingredients. Current ration formulations fail to provide significant differences in respect of key parameters.

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