A retrospective study of *in situ* and *in vitro* considerations in horse nutrition management

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BSc Agric

Submitted in partial fulfilment of the requirements of the degree of Master of Science in Agriculture

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2017

Abstract

The current changes occurring in equine feeding management to meet performance demands, do not take into consideration the repercussions on the digestive tract. The aim of this investigation was to determine the linkage between nutrient intake, gastro-intestinal pH and gut mucosa, the impact on digestibility with changes in nutrient intake, age and mucosal damage and whether the current IVGPT protocol is an accurate reflection of *in vivo* digestibility. *Post mortem* work was carried out on samples of 27 sport and leisure horses shot for mechanical failure and financial reasons for an *in silico* analysis of nutrient intake and digestibility. A relationship was found between stomach ulceration and gut pH. There is no correlation between ulceration and digestibility but rather between nutrient density and digestibility. This shows that by increasing the concentrate portion of the feed may improve performance but has health impacts on the horse. IVGPT experiments were also conducted with faecal inoculum of race and idle horses over 3 buffer pH levels using maize and Lucerne as substrates. The results showed the significance ($P<0.001$) of buffer pH levels across treatments which indicates that fermentation protocols in equine IVGPT need to adjust buffer pH from 7.2 to 6.5.

Candidate's Declaration

I, Catherine Lorna Wiid, 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. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then their words have been re-written, but the general information attributed to them has been referenced. Where their exact words have been used, then their writing has been placed inside quotation marks and referenced. This thesis does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the thesis and in the Reference section.

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CL Wiid

Supervisor's Declaration

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

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Dr NC Tyler

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

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Dr MB Young

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ACKNOWLEDGEMENTS

I would like to acknowledge the following people:

Dr Marion Young for your guidance and the wealth of knowledge you have imparted.

Dr Nicola Tyler for helping me climb the final hurdle.

Debbie Davies for always going the extra mile, helping me in the lab but most importantly for opening up your office and heart.

The tea clan, you know who you are, for all the good laughs.

My parents for all their love, encouragement and support over the past years. You have sacrificed so much for me to get to where I am today. I am proud to call you Mom and Dad.

My husband, Ben, you have been amazing. You have supported, encouraged, pushed and done whatever was needed to help me achieve this “M”. Your love and encouragement knows no bounds.

My Heavenly Dad, for allowing me to achieve the desires of my heart.

GENERAL INTRODUCTION

The reasons why and how horses are kept has changed. In the past century horses were used mainly as work animals and received by-products of millings and sweepings as their form of supplementation (Harris, 1999). Concentrate feed in this study is defined as bucket feed containing high levels of rapidly fermentable substrate and low levels of roughage. This century horses are seen more as recreational and performance animals with war, traction and agricultural uses being displaced (Harris, 1999). Performance horses require an increase in nutrient intake, as nutritional demands now exceed the provision of nutrients by grazing and forage based diets (Harris & Bishop, 2007). The change in feeding management to a more concentrate-rich diet has been linked to laminitis (Longland, 2007), ulcers (de Fombelle *et al.*, 2001) and other metabolic diseases (Kalck, 2009).

Studies have investigated the pH changes within the caecum of the horse fed a high carbohydrate diet (Willard *et al.*, 1977; Medina *et al.*, 2002). In this study a decrease in the pH in sections of the gastrointestinal tract (GIT) is anticipated with the increase in concentrate bucket feed proportions. This is expected to occur in the caecum because of the production of volatile fatty acids (VFA's) (Hintz *et al.*, 1971; Willard *et al.*,1977). The departure from an optimal pH along the GIT has not been studied concurrently with the changes that have been observed in the feeding strategies of the horse. The change in pH would certainly have an influence on the mucosa of the digestive tract, which could result in serious nutritionally related disorders. A horse is a trickle feeder so feeding large quantities of a nutrient dense feed at distinct meal times could have negative consequences on the health, behaviour and performance of the animal. It is known that a large amount of fermentable substrate is correlated to ulceration (Geor and Harris, 2007). The relationship between nutrient density, incidence and severity of ulceration in the foregut needs further research.

The digestibility of nutrients is important for optimal health and performance of the horse. There are many factors that have an impact on digestion. However, there is limited information available on how digestion is influenced in the different regions of the GIT, and how digestion in one region affects digestion in the next region (Julliand *et al.*, 2008). There is an over provision of nutrient-dense feed to performance horses. Race horses are receiving 80% of their diet as concentrate and a mere 20% as roughage (Hackland, 2007). Digestibility of nutrients in the small intestine is negatively affected when as little as 1g/kg body weight (BW) bucket concentrate is fed. An oversupply of bucket concentrate can lead to ulceration (Vasistas *et al.*, 1999a).

In vivo and *in vitro* digestibility studies are routinely performed in ruminant research. *In vitro* studies are more common than *in vivo* studies in equine research due to serious economical and health implications, such as colic, swelling and leakage associated with the cannulation required to perform *in vivo* studies (Lopes *et al.,* 2010). *In vitro* determinations of digestibility are also more economical, ethical, easier and quicker to use, although they may not fully replicate *in vivo* conditions. Reviews on the *in vitro* gas production technique (IVGPT) (Rymer, 1999; Dhanoe *et al*., 2000; Ouda, 2007) and previous work on horses (Lowman *et al*., 1996; Murray *et al*., 2003; Hussein *et al*., 2004; Murray *et al*., 2014), have determined that the high starch diets that horses receive have a negative impact on the GIT. The following are affected; microbial infrastructure, substrates that can be used effectively, products of digestion (i.e. VFA profiles) and the environment that is produced in the caecum (Willard *et al.*, 1977; Medina *et al*., 2002).

The departure from an optimal pH of 7.2 has not been studied in the GIT concurrently with the changes that have been observed in the feeding strategies of the horse. A decrease in the pH in sections of the GIT is anticipated with the increase in concentrate feed proportions. It is therefore important that the standard procedure that is being used in IVGPT be re-examined to determine if the digestive system of the horse is still being replicated correctly *in vitro*, or if alterations need to be considered for more accurate predictions of how to feed horses correctly in the 21st century.

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

To determine how pH along the digestive tract changes with concentrate feeding and the probability of ulceration and discolouration mucosa with nutrient density, age, breed and sex.

1) To determine if nutrient digestibility is affected by feeding level, age, ulceration or discolouration of the mucosa.
2) To determine the optimal buffer pH to be used in *in vitro* studies for accurate predictions of digestion in the horse's GIT.

This thesis aims to provide a better understanding of the impact that an overprovision of nutrients has on the GIT, whether directly or indirectly related to, and more importantly to relate the overprovision of nutrients to gastrointestinal arrangement and function in the hindgut fermenter.

CHAPTER 1

LITERATURE REVIEW

The horse is by nature a grazing animal that requires large amounts of fibre within the diet for correct gut function (Ellis & Hill, 2005). The important fact of a horse being a hindgut fermenter that requires large amounts of roughage is often overlooked when feeding management is considered. This is displayed in racehorse diets which comprise of up to 80% bucket concentrate feed instead of high quality roughage (Hackland, 2007). It is important that an understanding is developed regarding the impact that feeding management has on the digestive tract and how this correlates to performance.

In the past century horses were used mainly as work animals in war, agriculture and traction. Today they are seen more as recreational and performance animals with higher nutritional requirements. This has led to a concomitant change in production systems and feeding management. As hindgut fermenters, the main dietary requirement is fibrous plant material, however large amounts of concentrate feeds are usually incorporated into the diet to meet increased energy demands, which can result in nutrient requirements or the digestive capacity of the GIT being exceeded. This has a negative impact on the health and performance of the horse. Nutrient availability, feed and nutritional insults all need to be taken into consideration to optimize feeding management.

1 DETERMINATION OF NUTRIENT AVAILABILITY

1.1 In vivo and in vitro methods

Digestibility is the disappearance of nutrients in the digestive tract, as nutrients have been absorbed (Dryden, 2008). There are numerous factors that have an impact on the digestibility. There are animal factors such as species, breed, age, exercise and health as well as feed factors which can be comprised of the effect of feed processing, ration composition, chemical composition and level of feed intake. Digestibility is the end result of retention time and the degradability characteristics of an ingredient (Forbes, 1996). Digestibility is most commonly expressed as a percentage or coefficient. There are two types of digestibility coefficients, true and apparent. The apparent digestibility (AD) is the most commonly used due to its simplicity. However, it does not take into account the endogenous substances such as metabolic waste lost in the faeces. *In vivo* methods for the measurement of digestibility of nutrients are the most ideal. The *in vivo* technique is considered a superior method due to the digestibility being based on a live healthy animal, but due to the cost, skill, animal welfare and ethics involved, this method is used less frequently.

There are herbivore studies, including equine studies, that have used the *in sacco* technique for determination of digestibility (Bush *et al.*, 2001; de Fombelle *et al.*, 2004). *In sacco* digestibility and the mobile bag techniques have been used in horses (Moore-Colyer et al., 1997; Mc Lean *et al*., 1999; Bush *et al.*, 2001; de Fombelle *et al.*, 2001; de Fombelle *et al.*, 2004; Hyslop, 2006; Rosenfeld and Austbo., 2009; Silva *et al.*, 2009). The shortcoming of the *in sacco* technique is that it only takes into consideration the digestibility within the rumen of cattle or the caecum or ileum of horses, and it causes discomfort. Caecal cannulas do not separate the caecum and colon so a true reflection for each section cannot be obtained. The process of fistulation/cannulation is expensive and can lead to health impacts such as colic and anorexia as well as swelling, leakage rubbing and biting (de Fombelle *et al.*, 2004; Lopes *et al.*, 2010). This method also limits the amount of samples that can be digested at one time, is potentially hazardous and allows oxygen into the anaerobic environment when samples are collected

The *in vivo* method that is commonly used is the faecal collection method which gives a true result of the total tract AD as it takes into consideration the whole GIT (Miralgia *et al.*, 1999). This method involves feed intake and the faecal output over a 20-day period. There is a 14-day adaptation period and a six-day faecal collection period. All day-to-day occurrences with the horses need to be accounted for to ensure accurate results. The horses can either be stalled separately or in a group, if they are fitted with faecal harnesses for faecal collection (Crozier *et al.*, 1997). The feed intake is monitored and refused feed and faecal collection are stored either in a dried state or frozen to avoid changes in nutritional composition and mould, prior to analysis (Bush *et al.*, 2001). The AD can then be calculated using the following formula (Holland *et al.*, 1998; Pereitti *et al.*, 2006).

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

Crude protein (CP), crude fibre (CF), fat, ash, organic matter (OM), neutral detergent fibre (NDF), and acid insoluble detergent fibre (ADF) can each be determined from the following equation:

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

There are some cases where faecal collection is not possible such as in grazing or competition horses as their routine training would be upset (Goachet *et al.*, 2009). Where faecal collection proves to be a difficulty, markers can be used. Some feeds cannot be fed alone so they can be mixed with a basal feed that has a known digestibility in order for the digestibility of the sample feed to be determined. The proportion of test feed to basal feed needs to be taken into consideration for accurate results (Schneider and Flatt, 1975).

Apparent digestibility can also be calculated with the "digestibility by difference" method (Palmgren-Karlsson *et al.*, 2000; Lindsay, 2005). This method is used for supplements or feed that is not fed as a stand-alone but comprises a part of the diet. The digestibility of nutrients can then be determined using the following equation:

$$NDconcentrate = \frac{\left(TDN - \left(\left(\frac{Nforage\,\%}{Nconcentrate\,\%}\right) xNDforage\right)\right)}{\frac{Nconcentrate\,\%}{TDN}}$$

where ND= nutrient apparent digestibility in the concentrate or the forage, Nforage% is the amount of forage, Nconcentrate% is the amount of concentrate and TDN is the total apparent digestibility of the nutrient method (Palmgren-Karlsson *et al.*, 2000; cited in Lindsay, 2005).

In vitro techniques have been used in ruminant and monogastric nutrition studies (Lowman *et al.*, 1996; Lowman *et al.*, 1999; Rymer, 1999). In 1969, Appelgate and Hershberger showed that the *in vitro* rumen fermentation technique could be adapted to *in vitro* hindgut fermentation. The IVGPT has received much attention in ruminant research (Rymer *et al.*, 1999; Dhanoe *et al.*, 2000; Ouda, 2007). *In vitro* techniques are simple, cost effective, and require standard laboratory equipment and small amounts of test feed. The technique can be used as a rapid screening or indexing technique for a large amount of samples (Schneider and Flatt, 1975). The two-stage method was used by Tilley and Terry (1963), modified by Minson and McLeod (1972) and used by others (Murray *et al.*, 2003; Hussein *et al.*, 2004; Hackland, 2007). The pressure transducer method was developed by Menke *et al.* (1979) and refined by Theodorou *et al.* (1994). The method was modified to match how the digestive processes occurs in the horse, with microbial fermentation following acid digestion (Lindsay, 2005). The method of

France *et al.* (1993) was used by Murray *et al.* (2006) for curves to be fitted that experimentally derive gas accumulation profiles and Campos *et al.* (2004) was able to derive a model for two compartment fermentation kinetics. The kinetic data measures the appearance of fermentation gases such as CO_2, CH_4 and H_2 (Adesogan, 2002).The amount of gas produced affects the gas production profile and is an indication of the amount of soluble carbohydrate that is fermented (Fakhri *et al.*, 1998; Murray *et al.*, 2003). An excessive gas production in the caecum causes discomfort and irritability in the horse (Kohnke, 1998). Accurately being able to determine the change in fermentation that is occurring in the hindgut of horses, on a diet high in soluble carbohydrates, will provide a possible explanation for behavioural and performance problems.

The method of McDougall (1948) is still followed for the preparation of buffer solution in IVGPT. The buffer pH that is obtained in conventional horse assays is 7.2 (Bailey *et al*, 2002; Young, 2011). The function of the buffer is to resist minimal changes in pH (van Slyke, 1922). This is necessary for optimal microbial activity. The IVGPT method has already been adapted and refined (Tilley and Terry, 1963; Minson and McLeod, 1972; Murray *et al.*, 2003; Hussein *et al.*, 2004; Lindsay, 2005; Hackland, 2007) to mimic the digestive system of the horse, but with current feeding regimes changing to incorporate increased proportions of bucket concentrate, it is worth considering whether the method needs to be re-evaluated for accurate prediction of degradability. With an increase in bucket concentrate feeding there is an increase in the production of VFA's which decreases the pH (Hoffman, 2003). A decrease in pH will lead to a change in the microbial population (Ellis and Hill, 2005) and with this a change in the degradability of feedstuffs, thus, it is important that the standard buffer pH that is currently being used in degradability researched is investigated.

The IVGPT technique can be used in horses for the evaluation of feedstuffs (Murray *et al*., 2005; Murray *et al*., 2006; Murray *et al*., 2009). The inoculum can provide the greatest source of variation (Rymer, 1999) particularly in respect of the feeding vs collection time of the inoculum donor animals. In horses, faecal inoculum could be used as an alternative to caecal inoculum (Murray *et al.*, 2014) as microbial fermentation occurs after gastric digestion (Lindsay, 2005). The proportion of inoculum used in the solution can lead to a higher gas production (Wood *et al.*, 1998), an increase in the rate of gas production and a decrease in lag time (Pell & Schofield, 1993). Therefore, the amount added with the buffer needs to be kept constant to reduce variation, while blanks need to be measured to account for atmospheric pressure and residual fermentable organic matter in the inoculum (Pell & Schofield, 1993). The ration that the inoculum donors are on needs to be kept constant as feeds with different nutrient contents will vary in the amount of gas produced as well as the microbial activity being influenced with a change in animal diet. Straining of the inoculum needs to be done carefully so that faecal contents does not mix with the sample as this will have a negative impact on the lag time and a positive impact on the rate of fermentation (Mertens and Weimer, 1998; cited in Hervas *et al.*, 2005). Straining also has a physical impact on microbial activity so lag time, time taken for microbes to acclimatise, may be increased (Harris, 1996). In contrast to ruminants, where faecal inoculum follows gastric digestion (Konke, 1998), caecal or faecal inoculum in hindgut fermenters are considered similar therefore faecal inoculum can replace that of caecal inoculum (Lowman *et al.*, 1999; Murray *et al.*, 2003; Lindsay, 2005; Hackland, 2007). When samples are being prepared it is important that there is an accuracy in weighing, consistency of particle size, anaerobic conditions from collection to placement in the chamber, temperature from collection to placement in the chamber and the correct pH is being maintained as this has an influence on replication of samples in the experiment.

1.2 Flow rate

Rate of passage (ROP) is a time-dependent process (Moore-Colyer, 2000; Moore- Colyer, 2003), which quantifies the time that digesta remains in the gut for mechanical mixing, digestion, microbial fermentation and absorption (Kern *et al*., 1973; Cuddeford, 1996; Hyslop, 2006). Mean retention time (MRT) is the flow of digesta through the entire tract per unit of time (Robbins, 1993). Castle (1956) was able to determine the passage rate through the digestive system of a goat that enabled him to calculate foregut and hindgut digestion as being 5% and 95% respectively. This proportional split was then incorporated into models used by Blaxter *et al.* (1956) and Grovum & Williams (1973). Mean retention time (MRT) in the foregut and hindgut is an important determinant in the extent of feed digestion and the efficiency of microbial metabolic activity (Castle, 1956). Factors that influence MRT are animal weight, latter stages of gestation, lactation, work, type of diet, feeding level and disease status (van Weyenberg *et al.*, 2006). Fibre in the diet of a hindgut fermenter is critical as it plays a role in passage rate, microbial species and the integrity of the intestinal mucosa. A faster passage rate is associated with an increase in feed intake or the level of readily available substrate in the feed (van Weyenberg *et al.*, 2006). Diets with higher roughage to concentrate ratios have a faster ROP through the GIT (Warner, 1981; Pagan *et al*., 1998), this is due to a higher volume intake (Drogoul *et al.,* 2001). Restricted feed intake increases MRT, especially in the colon (Medina *et al,* 2002).

Digesta moves through the small intestine at a rate of 30cm/min (van Weyenberg, 2006). The motility of the mucosal villi is under neural and hormonal control. Transit time from the stomach to the caecum is much shorter after a fast (van Weyenberg *et al.*, 2006). The passage rate is influenced by the physical form of the feed (van Weyenberg *et al.*, 2006). Pelleted diets have a faster rate passage than chopped hay. Fresh grass has a faster transit time than that of hay. This is due to the amount of rapidly digestible substrate (van Weyenberg, 2006). An increase in meal size increases ROP in the small intestine, but not in the large intestine. The MRT of 18 month old horses given diets with 40% and 100% roughage was 45.7 hours and 42.3 hours respectively while ROP was 2.2% per hour and 2.4% per hour for the solid phase (Chiara *et al*., 2003). These results are controversial to the general census that a higher proprtion of concentrate to hay decreases MRT (Miralgia *et al.,* 2003). In older horses the MRT of a hay diet was 21-40 hours. Dental structure and occlusal surface will negatively impact chewing ability and thus increase MRT in horses (Miralgia *et al.*, 2003). A decrease in MRT reduces digestibility (Frape, 2010) and this needs to be taken into consideration given current horse production systems increasing the amount of supplementation.

The flow of nutrients in a hindgut fermenter differs from that of a monogastric. A hindgut fermenter utilizes both enzymatic hydrolysis and microbial fermentation when processing different nutrient classes that are absorbed in varying areas of the digestive tract (Figure 1; Young 2011).

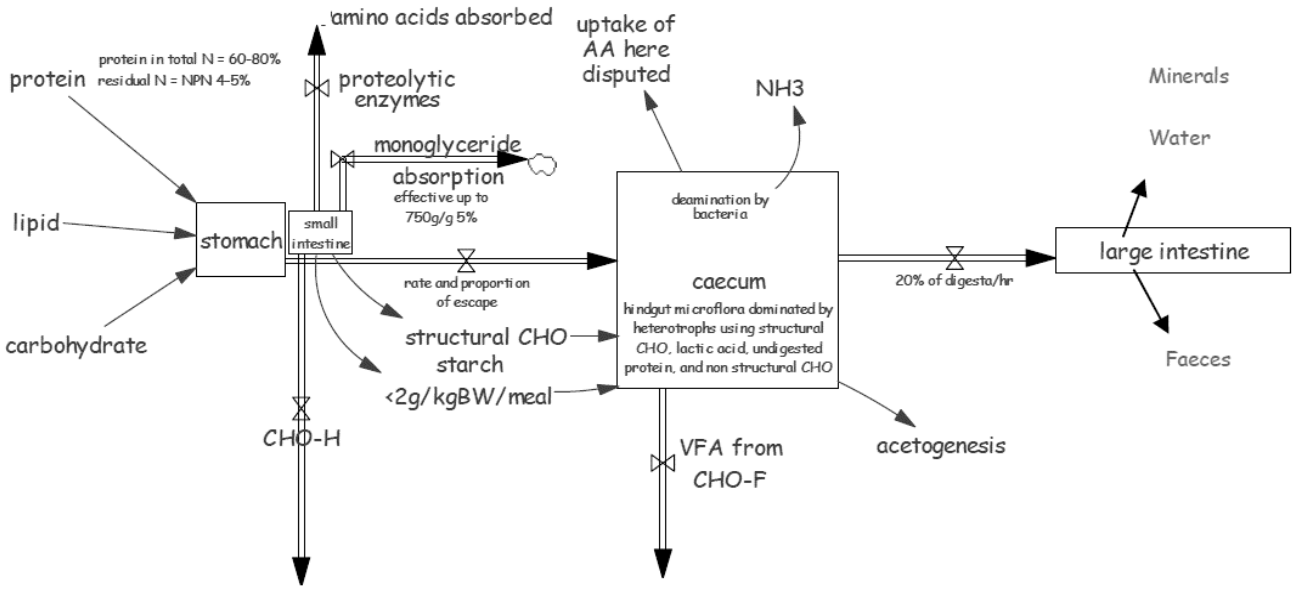

Figure 1: Schematic representation of nutrient flow in the horse with monogastric and caecal activity (Young, 2011). Abbreviations: N = Nitrogen, NPN = non-proteinic nitrogen, CHO = carbohydrate, CHO-H= hydrolysable carbohydrate, BW = body weight, AA = amino acids, NH_3, VFA = volatile fatty acids, CHO-F= fermentable carbohydrates.

Digesta flow can be determined with the use of totally indigestible and non-absorbable markers. Markers can be chemical constituents of feed particles that are fed (internal) or they can be markers that are not associated with the feed that is being fed (external). What is important is that they are nutritionally inert. There are many different types of markers. Some of the more commonly used ones in horses are chromium mordanted fibre (Cuddeford and Hughes, 1990), cobalt ethylenediaminetetraacetic acid (Co-EDTA) (Medina *et al.*, 2002) and acid insoluble ash (AIA) (Miraglia *et al.*, 1999: de Fombelle *et al.,* 2003). The caecum, right ventral and right dorsal colon are the main mixing compartments (Moore-Colyer *et al*., 2003). There are time-dependent and time-independent models of digestion, with the former being more popular in equine research. This is due to the passage rate of digesta in the horse being a time-dependent process (Moore-Colyer *et al*., 2003; Austbo & Volden, 2006). With the use of markers and models, fractional passage rate of the hindgut and MRT can be calculated. This aids in developing an understanding of passage kinetics, nutrient utilisation and absorption in the horse.

1.3 Nutrient digestion and absorption

1.3.1 Fat

Dietary lipids can be used as an energy source and provide essential fatty acids to aid in coat and skin condition (Oldham *et al.*, 1990). It has been proposed that using lipids as an energy source instead of fermentable carbohydrates (CHO_f) will spare glycogen (Geleen *et al.*, 2001), lower the heat increment of digestion, while still supplying sufficient energy (Kronfeld, 1996) with a positive effect on condition (Ellis and Hill, 2005) and temperament (Holland *et al.*, 1996). However, the decrease in microbial efficiency that varying levels of fat have on rumen fermentation is well documented (Onetti *et al.*, 2001) and this cannot be disregarded when it comes to hindgut fermentation in the horse (Young, 2011). In horses there is a negative impact on caecal fermentation and fibre digestibility when fat, particularly soybean oil, is included at the 5% level (Jansen *et al.*, 2007a, 2007b). Microbial activity, especially that of cellulolytic bacteria is vital for optimal fibre digestion (Jansen *et al.,* 2000; Jansen *et al.,* 2007a). Fibre digestibility is reduced at the 25-35% NDF level (Bragal *et al.*, 2008). Sales (2010) reported that there is a decrease in the digestibility of ADF when content ranges from 125-432g/kg DM of total diet. However digestibility is not affected when NDF content ranges from 317-586 g/kg DM of total diet.

Horses exhibit aerobic endurance when fed 10% fat in the diet (Oldham *et al*., 1990). The supplementation of fat in the diet helps to decrease resting heart rate and nervousness (Holland *et al.,* 1996; MacLeay *et al.,* 2000; Mckenzie *et al.,* 2003). This type of diet is useful for endurance horses as fat-adapted horses have a higher chance of meeting energy demands for endurance training (Treiber *et al*., 2006). Higher levels of fat in the diet improve total lipase, lipoprotein lipase and skeletal muscle citrate synthase activity (Orme *et al*., 1997; Geleen *et al.*, 1999; Dunnett *et al*., 2002). This is important in endurance horses as stamina is increased (Kronfeld *et al.*, 2001). An increased activity in these enzymes is an indication of a higher oxidative capacity of horses in training. Fats reduce fatigue and digestive disorders caused by a high carbohydrate diet (Hambleton *et al*., 1980). Blood glucose and glycogen depletion is delayed, maintaining higher muscle reserves for extended exercise (Frape, 1986; Hyyppa, 2005). Reduction in starch and not an increase in fat is recommended to avoid post-prandial glycaemic and insulinaemic responses (Verveurt *et al.,* 2009). Endurance horses have a lean Body condition score (BCS) as the fat reserves are used during extended exercise and excess fat has a higher maintenance requirement (Frape, 2010). The impact that dietary fat levels of 5% have on fibre digestibility will also have an impact on body condition (Jansen *et al*., 2007a; Jansen *et al*., 2007b). There is much controversy on dietary fat inclusion levels. High levels of dietary fat of up to 20% still have a 76% to 94% digestibility (Hambleton *et al.,* 1980; Pagan, 2000; Pagan, 2002) and improve performance (Holland et al, 1998; Kronfeld *et al.*, 2001; Lindberg & Jansson, 2010) but decrease fibre digestibility (Sales & Homolka, 2010; Zeyner, 2002). High fat levels increased the colonization time of the microbial population (Murray et al., 2006) and substrate digestion was lower (Young, 2011) which indicates that an increase in dietary fat content decreases microbial efficiency.

1.3.2 Protein

Protein digestion occurs in the small intestine (Slade *et al.*, 1971; Wootton and Argenzio, 1975; Cuddeford, 1996; Evans, 2000) while the deamination of excess protein occurs in the hindgut where exogenous D- amino acid oxidases convert amino acids to keto acids, H_2O_2 and NH_3. The H_2O_2 is converted to water and oxygen with the use of a catalase (Ellis & Hill, 2005). The three major pathways of amino acid metabolism all have NH_3 as an end product, which needs to be cleared rapidly (Ellis and Hill, 2005). Excess nitrogen that enters the large intestine will be utilised for the production of non-essential amino acids, utilised by the microbes or deaminated (Potter, 2002). There are detrimental effects when the protein that is supplied in the diet far exceeds the requirements and is beyond the capacity of the urea cycle (Miller-Graber *et al.*, 1991). The nitrogen retention for horses at maintenance plateaus at 0.202g N.kg BW^{-1} or at 1.26g CP kg BW^{-1}(NRC, 2007). Excess intake increases water intake and NH_3 excretion in urine (Hintz, 1994), which can lead to respiratory problems for stabled horses. Racehorses are commonly fed diets of 80:20 concentrate to roughage ratio's (Hackland, 2007). A diet with a 10% protein variation has shown no detrimental or beneficial effects in young horses in intense training. However, there is an increase in metabolic and gastrointestinal disorders in horses under these sorts of feeding conditions (Kalck, 2009) indicating that if not protein, perhaps its relationship to other nutrients, may well be worth investigating if it is causing disorders.

Nitrogen forms other than NH_3 are needed for microbial growth and fibre digestion within the caecum (Maczulak *et al.*, 1985; Carro and Miller, 1999; Hainze *et al.*, 2003). Caecal bacteria are responsible for the fermentation of the structural and non-structural carbohydrates which also has an effect on the extent of absorption and excretion of NH_3 and urea (Reynolds and Kristensen, 2008). When bacteria utilise structural carbohydrates, NH_3 production is lower than when non-structural carbohydrates are fermented. Nitrogen and ammonia production is damaging to microorganisms as 79.5% of caecal organisms have the inability to grow with NH_3 as the sole N source. This indicates that other forms of N are needed for optimal health and production (Mair *et al.*, 2002; Medina *et al.,* 2002). A diet that is balanced in terms of NDF/starch along with a probiotic has a beneficial effect on the equine intestinal ecosystem (Medina *et al.*, 2002). Provided that energy content is sufficient racehorses can perform adequately on feed that contains a CP content of 12-14% (Young, 2011). This then raises the question of protein to energy ratios in performance horse diets. Higher quality protein is beneficial to young horses in training as it aids in blood cell and muscle mass production and tissue repair post-race (Konkhe, 1998). Non-protein nitrogen (NPN) can be tolerated by the horse as it is converted to urea. However, due to no protein absorption occurring in the caecum (Schmitz et al., 1991), it can enter the bloodstream where it will be converted to NH_3 which is toxic thus having an effect on athletic performance (Slade *et al.,* 1971). Excessive NH_3 production in the large intestine is a possible etiology of gut mucosa in horses (Mair *et al.*, 2002) due to the association of primary hyperammonemia and signs of gastrointestinal disease.

1.3.3 Carbohydrates

Energy is provided in the form of carbohydrates, lipids and proteins. The nutritive value of a feed depends largely on the amounts of soluble, insoluble, degradable and undegradable fibre fractions of the feed (Getachew *et al.*, 2005). Carbohydrates are the main source of energy for the horse (Hoffman, 2001). Hydrolysable carbohydrates yield mainly glucose while CHO_f yield acetate, propionate and butyrate. Fermentable carbohydrates are categorised into a rapidly and slowly fermentable carbohydrate.

Glucose is the main soluble carbohydrate that is absorbed by the foregut when a horse is given a high grain diet, while VFA's are the main energy source for horses on a high roughage diet (Ellis and Hill, 2005). Glucose and propionate are stored as glycogen in the liver while acetate and butyrate are stored in the form of fat. Glycogen stores in the horse are estimated at 4.7kg in a 500kg horse with 90% being found in the muscle, 8% in the liver and the remaining 1 to 2% in the blood in the form of glucose (Mc Miken, 1983). The normal level of blood glucose is 4-6 mmol/L in horses. Blood glucose levels are known to increase post-feeding. Peak plasma glucose levels are 90-120 minutes post-feeding and return to normal five to six hours later (Mc Miken, 1983). Insulin is released from pancreatic beta-cells with an increase in blood glucose concentration. Insulin responses mirror the glucose response (Rodiek *et al*., 1991; Williams *et al*., 2001; Rodiek and Stull, 2007). Insulin stimulates the uptake of blood glucose by the skeletal muscles and adipose tissue. This returns the blood glucose to normal. Excess glucose is stored in adipose tissue in the form of fat (Hyyppä, 2008). Horses that are fed a high roughage diet will have a more stable blood glucose level than horses that are fed a high grain diet. A horse that is on a high grain diet will have higher peaks and lower troughs of blood glucose than a horse that is on a high roughage diet (Lawrence *et al.*, 1993). Frequent, smaller meals will aid in stabilizing the blood glucose levels (Frape, 1986). Ponies have a lower insulin sensitivity, compared to Thoroughbreds, which means that they can go for longer periods between feedings and are less excitable than Thoroughbreds after a meal (Frape, 1986). The delayed insulin response needs to be considered when planning to exercise as the chance of nervy behaviour increases if a high grain diet has been consumed within four hours (Kohnke, 1998). Horses that are accustomed to high starch diets have a higher insulin activity and are more likely to go into hypoglycaemic shock when they are fasted (Williams *et al.*, 2001). Glycaemic and insulinaemic responses are affected by: feed type, feed processing, feed quantity and rate of intake (Jenkins *et al*., 1990; Verveurt *et al*., 2007).

Sources of carbohydrates have an impact on the pre-ileal starch digestibility (Meyer *et al.,* 1993). Starch molecules all differ in structure depending on variety and species. Starch sources such as oats and barley are small granules and are easily digested. Oats starch has a pre-ileal digestibility of up to 90% while that of whole or crushed maize is only 30% (Meyer *et al.,* 1993). Recommendations for starch levels are 3.5-4g starch.kg BW^{-1}.$Meal^{-1.}$ (Potter *et al.*, 1992), 3.5g starch.kg BW^{-1} (Medina *et al.*, 2002) and less than 1.1g starch.kg BW^{-1} $Meal^{-1}$ (Vervuert *et al.*, 2009c). Non-structural carbohydrate (NSC) content in performance horses can be as much as 32-36% (Coleman, 1999). Pre-caecal starch digestibility was increased by 31% when grass hay was used as a roughage source instead of ground lucerne as the passage rate of the substrate was decreased (Willard *et al.*, 1977; Meyer *et al.*, 1993; Palmgren Karlsson *et al.*, 2000). High-energy diets can have an effect on the digestion of the structural carbohydrates in the hindgut (Julliand *et al.*, 2004). Glycogenic (propionic) and lipogenic (acetic, butyric) VFA's are products of carbohydrate degradation in the caecum caused by an oversupply of starch. This creates a favourable environment for lactobacilli, which decreases the pH.

It is known that high starch diets lower caecal pH, fibre digestion and VFA production (Willard *et al.,* 1977; Palmgren Karlsson *et al.*, 2000; Hoffman, 2003; Hussein *et al.*, 2004). A 24 hour *in vitro* study showed that the pH of caecal fluid decreased to 1.5 when starch was added (Bailey *et al.*, 2002). The type of forage and the time it is fed in relation to grain intake can have a huge impact on the pre-caecal digestion of starch (Pagan, 1997a; Vervuert et al., 2009b). Incorrect management of grain in the diet can predispose horses to metabolic disorders such as colic, laminitis and post feeding acidemia (Hoffman, 2003; Hussein *et al.*, 2004). A pH of 6.0 indicates subclinical acidosis with less than 6.0 indicating clinical acidosis, a common problem in the horse racing fraternity (Richards *et al.*, 2006). Increasing the hydrolysable carbohydrates over 0.4% of BW per meal will result in a low pH (Hoffman, 2003). The drop in the hindgut pH from rapidly CHO_f has also been linked to behavioural problems. Excessive gas in the hindgut causes discomfort to the horse (Willard *et al.*, 1977; Rowe et al., 1995; Ellis and Hill, 2005), which exhibits with the desire to chew wood, wind suck or practice coprophagy (Rowe *et al.*, 1995).

The balance of nutrients within a diet can play a major role in fore and hindgut digestion (Geleen *et al.*, 1999). A full understanding of the equine GIT and overprovision of nutrients is still needed in the horse fraternity. If this can be achieved, conditions such as glycosuria, glucose present in the urine and other metabolic disorders can be avoided.

1.4 Nutritional Modelling

Nutritional modelling plays an important role in equine nutrition research. Computer models allow the prediction of outcomes and point out areas that are in need of additional research. There are many different computer models that are focused on nutrition and metabolism in species such as bovine, swine and ovine (Tylutki, 2011). Models vary according to the objectives and implementation of the model. The underlying objectives must be clearly understood when a model is selected. Some are modelled at an animal integration level while other models that are more complex may have additional sub levels (Tylutki, 2011). An example of this is data that are collected from a model predicting occurrences of amino acid flux in the liver, cannot necessarily be used to predict the overall digestive function of the animal. All models can be categorised as dynamic, static, mechanistic or empirical models. A dynamic model is a model that will be able to predict an outcome from a metabolic pathway or production cycle whereas a static model is only able to represent one point at a time. The main structure of an empirical model is a series of equations that have been derived from experimental data with the aid of regression analysis. A mechanistic model is one that contains theoretical components that will produce outputs according to the inputs that it has been given. An example of a mechanistic model is, asking the model to predict BCS of a mare at foaling on different types of diets (Tylutki, 2011).

There are limited models that have been applied to equine nutrition such as deterministic models for rates of passage (Cuddeford, 1999) and some empirical models for growth rates (Staniar *et al.*, 2004). The National Research council (NRC, 1989) used some empirical modelling to provide recommendations and energy requirements. The NRC model for nutritional requirements has been partially updated (NRC, 2007), which allows a choice of three energy levels and concomitant nutrient requirements. It has been determined that protein requirements do not increase at the same rate as energy requirements (van Saun, 2006) yet the NRC (2007) still follows the principle of protein requirements increasing at the same rate as the energy requirements. Nutrient requirements for horses have seen very small changes in the absolute requirements as seen in the NRC (Harris and Bishop, 2007). In contrast, the poultry industry has seen huge advancements in the last 30 years in the quality of the approach to bird nutrition and a large contributing factor could be making feed intake an output rather than an input in the growth model (Gous, 2007). Van Saun (2006; cited in Young, 2011) mentions that microbial fermentation needs to be taken into consideration when predictions of nutrient provision are being made in horses. Currently feed intake is an input in equine nutrition models but when the theory of desired food intake, constrained food intake and the first limiting nutrient by Emmans and Oldham (1988) is taken into consideration, feed intake should rather be seen as an output (Gous, 2007). This type of modelling can be used in horses. The digestive tract of birds and horses both undergo chemical digestion prior to mechanical digestion. The digestive system of the horse does contain an extended caecum for fermentation, which is absent in a bird. Therefore, this type of model would allow the prediction of pre-caecal nutrient requirements. This could aid in reducing nutritional insults that are currently being imposed on the caecum.

Nutrient synchrony is defined as the co-existence and availability of nutrients (Hersom, 2008). Nutrient requirements, nutrient synchrony and other factors influencing performance need to be considered for a horse according to life stage and activity level. Correctly balanced nutrients and feeding management should be the determining factor of feed intake (Young, 2011). However, feed intake is altered by nutrient inadequacy of the feed, be it a deficiency or an excess which leads to an increase in metabolic disorders (Kalck, 2009). This problem can be solved with the use of empirical modelling to determine requirements and animal responses. Efficiencies for utilizing and modelling the partitioning of nutrients in the hindgut has been calculated (Vermorel *et al.*, 1997). Energy requirements can hence be accurately modelled for the prediction of feed intake and performance. The calculated nutritional constants (Vermorel *et al.* 1997) can be used within effective energy models (Emmans, 1994) which in essence will give a more accurate effect of thermic load. Utilization can then be determined for the improvement of specifications and raw materials that are being used in feed formulations.

The gas production model of Campos *et al.* (2004) is used to represent the flow of digesta kinetics in the horse (Rosenfeld *et al.*, 2006). Passage rate and MRT can be calculated with the use of mathematical modelling. This is done with the use of faecal excretion data and external markers. Mathematical models are comprised of time-dependent (Grovum and Williams, 1973; Dhanoa *et al.,* 1985) and time-independent models (Moore-Colyer *et al.,* 2003). Time independent models assume that digesta is one directional when passing through the compartments of the GIT (Lalles *et al.,* 1991). Time-dependent models accurately predict faecal marker excretion and fit equine faecal excretion data far better than time-independent models (Dhanoa *et al.,* 1985; Dhanoa *et al.,* 2000; Moore-Colyer *et al.,* 2003).

If the modelling of nutrient intake and utilisation in equines is able to provide sufficient data to feed according to what the digestive tract can handle while performance is optimised, then there would be less horses suffering from digestive upsets due to an overprovision of nutrients.

2 FEED PROCESSING AND FEEDING

2.1 Feed processing

The horse is a free ranging herbivore that needs large volumes of highly fibrous feeds and is able to preferentially select feeds that will meet their nutritional requirements (Dulphy *et al.*, 1997a, 1997b). Horses are unable to follow natural habits if they are kept in a stabled environment and they receive large portions of bucket concentrate vs roughage and the roughage that is provided is of poor quality. Roughages are categorised into different classes according to the type of grass, how they were grown and how they were processed from the field to the feed bucket. Higher energy and protein feeds are supplemented to meet the more demanding work level of performance horses (NRC 2007).

The control of dry matter intake in horses differs greatly from that of ruminants (Hill, 2002). A horse is a trickle feeder that will eat for 16-18 hours/day compared to a ruminant that eats large quantities at one time. The two different types of eating methods influence the physical characteristics of the feed that is being consumed. There has been some controversy with processed feeds increasing colic occurrences (Morris *et al.*, 1989; Tinker *et al.*, 1997b; Bulmer *et* *al.,* 2015) or having no impact (Little & Blikslager, 2002) in comparison to wholegrain feeds. The bucket concentrate feeds that are fed to the horse are formulated with a variety of ingredients that are attractive to the human consumer. Visual appeal in packaging and branding determines purchase by the horse owner and not necessarily what is in the bag (Young, 2011). One way to improve the look of the food that is in the bag as well as digestibility is by using feed processing techniques.

Feed processing alters the physical or chemical form of an ingredient. This should result in a higher nutrient availability without being detrimental to the feed quality (Jackson, 2000). Mechanical, thermal and thermo-mechanical are feed processing techniques. Mechanical processing includes rolling, crushing or grinding of ingredients. The reduction in particle size is the most frequent processing method that is used. There are advantages and disadvantages of decreasing particle size such as a shorter shelf life of cereal grains, due to the starchy endosperm and seed lipids being exposed to oxidation once the seed coat is broken. Crimping or rolling of oats in equine feed increases the digestibility by 5% which may not be high enough to outweigh the extra milling costs, however, there are other ingredients such as barley, wheat, milo and maize that have a better response (Jackson, 2000). Pre-ileal digestibility of whole or cracked maize is 30%, ground maize is 51% and popped maize is 90% (Meyer *et al*, 1993). The surface area that is exposed to the digestive enzymes influences the digestibility. In the case of the popped maize, the gelatinization of the starch molecule is what increases digestibility (Meyer *et al.*, 1993). Thermal processing includes roasting or micronizing of the ingredients and the combination of the two is referred to thermo-mechanical processing, which is the process of flaking, popping or extrusion. These techniques can be done under dry or wet conditions and are then referred to steam rolling, steam crushing, steam flaking, pressure toasting, steam extrusion and pelleting (Julliand *et al.,* 2006). The digestibility of maize was increased by 21%, when the maize was ground and extruding maize can increase the digestibility up to 90% (Kohnke, 1998). The objective of feed processing is to improve the nutritional value of the feed by increasing the availability of starch that is present as starch intake is inversely proportional to small intestine starch digestibility (Julliand *et al.,* 2006).

Horses in the wild are able to select feeds that will provide them with the nutrients that they need, without digestive disruptions or toxicity. When a horse is housed in a closed environment, its nutrient ingestion is restricted and regulated by owner management. If a diet is lacking in fibre (less than 10g/kg body weight), a horse will tend to make up for the shortages with other items that are present in its current environment such as ingesting wood shavings or practicing coprophagy (Ellis & Hill, 2005). The main dietary challenge for a horse is a change from a low energy, high fibre feed that can be consumed for long periods of time to a high energy, low fibre feed that is consumed in small quantities and at restricted times (Ellis & Hill, 2005). Pre-gastric factors and the novelty of the feed ingredients are the most direct regulators of food intake and chewing in horses (Ralston, 1984; Hill, 2002). Supplying feeds containing processed ingredients improves digestibility and may decrease the amount of starch passing into the caecum (Julliand *et al.,* 2006) to enable a reduction of feed intake of bucket concentrate without influencing performance.

2.2 Sites of Digestion

The GIT of the horse is made up of different sections with each section having different nutrient contents and functions configured by the arrangement of the GIT within the abdominal cavity. Mechanical and physical digestion occurs as the food enters the mouth. The teeth are the source of mechanical digestion while the saliva provides amylase for chemical breakdown. Although there are differences in ruminants and equines with regard to amounts of saliva, buffering capacity and the overall functioning of the glands, the main function of saliva on pH regulation remains the same. The bicarbonate content of saliva provides the buffering capacity. Saliva secretion is stimulated by the physical presence of feed material (Frape, 2010). Feed that requires more chewing will have a different buffering capacity (Bailey & Balch, 1991; Ellis & Hill, 2005) in comparison to a feed that has been processed prior to it being given to the horse. Horses fed a diet consisting of hay chewed four times more than horses on a concentrate diet, thus an increase in buffering capacity (Houpt *et al*., 2004). The ingesta on a high roughage diet will have a higher pH by the time it enters the stomach in comparison to a high carbohydrate (CHO) diet. The continuous supply of saliva during eating buffers the digesta in the non-glandular region of the stomach. This allows for some microbial fermentation to occur with lactate production (Frape, 2010). The dental structure of the horse is also important for feed intake (Ashley *et al*., 2005) as well as mechanical digestion (Ralston *et al*., 2001) as it affects particle size that enters the stomach and is excreted. Up to 83% of horse owners and trainers ensure that the horses receive dental check-ups (Dixon *et al.,* 2004). Carmalt *et al.* (2005) found that if the angle of the occlusal surface was between a gradient of 6% and 19%, then digestibility and faecal particle size is unaffected, but as soon as this gradient exceeds 19%, digestibility is lowered (Ralston *et al.,* 2001). Dental floating and rostrocaudal mobility had no effect on digestibility (Carmalt *et al.,* 2004; Carmalt and Allen*,* 2006). Factors such as chewing intensity increased pre-ileal starch digestibility (Meyer *et al,* 1995) while an increase in chew time reduced rate of passage and nutritionally-induced disorders (Ellis *et al.,* 2010; Ellis *et al.*, 2015).

The foregut in the horse represents 10% of the overall GIT, which is about 15L in a 500kg horse unlike that of the ruminant, which represents 67% of the GIT (Pond *et al.*, 1995; Frape, 1998). The stomach in the horse is in the form of a 'J' and lies between the small intestine and the oesophagus. The curvature is the main reason for the variety in acidity and physical characteristics of the digesta. The *margo plicatus* serves as a delineation between the cardiac and pyloric regions of the stomach. A distinct difference can be seen in the stomach lining of these two sections. The buffering capacity of the saliva decreases the rate at which the pH of stomach contents falls. This action along with continuous flow of ingesta leads to the stratification of pH in the two regions (Frape, 2010). The pH in the cardiac region is about 5.4 while that in the pyloric region drops to about 2.6 (Frape, 2010) Gastric juices along with hydrochloric acid (HCl) and pepsinogen are secreted in the cardiac region. The chyme passes into the pyloric region along with the gastric juices and reduces the pH to as low as 1.8 (de Fombelle *et al*., 2003). The time period that feed resides in the stomach will have an impact on the chemical compositions. There is a large diverse microbial population within the *saccus caecus*. These communities are likely to be dominated by the epiphytic micro-flora that are present in the feed (Morris, 2003). There is also a large variety of microbes in the cardiac region, mostly populations of *lactobacilli, streptococci* and lactate-utilising bacteria. Cellulolytic bacteria are present but in low quantities (de Fombelle *et al.*, 2003). Fibre in the diet plays an important role in maintaining the phytochemical environment that is present in the horse. A higher ratio of soluble to insoluble fibre that enters the stomach increases the viscosity of digesta, water holding capacity and the rate of passage (Bach-Knudsen, 2001). The feed entering the stomach and the distribution thereof controls voluntary intake, flow and utilisation of the feeds along the GIT (Ellis & Hill, 2005).

The small intestine is 21- 25m in length in a 500kg horse (Frape *et al.*, 1998) and is where the majority of the absorption occurs within the GIT. The small intestine is made up of three sections, the duodenum, jejunum and the ileum. It has been said that the pH of the digesta that enters the small intestine ranges from 2.5 to 3.5 and is buffered to approximately 7-7.5 by bile and the pH is again raised to 7.8-8.2 in the jejunum and ileum (Meyer *et al.*, 1993). The absorption of nutrients in the small intestine is not uniform especially in the case of minerals, of which 50% pass into the large intestine (Argenzio and Stevens, 1975). The proximal jejunum is responsible for majority of the water absorption (Singer, 1998). The small intestine is the site for the breakdown and absorption of sugars, starch, amino acids and lipids with the absorption of nutrients being influenced by flow rate. It was suggested by Varloud *et al.* (2004) that the digestion of starch in the jejunum and Ileum is as high as 88%. However, Hintz *et al.* (1971) and Brown (1987; cited by Potter *et al.*, 1992) found the result to be lower by 14-16%. Starch utilisation is determined by the degradation of pancreatic amylase, brush border disaccharides and limited microbial utilisation. High starch diets are not suited to equines as they battle to adapt due to the limited absorption capacity of the small intestine (Richards *et al.*, 2003). The ileum is responsible for the majority of the protein digestion and the absorption of the amino acids. There is a large microbial population in this section of the GIT of which most are proteolytic in activity (Kern *et al.*, 1973; Mackie & Wilkins, 1988). This process is dependent on flow rate. If the residence time of the digesta is not sufficient then some of the more complex sugars and starches pass through into the large intestine (Hoffman, 2003).

The large intestine comprises the caecum, the large colon, the small colon and the rectum. In a 500kg horse, the caecum is about 1.25m in length with a holding capacity of 25-30L and is 16% of total tract volume. The colon is in four compartments and holds 50 to 60L and is 10m in length and along with the rectum makes up 45% of the total tract volume. The sternal, pelvic and diaphragmatic flexures split the colon up into the right ventral colon, right dorsal colon, left ventral colon and left dorsal colon (Frape, 2010). These flexures constrict the large intestine, forming compartments that split the colon into four sections that are important in the flow of digesta (Ellis & Hill, 2005). The flexures reduce the backflow of digesta. In each compartment digesta is thoroughly mixed with limited mixing occurring between compartments (Cuddeford, 1996). Although the flexures benefit the digestive efficiency of the horse, impaction in these bends can lead to death (Cuddeford, 1998).

The microbial population differs between the compartments. Microbial fermentation and absorption is the main function of the large intestine (Meyer *et al.*, 1997). All products that escape absorption in the small intestine pass through into the large intestine. The sugars and other soluble dietary fibre that pass through into the caecum, undergo rapid fermentation (Ellis and Hill, 2005; Frape, 2010). The insoluble fibres also undergo degradation and fermentation. This is dependent on microbial activity (Frape, 1998; Julliand *et al.*, 2001). Microbial communities in the large intestine have the ability to degrade fibre to the same extent as ruminants if the flow rate is optimal (Hyslop, 1998). The retention time of the caecum is 9 hours for liquid and 10 hours for fibrous particles (van Soest, 1994) while the outflow rate has been measured at 0.2 of the total content per hour (Hyslop, 1998). A ruminant can release gases through eructation. This mechanism of gas release is not present in the horse. The horse therefore needs a limited amount of fermentation to occur or the microbial population must have an adaption that allows them to produce low levels of methane (Russel & Gahr, 2000). Large amounts of gases do occur in the large intestine and this is mainly due to an abrupt change in the diet or the animal is being fed a diet that contains a large amount of rapidly fermentable substrate (Drougal *et al.*, 2000). The large amount of gases that have no escape route can cause the horse a huge amount of discomfort and thus behavioural problems. It is assumed that there is a 100% digestibility and absorption of starch in the entire digestive tract. There can be an overflow that enters the large intestine, which is determined by the quantity that is being fed, and the type of feed processing. The passage rate of digesta through the caecum is 20%/hour as compared to 2- 8% in the rumen (Hintz, 1990). Digesta entering the caecum increases the volume and motility, which allows for more thorough mixing of digesta and microbes (Frape, 2010). Caecal bacteria that are adapted to a grain diet are not as efficient at fibre digestion as those found in a horse on a hay diet (Frape, 2010). This is because there are different populations of bacteria. If hay-adapted caecal microbes are subjected to a grain overload, impactions, colic and laminitis can occur (Frape, 2010). There is a faster rate of microbial degradation of substrate in the caecum and ventral colon than the dorsal colon. The rate increases with an increase in dietary starch. The change in concentrate to roughage ratio leads to changes in the proportions of acids produced (Hoffman, 2003; Young, 2011). There is an increase in propionate production in the caecum and ventral colon with an increase in starch. This increases the risk of colic. Products of fermentation, water and minerals are absorbed in the caecum and colon. Ninety-six percent of the sodium and chloride and 75% of the soluble potassium and phosphate that enters the large intestine is absorbed (Frape, 2010).Ammonia is readily absorbed in the proximal colon. The small colon is about 3m long and is the main site for water and mineral absorption (Kohnke, 1998). The rectum is 30cm in length and is used as a storage chamber for the expulsion of faeces, which has an approximate DM of 15-25%.

2.3 Nutrient Synchrony

Nutrient synchrony is the corresponding existence of nutrients to be consumed or to be present in the diet for optimal performance and microbial efficiency (Hersom, 2008). Anaerobic fermentation whether in relation to soil, ruminant ecology or the equine caecum requires a ratio of nutrients to be available to them in a favourable environment for substrate degradation. The relationship between nutrients in the digestive tract is of vital importance, especially in the hindgut of the horse. The microbial population found in the caecum is altered with a slight change in the microclimate (Bailey *et al.,* 2002; Ellis and Hill, 2005). When a low carbon to nitrogen ratio is present, N is converted to NH_3 at a faster rate to what methanogenesis can utilize the NH_3 (Ghasimi *et al.,* 2009). Horses have a lower capacity for methanogenesis than acetogenesis (Ellis & Hill, 2005). The optimal range of non-lignin carbon to nitrogen is 25 to 30 (Hills & Roberts, 1981), anything in excess will decrease the pH (Ghasimi *et al*., 2009). The bucket concentrates that horses are currently getting are far exceeding the optimal level of a carbon to nitrogen (Young, 2011). Increasing the carbon to nitrogen ratio or fat content (Geleen *et al*., 1999) will impact the cellulolytic bacteria present (Jansen *et al.*, 2000; Jansen *et al.*, 2007a, 2007b). This in turn will have a negative impact on cellulose digestibility. Bragal *et al.* (2008) also found that digestibility was affected when fibre with an NDF content of 25% and 35% was fed. An improvement in cellulose digestibility was found when the ratio of fibre to energy was favourable (Miralgia *et al.*, 2006). The ratio of cellulose to hemicellulose to lignin can accurately indicate the structural fermentable substrates in horse feeds (Miraglia *et al.,* 2006; Bragal et al., 2008). This allows for the degradation potential of feeds to be determined which gives an indication of anaerobic activity. Biodegradable carbon can be calculated as follows (Richard, 1996; cited in Richards 2005).

$$(C_{biodeg}) = C_{total}\ (NDF\%/100)\ (1\text{-}0.054(lignin\ \%/(NDFx0.01))0.76) + C_{total}\ (1\text{-}NDF/100$$

Substrate source, usage and product yield effectiveness can be disrupted by intrinsic properties, interactions, transformations and passage (Hall & Huntingdon, 2008). These changes in the horse and in the digestive tract need to be balanced with nutrient synchrony.

2.4 Microbial health

There is a huge diversity of micro-organisms within the equine GIT including virus, fungi, protozoa and bacteria groups. In each of these groups there are two types of microbes, autochthonous and allochthonous. Autochthonous microbes are those that colonize the gastrointestinal tract natively whereas allochthonous microbes are those that can only exist under abnormal conditions (Mackie *et al.*, 1999). Microbial activity, correct gut function and digestion is vitally important to equine nutrition. The primary role of the micro-organisms is to digest fibrous plant material in the hindgut. This process is driven by hydrolytic and fermentative micro-organisms that produce VFAs in the process which are absorbed across the intestinal wall and provide energy for the animal (Argenzio *et al.*, 1974; Argenzio & Stevens, 1975; Mac Bee, 1977). Every disturbance that either disrupts or breaks down the balance within the microbiome can lead to a decrease in digestibility and even health implications for the horse. The microbial population is altered by feeding grasses of different types and maturities (Muhonen *et al.,* 2010). Fibre can have a protective action on the pH of the caecum by binding protons, thus preventing a drop in caecal pH (Brokner *et al.,* 2010).

Faecal pH has been used in several studies as a determinant of gut health (Hussein *et al.*, 2004; Williamson *et al.*, 2007). Due to a horse being a hindgut fermenter, it depends on cellulolytic microbial activity in the caecum and colon for the degradation and fermentation of fibrous particles to produce VFA's. The optimal pH range is 6-7 in the large intestine (Sjaastad *et al.*, 2003). A pH of 6.1 has been previously reported when 2g starch from barley/kg BW was fed (Austbo, 2005). Fibre digestion can be severely decreased if amylolytic bacteria are replacing the cellulolytic bacteria. A caecal pH of less than 6 can result in a horse being classified with sub-clinical acidosis, which leads to an increase in metabolic conditions such as colic (Austbo, 2005). Microbial health can be greatly influenced by internal environmental conditions such as gut pH. There are some conditions that are favourable to some micro-organisms while fatal to others or there are conditions that are so severe all micro-organisms are negatively impacted. It can be one environmental factor or an interaction of factors that will have an effect on the propagation or fatality rate of the microbial population. In nature these factors cannot be controlled, due to some species co-existing and population shifts due to varying growth rates. The acidity of the environment also has an effect on the health of the organisms. Most bacteria survive in the pH range of 5 to 9; fungi can exist in the range of 6 to 9 while acidophilic bacteria thrive in the pH range of 1 to 3. Pressure can play a role in the survival of micro-organisms as well. When the pressure becomes too high it becomes fatal for the organisms.

Temperature is one of the most important factors that have an influence on microbial health. Each species has a different genetic composition so some species will be better adapted to certain temperature ranges. The optimal temperature range for most organisms is the body temperature of their host.

Micro-organisms are categorised into different groups depending on the concentration of oxygen they can be subjected to without having a negative effect. The majority of micro-organisms present in the hindgut or in the rumen are anaerobes, which grow in the absence of oxygen either by fermentation or anaerobic respiration. These anaerobes are grouped into classes; obligate anaerobes, facultative anaerobes and microaerophiles with anaerobes being the most popular within the hindgut. The obligate anaerobes cannot survive in the presence of oxygen while facultative anaerobes can reproduce and survive in the presence or absence of oxygen and microaerophiles require a certain amount of oxygen but have maximised growth rates in very low concentrations of oxygen.

When IVGPT work is done, these conditions are taken into consideration. The temperature is maintained at 37°C, the inoculum is placed into the bottles under a stream of CO_2 and the bottles are also flushed with CO_2, the pH is maintained with the aid of a buffer and the size of the bottle is proportional to the amount of substrate that is added so that the pressure build up has no impact on the micro-organisms (Atlas, 1995). To optimise microbial health a balanced diet needs to be given. Nutrient synchrony and C to N ratios are important as excess nutrients passing into the caecum have a negative impact on microbial health.

3 NUTRITIONAL INSULTS

3.1 What constitutes a nutritional insult?

A nutritional insult is disrespect of the requirements of the GIT, or ignorance of such requirements. A horse is by nature a free ranging animal that grazes throughout the day. The GIT is not adapted to large quantities of highly fermentable substrate, which is commonly given to the working horses or recreational animals in intensive production systems. An excess, a deficiency or an imbalance of nutrients or minerals can constitute a nutritional insult. A lack of nutrients can cause severe problems. These are more commonly seen in rural horses where grazing is at a premium and horses are worked, but not supplemented nutritionally. Whether an animal is receiving a lack of nutrients or excess, it is still being subjected to a nutritional insult. Like all animals a horse has nutrient requirements that need to be met for correct function. Horses in different life and exercise stages have varying requirements and this need to be taken into consideration with feeding management.

3.2 Nutritional Insult Solutions

Meals should be fed in smaller amounts more frequently throughout the day with at least 1% BW/kg of roughage (Zeyner *et al.*, 2004). Regular feeding times are important. There is an increased risk of gastrointestinal problems occurring if the horse's normal routine is abruptly changed. Some ingredients must be at the optimum inclusion rates; as certain ingredients cannot be tolerated above a specific level. Hindgut function should be maintained with feeding, therefore forage contribution should be maximised and carbohydrate content should be minimised. The storage and procurement of feed should be continuously checked to prevent mould and the loss of feed quality. It is important that quality of the ingredients/feed is maintained to eliminate problems such as fusarium in maize, mycotoxins and wheat mould (Akande *et al*., 2006). All feeds have different densities so it is important that feed is fed by weight rather than volume to ensure the horse is receiving the right amount. Therefore, it is important that exercise is done on a daily basis and feed intake is adjusted according to the body condition of the horse. Dentistry and worms plays a major role in nutrition. Regular teeth check-ups by an equine dentist should be done and a worming programme needs to be established so that the maximum feed utilisation is obtained with minimal feed usage (Jackson, 2000). Potential problems such as metabolic disorders need to be considered (Beyer, 2000) when an attempt is made to optimise performance by feeding the highest quality rations. Nutrients such as starch, roughage, fat, protein and minerals being fed at the incorrect levels all constitute a nutritional insult. The ratio of roughage and starch is the most common insult that is prevalent in the horse industry today. Although the correct level of fat in a diet is of importance for microbial gut health and the balance of minerals is vital for optimal nutrient absorption, the ratio of roughage and starch should be the main focal point.

The Kentucky Equine Research Unit recommends that daily roughage intake should amount to between 1 and 1.5% BW on a dry matter (DM) basis. Konkhe (1998) states that for correct gut function, roughage intake should be a minimum of 50% of the total caloric intake. High performance horses have higher energy and other nutrient demands. For these nutrient requirements to be met, the owner increases the amount of concentrate provided in bucket feeds in the stable. Two meals a day are often provided, and the extra feed is split between these feeds. Starch provided in excess of 0.2-0.4% BW that is common in horses (Radicke et al., 1991; Kienzle et al., 1992; Potter et al., 1992), depending on the type of starch, will pass through the digestive tract and can lead to metabolic or behavioural problems (Willard *et al*., 1977; Hillyer *et al.,* 2002; *Nicol et al*., 2002; Cooper *et al*., 2005). When the amount of starch that is fed exceeds the digestive capacity of the small intestine, there is a negative impact on microbial health and activity within the caecum and colon (De Fombelle *et al.*, 2001; Drougal *et al.*, 2001; Hussein *et al.*, 2004; Lopes *et al.*, 2004). Varloud *et al.* (2004) reported that starch entering the large intestine increased from 8g to 37g/100kg BW when horses were given a high starch diet in comparison to a high fibre diet, this leads to severe microbial and biochemical dysfunction

(Julliand *et al.*, 2001; Bailey *et al.*, 2002). High starch diets will decrease the pH stratification while promoting fluidity and gastric content mixing with limited fibre thus exposing the squamous mucosa to highly acidic gastric secretions.

Pre-caecal digestibility may be affected by the high volume of chyme that is linked too high starch feeds (Clarke *et al.*, 1990; Drougal *et al.*, 2001; Metayer *et al.*, 2004). The increase in glucose concentration from starch degradation is absorbed across the intestinal mucosa so it can be transported around the body. Excess glucose is utilised by micro-organisms with VFA's as by-products. There is also some evidence for the consequent intestinal damage from excess ammonification with high protein diets (Mair *et al*., 2002; Frape, 2010).

3.3 Nutritionally related disorders

There are disorders that can be directly or indirectly nutritionally related. Directly related disorders include obesity, white muscle disease, developmental orthopaedic disease, mycotoxicosis and nutritional secondary hyperparathyroidism. Disorders that are indirectly related include Hyperkalemic Periodic Paralysis Disease (HYPP), insulin resistance, enterolithiasis and polysaccharide storage myopathy. Nutritionally related disorders can also be indirectly or directly related to nutrition. Examples include colic, laminitis and gastric ulcer syndrome.

3.3.1Colic

Colic can be classified as any abdominal pain that is related to the anatomy of the GIT as well as the balance of micro-organisms that are present within the GIT (Beyer, 2000). Symptoms of colic include tail twitching, pawing the ground, restlessness, playing with food and water, blowing bubbles in water, loss of appetite and the head is turned towards the flanks. In extreme cases, the horse will roll around and thrash risking further injury. Symptoms and severity of colic vary between horses. Heart rate, respiration rate, sweating and fever are dependent on severity. Heart rate can increase from 38bpm to between 68 and 92 bpm in moderate colic and can rise above 100bpm in acute cases. Respiration rate increases from 12-24/min to 72/min with an increase in temperature (Frape, 2010).

All cases of colic cause acute abdominal pain and this is due to the distension and stretching of the mesentery and visceral peritoneum from an obstruction. There is an 80% recovery rate of colic within 4 hours. In the remaining 20%, a mild disturbance can be fatal. Colic is associated with an increased lactate concentration in the blood. Lactate concentrations also increase in the peritoneal fluid if the cause of colic is not impaction (Frape, 2010). Compaction colic is 30 to 50 times more common in stabled/confined horses than farm horses (Beyer, 2000). There is a 10.6% chance a horse will get colic in its lifespan with a 0.7% chance of mortality (Tinker *et al*, 1997a; 1997b), with mortality rate as high as 25% in Israel due to surgery not being as successful as in the USA or UK (Sutton *et al*., 2009).

Colic occurs more commonly in horses that have either had a change in diet, been transported with limited water or received antibiotics that have altered the gut micro-organisms (Cohen, 1995; Cohen and Peloso, 1996; Cohen *et al*., 1999). The incidence of colic is greater in horses between two and ten years of age. Arabian horses have the lowest incidence (Cohen and Peloso, 1996) while Thouroughbreds (TB) have the highest (Mair and Hillyer, 1997). The highest risk of colic comes from a change in feeding management (Tinker *et al.*, 1997b; Wood *et al.*, 1998; Cohen *et al.*, 1999; de Fombelle *et al.*, 2001; Hudson *et al.*, 2001).

There have been several studies that have linked the consumption of bucket concentrate feeds and cereal grains by horses to an increased risk of colic (Tinker *et al.*, 1997b; Hudson *et al.*, 2001; Kaya *et al.*, 2009). Horses receiving between 2.5-5kg of concentrated bucket feed were at a five times greater risk while horses receiving more than 5kg increased the risk of developing colic more than six fold compared to horses that were grazing (Tinker *et al.,* 1997b). An association has been made between the different types of colic and the feeding management system of the horse (Hillyer *et al.*, 2002; Lopes *et al.,* 2004; Cohen *et al.*, 2006; Cox *et al.*, 2009). An increase in grazing is strongly associated with a decreased risk of colic (Hudson *et al.*, 2001; Hillyer *et al.*, 2002; Cox *et al.*, 2009). However, some studies have not come to the same conclusion. This may be due to the different types of colic not being considered separately (Cohen and Peloso, 1996; Kaya *et al.*, 2009).

Ileal impactions are intestinal obstructions with the accumulation of gas and liquid at the front of the obstruction. The consequence is loss of smooth muscle activity and peristaltic movement of digesta, which can prove fatal if not corrected. About 30% of colic's are impactions within the GIT, with majority occurring in the hindgut (Mair & Hillyer, 1997; Frape, 2010). Large colon impaction, displacement and torsion are the most common gastrointestinal causes of colic (Steel and Gibson, 2001). Common sites of obstruction are where the intestine changes in diameter at the flexures. The closer the impaction happens to the ileum, the more dangerous as water absorption in the caecum and ventral colon is severely restricted. This leads to dehydration and hypovolemic shock. Worms can cause extensive damage to the lining of blood vessels, especially the anterior mesenteric artery and the branches. This can cause severe problems with blood flow. Thromboembolism can cause loss of blood flow to the GIT. This can lead to the death of a section of the GIT and obstructive colic. Tapeworms locate themselves around the ileo-caecal valve which causes problems with digesta flow and leads to ileal impaction colic (Proudman *et al.*, 1998; Goncalves *et al.*, 2002). Anthelmintic treatment must be done regularly to avoid colic (Cohen *et al*., 1999; Frape, 2010).

Gas colic is extremely painful and is often secondary to impaction colic. Gas colic is caused by a starch rich diet, lush legumes or the consumption of grass cuttings. Quick action is essential with gas colic as ruptures and twisting of the stomach and intestine is common. Torsion, volvulus and interssusceptions of the terminal ileum into the caecum require surgery, as do nephrosplenic colics, which can be fatal (Frape, 2010).

In spasmodic colic, there is a change in frequency of peristalsis. Feeding management such as large meal sizes and forage quality have been linked (Meyer, 2001). Spasms can last for a few minutes or up to 30 minutes. Recovery of this type of colic occurs without treatment. Medication such as spasmolytic drugs can be administered for the relief of pain.

Sand colic accounts for 30% of colic occurrences (Husted *et al*., 2005). Sand colic is common in horses that live in areas with sandy soils and have a lack of grazing. The accumulation of sand in the intestine leads to chronic inflammation and poor motility of the intestine, as well as sedimentation in the tract, altering position. The common location for sand accumulation is the cranioventral abdomen. The sand can be eliminated from the digestive tract with the consumption of hay over a period of time (Lieb and Weise, 1999; Weise and Lieb, 2001).

Feed inconsistency and moisture content can also cause colic as impactions can occur from lack of moisture or too much coarse material. Uninterrupted supply of clean drinkable water is critical, particularly in changes of season. Absorption abnormalities from mucosal damage due to high amounts of starch and a decrease in pH can also be a cause of colic (Frape, 2008). Diets with high amounts of starch increase the risk of colic due to an imbalance of microflora present in the GIT (Hoffman, 2009; Dicks *et al.,* 2014). The reduction of starch in performance horse diets would optimise digestion and metabolic nutrient utilisation (Julliand *et al.,* 2008). Colic can have huge implications for the future and intended performance of the horse (Tinker *et al.*

1997a). Correct nutrition can eliminate huge proportions of colic cases (Beyer, 2000; Julliand *et al*., 2008).

3.3.2 Laminitis

Laminitis is a manifestation of a serious metabolic problem observed from the inflammation of the laminae that interdigitate between the hoof wall and the third pastern. The toxins enter the blood stream and the capillary arterial supply in the distal extremities is altered with the digital laminae being compromised due to endotoxins in the circulating blood (Frape, 2010). There are four degrees of laminitis ranging from a mild case of no visible lameness to severe cases where the horse will only walk if forced (Frape, 2010). The three theories for the aetiology of laminitis are: (1) vascular haemodynamic, (2) toxic, metabolic or enzymatic and (3) traumatic or mechanical overload (Bailey *et al*., 2004). All three theories concur that laminitis is usually due to carbohydrate overload.

Threshold levels for starch-induced laminitis can range from 24g starch/100kg BW to over 1000g starch/100kg BW (McLean *et al*., 1998). Low intakes of starch meals reduce overflow into the large intestine. Horses receiving two to three concentrate bucket meals a day should only receive 0.4% of BW per feeding (Potter *et al*., 1992).

There is an association between laminitis and fructans (Frape, 2010). Horses fed on pasture can have a fructan intake of 2.5-10.5g/kg BW (Longland *et al*., 1999). The upper level has been known as the dosage for fructan induced laminitis (French and Pollitt, 2004). Horses that have previously had laminitis have higher glucose intolerance (Jeffcott *et al*., 1986) than horses that have had no laminitis. Laminitis can follow colitis which affects the gut mucosa, which allows for endotoxins to be absorbed from the GIT into the blood stream.

There are numerous treatments. However, not one has proved more successful than the other thus showing the importance to prevent rather than treat (Beyer, 2000). Moderate regular exercise can be used as a preventative for laminitis and insulin resistance (Frape, 2010).

Low dosages of fructo-ogliosaccharides as a prebiotic can be used during dietary changes as a protective mechanism (Respondek *et al*., 2008). Laminitis caused by grain overload can be treated with mineral oil being administered by a nasogastric tube. This slows down the absorption of the endotoxin. Shoeing, sole support or trimming can also be done in chronic cases. If pedal- bone displacement and rotation is visible the horse should be placed on soft surfaces like sand or mud or have frog supports that are bandaged to the sole.

3.3.3 Gastric Ulcer Syndrome

Equine gastric ulceration syndrome (EGUS) can be distinguished by the presence of ulceration in the terminal oesophagus, proximal and distal stomachs and the proximal duodenum (Videla and Andrews, 2009). Stereotypies such as crib biting and wood chewing can result from incorrect feeding management (Pagan, 2000; Freire *et al.*, 2009; Ellis *et al.*, 2015) and may be associated to gastric ulceration syndrome. Symptoms such as colic, dull coat, difficulty maintaining weight and poor performance may also be indicators of gastric ulceration. Gastric ulcers have become widely recognised as a clinical problem in young horses due to performance demands. The reason for ulcers in young racehorses has been linked to feeding management and stress. Type of feed is linked to ulceration, as post-prandial serum gastrin levels are higher in horses after consuming grain instead of hay (Smyth *et al.,* 1989). Horses that are fed grain increase the gastric acid production in the stomach in comparison to those that have free access to hay (Murray, 1996). Many horse owners feed 60% of the diet in two, bucket meals of concentrate feed per day. Racehorses receive a diet with an 80:20 concentrate to roughage ratio (Hackland, 2007) and two to three large feedings of concentrate per day (Beyer, 2000). A study by Murray *et al.* (1996) showed that the number was as high as 91% in racehorses that were in training and this number increases to a 100% if the racehorses were running races. A *post mortem* survey that was conducted in Hong Kong determined that 66% of different class horses and 80% of racehorses in training had ulceration (Acland *et al.,* 1983). The presence of ulcers leads to discomfort and thus has a negative effect on performance (Vasistats *et al.*, 1999). Up to 37% of horses that are fed concentrates develop gastric squamous mucosal ulcers in comparison to horses that consume grass only (Coenen, 1990).

3.4 Consequences and duration of nutritional insults

The quality, quantity, pattern of ingestive behaviour or restriction of the diet can all lead to metabolic disorders (Durham, 2008). An insult due to an excess provision of nutrients is in some cases related to owner lack of knowledge but more commonly it is linked to the desire for improved performance. Improvement can be short lived as the horse may begin to develop metabolic or behavioural problems, which may in turn hinder performance. Although lack of performance can indicate an underlying problem, there are also other indicators such as aggression, difficulty to ride and other behavioural problems that may manifest due to the discomfort that a metabolic disorder is placing on the animal (Beerda *et al.*, 2003). Temperament of the horse can be linked to carbohydrate fermentation in the hindgut as it causes caecal distension (Cuddeford, 2000; Young 2011). Pagan (1997a) has found that there is a correlation between horses with a poor temperament and a rapid glycaemic response. Nutritional insults over a prolonged period can lead to metabolic disorders, behavioural problems and in some cases other health problems (Ellis, 2010). The duration as well as the level of insult that the GIT is under will determine the extent of the damage. In a period of three months there was a significant increase in coprophagy and wood shavings being eaten.

The majority of metabolic disorders develop over a prolonged period. This period can vary drastically according to breed, age, sex, exercise level and the extent to which it is being insulted. Severe short-term insults can have long-term implications. This is seen in organic acid absorption in small ruminants (Kriebel *et al*., 1995) and in racehorses (Medina *et al*., 2002). Racehorses are fed diets that comprise 80% bucket concentrate (Hackland, 2007) and the GIT is subjected to this nutritional insult for the time period they are kept in the racing industry, this can be for as little as two years or up to seven years depending on performance. It is for this reason that off-the-track TB's take between two and twelve months to rehabilitate (Young, 2011).

Conclusion

Although the purposes of horses may have changed over the decades, care and devotion to the horse as a companion animal have not. With the advent of technological systems for nutritional evaluation, and a myriad of *in vivo, in vitro* and *in silico* determinations, knowledge has grown. In addition to novel ingredient selection, there is processing and feeding management to closely scrutinise. *Post-mortem* investigations are invaluable to determine the effect of new feeding strategies on the actual transit times and influences on pH, pathology and digestibility within the gastrointestinal tract. Such investigations are crucial in allowing for nutritional evaluation protocols to be made relevant to what is currently being seen in the field.

CHAPTER 2

POST MORTEM INVESTIGATIONS OF HORSE GASTROINTESTINAL pH, NUTRIENT CONTENT AND MUCOSAL HEALTH

Abstract

The type and quantity of feed provided to horses is often specific to discipline . A horse is a hindgut fermenter and functions optimally on a diet high in good quality roughages, but because of the various performance requirements, horses are often provided nutrient dense feeds. The aim of the study was to investigate the linkage between nutrient intake, gastro-intestinal pH and gut mucosa. Quantitative and qualitative data were collected from 53 horses *post mortem* that were shot due to mechanical injuries or financial reasons. Zones of the gastrointestinal tract (GIT) were examined and pH levels were recorded from each location. PH levels in sections of the tract altered significantly ($P<0.05$) with the nutrient density categories. There was a significant ($P<0.05$) difference in the incidence of ulceration in relation to the age and nutrient density levels, with an increase in ulceration of the stomach in older horses and horses receiving high concentrate feeds. Ulceration and discolouration were highly correlated to nutritional parameters. This study shows that although diets are altered to improve performance, the overall health of the horse is negatively affected.

Keywords: Equine gastrointestinal tract, ulceration, nutrient density, pH

Introduction

The changes in the production systems of the horse and the raw materials and composition of rations over the last two decades (Harris & Bishop, 2007) has meant that there is a concomitant change in the response of horses to the way that they are kept. Horses are managed according to type of discipline they are used for, where and how they are kept, time of year, breed, age and financial circumstances (Harris, 1999). In the 20th century the production system of horses differed to the current production systems. In the 20th century 54% of horses that did light work were not supplemented with concentrate, the remaining 46% of horses in light work were fed a form of concentrate with 69% of horses spending more than 50% of their time grazing, 10% permanently grazing and only 2% that never grazed (Harris, 1999). In the 21st century horses are more commonly let out to graze on lush pasture with protein contents as high as 28% and an energy content equivalent to that of competition feeds. But, grazing on lush pastures can cause the same peaks and troughs of blood glucose and insulin as found in horses fed high starch and sugar concentrates (McIntosh, 2007). The change in feeding management may cause laminitis (Longland & Byrd, 2006) and possibly other metabolic diseases (Kalck, 2009).

There is limited research regarding how the equine gastrointestinal tract responds to the amount and type of feed being given (Harris and Bishop, 2007). Ulcers, colic and metabolic disorders such as insulin resistance and laminitis are on the increase (de Fombelle *et al.*, 2001; Kalck, 2009) and these have been linked to dietary management (Harris & Bishop, 2007; Kalck, 2009; Glunk *et al.*, 2013). The health and behaviour of the horse is of utmost importance irrespective of whether the horse is used as a recreational or performance animal. The extent to which the increase in nutrient demand for the expectation of performance is being regulated by the increase in feed intake needs to be questioned. Oftentimes, an inadequacy in the feed is countered by the provision of more of it (Young, 2011, *pers.comm.*). Dietary starch in excess of 4g starch per kg BW of the horse passes into the caecum and colon and is fermented by gram positive bacteria to produce lactic acid (Potter *et al.*, 1992). Hoffman *et al.* (2009) found a threshold of 0.296 unit NSC intake/ kg BW for the utilisation of CHO_f. In fact, many mechanisms of pre-caecal digestibility are precluded by high volume through-put rates (Potter *et al.*, 1992; Kienzle, 1994; Harris & Bishop, 2007), leaving many nutrients to be digested in the fermentative environment of the hindgut. Studies have been performed on the pH changes within the caecum of the horse on a high carbohydrate diet (Willard *et al.*, 1977; Medina *et al.*, 2002), but departure from an optimal pH along the entire GIT has not been studied concurrently with the changes that have been observed regarding quantity of nutrient dense feed, time spent grazing and the overall health of the horse. A response in the pH in sections of the GIT is anticipated with the increase in concentrate bucket feed proportions, with an expected decrease of pH in the caecum due to the production of VFA's (Hintz *et al.*, 1971; Willard *et al.*,1977).

There is a high prevalence of digestive disorders in horses that are kept in stalls, especially those of performance horses where high energy-dense feeds are used and forage intake is limited. Different ingredients within the diet can elicit significant changes within the micro-flora (de Fombelle *et al.*, 2001). Recent research has shown that feeds high in starches and sugars can be substituted with feeds high in fibre and fats. A study on foals with a fat and fibre supplement vs a starch and sugar supplement showed that foals that received the fat and fibre supplement were significantly less stressed at weaning and more settled than the group receiving the starch and sugar supplement (Nicol *et al.*, 2005). There has been a link shown between horses beginning to crib bite at weaning if they have been on a high concentrate diet (Nicol *et al.*, 2005).

A video endoscope showed that the stomach of crib biting horses was more inflamed and ulcerated. It has been proposed that crib biting is performed to increase the production of saliva which in turn buffers the stomach acid. Nicol *et al.* (2002) found crib biting horses to have a lower faecal pH than non-crib-biting horses thus showing that additional saliva production from crib-biting does not buffer to the extent that is required. When the energy source is derived mainly from fats and fibres, 33% of the horses stopped crib biting within a three-month period as stomach pH is higher than on a carbohydrate diet (Nicol *et al.*, 2002). A study by Murray *et al.* (1996) found racehorses with ulcers by the age of two years, which increased in severity and quantity the longer the horse was subjected to a high concentrate diet, intense exercise and stabled for long periods of time. It is important that an understanding is developed regarding the impact that feeding management has on the digestive tract and how it can be corrected without negatively impacting performance. Horses continually secrete acid whether they are being fed or fasted (Pagan *et al*., 1997). Horses that receive a higher roughage content secrete as much as twice the amount of saliva as horses that are fed grain (Meyer *et al.,* 1985). It is suspected that horses that are on a high fibre diet should have a higher pH along the GIT as saliva production is stimulated. Pellets and grain concentrates increase the production of gastrin which in turn stimulates gastric acid secretion (Smyth *et al.*, 1989) so not only do horses that are fed nutrient-dense diets with limited hay have a lower saliva secretion to buffer the acid (Pagan, 1997a), but they also over-secrete gastric acid. Intensively trained horses are generally fasted prior to exercise thus allowing an accumulation of acid in the stomach that is continuously washed up against the mucosa during exercise (Pagan, 1997a).

The objective of this experiment was to investigate the link between nutrient intake and pH in sections of the equine GIT and to consider mucosal gut health by performing *post mortem* analysis on a population of KwaZulu-Natal (KZN) horses shot for non-disease related issues.

Materials and Methods

Post mortem samples were collected from 27 horses in the KZN midlands that were shot for mechanical injuries or financial reasons. A spreadsheet was constructed where demographic and qualitative data were described for each horse. Demographic and quantitative data were collected for every horse and these included sex (male/female), body weight estimation (Carol & Huntingdon, 1988), age estimation (Evans and Jack, 2007; Parker, 2013) and breed. Horses were assigned to body weight categories as (1) less than 400kg (2) 400-525kg and (3) greater than 525kg. Teeth were used to age the horses into five year intervals: (1) less than five years

(2) 6-11 years (3) 11-18 years and (4) 18 years and above. Breed categories were (1) Thoroughbred (2) Warmblood (3) Arab or (4) other.

pH readings were measured directly *post mortem* at eight sections of the GIT: the cardiac and pyloric region of the stomach, duodenum, jejunum, ileum, caecum, transverse colon and rectum. Grab samples of the stomach, caecum, ileum, colon and rectal contents were also collected. The grab samples were elucidate, homogenous mixtures, taken as a representation of half a days feed intake to allow accurate segregation of horses into the respective nutrient density categories. The stomach was rinsed and the stomach lining was inspected for abnormalities such as ulcers and discolouration and presence or absence of each was recorded. Photographs were taken for evaluation of extent of the discolouration and ulceration. Ulcer severity was classified as follows: no lesions (0); one or two localized lesions (1); three to five localized lesions (2); five to ten localized lesions (3); and greater than 10 lesions or a large area of diffuse loss of surface epithelium (4) (Andrews *et al.*, 2002). Discolouration was classified as follows: none (1); less than 30% surface area (2); 30–50% surface area (3); 50-70% surface area (4); greater than 70% surface area (5)

Horses were separated into three classes based on the percentage (%) concentrate in stomachs: <30% (low), 30-50% (medium), >50% (high), and each class had 9 replicates. A selection of 54 samples collected for this study were sent to Cedara for nutrient analysis and used to calibrate the Near-infred spectrometer NIR. All the GIT content samples underwent NIR testing at UKZN. The NIR analysis was then used to define eight classifying variables: Crude Protein (CP), Ether Extract (EE), Digestible Energy (DE), Ash, Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), Acid Detergent Lignin (ADL) and Non-structural Carbohydrates (NSC) (Equation 2.1) that were used in a hierarchical cluster analysis (HCA) (Genstat® version 14, 2014) to produce three distinct groups that described the nutrient intake in each horse as high, medium or low density. This was on account of the fact that CP, DE and NSC were from highest for high nutrient density classes and lowest for the low nutrient density classes (table 2.1). Given that stomach samples were homogenous and stomach transit time is 6 hours (van Weyenberg, 2006) one can safely assume that reflects intake over foraging and meal time.

$$NSC\ \% = 100 - moisture\% - CP\% - EE\% - NDF\% - Ash\% \qquad 2.1$$

Statistical analysis

An unbalanced ANOVA (Genstat® version 14, 2014) was performed on the pH based on the nutrient density, age and region of the GIT. A chi-squared analysis and a binomial logistic regression model (Genstat® version 14, 2014) (Mc Conway *et al.*, 1999) was used to link each factor (X) (nutrient density, age category, sex, breed and body weight category) to the probability of having an effect on the presence of discolouration or ulcers. The factor score status was given either a 1 or a 0 depending on whether there was discolouration or ulcers (0 for "no ulcers" or "no discolouration" and 1 for "ulcers" or "discolouration") (Kleinbaum and Klein, 2002).

The model for the logistic regression used to determine probability of occurrence is given by the equation

$$Ln\ (P_1/1-P_1) = \alpha + \beta X_1 \ldots \beta_i X_i \qquad 2.2$$

Where

P_1 = probability of having "ulcers" or "discolouration"

$1-P_1$ = the probability of not having "ulcers" or "discolouration"

$P_1/\ (1-P_1)$ = Odds ratio: the ratio of the probability the presence of ulceration or discolouration (=1) over the probability of no ulceration or discolouration (=0)

X = the value of the predictor variable

α and β = parameters of the model

LN $(P_1/(1-P_1))$ is known as the logit or logistic link function (Mc Conway *et al.*, 1999) and was used to calculate whether nutrient density, breed, age, sex or body weight would increase or decrease the probability of ulcers or discolouration being present, given that the explanatory variable is placebo in nature (=0) and that adding the explanatory variable (=1) to the model will increase the probability of a successful outcome (ulcers or discolouration). It was thereby possible to assess whether the explanatory variables; nutrient density, age, breed, sex and bodyweight would increase the probability of discolouration and ulcers.

Results and Discussion

Nutrient content of the high, medium and low nutrient density categories are given in Table 2.1 above. In general race horses were assigned to the high density category and grass horses were assigned to the low nutrient category. Ether extract (EE) content from horses on a medium nutrient diet were close to double the content in the high and low nutrient diets. The ash content of the high and low nutrient diets were similar. The ADL content was highest (P=0.002) in the high nutrient diet, while the NSC was highest (P<0.001)in the high nutrient category.

Table 2.1 Segregation of horses into categories fed high, medium or low nutrient densities from the Hirachial Cluster Analysis (HCA).

Nutrient Density	CP%	EE%	DE%	Ash%	NDF%	ADF%	ADL%	NSC%
High	15.02^{b}	1.74^{b}	10.07^{c}	8.20	47.06^{a}	27.16^{a}	10.42^{b}	27.99^{b}
Medium	14.16^{ab}	3.02^{c}	9.65^{b}	6.96	54.77^{b}	27.06^{a}	6.15^{a}	21.22^{a}
Low	12.89^{a}	1.23^{a}	8.74^{a}	8.23	57.23^{b}	33.92^{b}	9.46^{a}	19.67^{a}
SED	0.781	0.205	0.179	0.642	1.986	1.727	1.044	1.427
LSD	1.628	0.427	0.375	1.346	4.156	3.615	2.179	2.987
Fprob	0.022	<0.001	<0.001	ns	<0.001	<0.001	0.002	<0.001

The pH measurements taken in this study from the cardiac stomach, pyloric stomach, duodenum, jejenum, ileum, caecum, colon and rectum changed in horses on different levels of concentrate, and in different regions of the GIT (Table 2.2). There was a higher pH in the pyloric region of the stomach of horses that are on a high nutrient dense diet in comparison to the pyloric region of the horses that were on a low and medium nutrient diet, this is contrary to what was expected. The pH of both the cardiac and pyloric regions of the stomach was lower (P<0.05) than all the other regions within the GIT, this was to be expected. The pH was lower (P<0.05) in the pyloric region of the low and medium nutrient dense diets than the pyloric pH on a high nutrient dense diet, this was not expected as horses fed hay are expected to have a higher pH because forage stimulates saliva production (Meyer *et al.*, 1985). Saliva production is double in horses that consume hay or fresh grass as compared to grain fed horses (Meyer *et al.*, 1985).

The 2 regions of the stomach, pyloric and cardiac, separated by the *margo plicatus* can be seen clearly in Figure2.1. In this study, the pH of the cardiac region deviated from the optimal pH of 7 by 2 points, while the pH in the pyloric region was within the optimal range of 1.5-4. The cardiac region consists of squamous epithelial cells, which have no protective mucosa layer and rely on the buffering capacity of the saliva. (Pagan, 1997b). It is therefore important that the pH remain as close to neutral to avoid mucosal damage, which was not reflected across the nutrient density levels. Andrews & Nadeau (1999) described similar results while Nadeau *et al.* (2000) showed horses fed on a diet high in protein and calcium comprising of alfalfa hay and grain had a higher stomach pH than horses on a low protein and high calcium diet.

Figure 2.1 The cardiac region with a slight degree of discolouration, pyloric region and *margo plicatus* is seen on the stomach lining of a three year old colt off the racetrack on a high nutrient dense diet.

The pH of the ileum was the highest between the regions for all nutrient density levels. There was less pH stratification visible between regions of the GIT in horses on a high nutrient dense diet. The pH of the caecal contents in the current investigation was slightly lower in horses that were on a high nutrient diet, but not significantly so (Table 2.2). It has been found that starch additions to caecal contents decrease the pH as fermentation occurs (Bailey *et al.*, 2002). The results in this study and by Bailey *et al.* (2002) were also similar to those found by Moore *et al.* (1979), where not only was there a decrease in pH of caecal contents of horses on a high starch diet, but there was also an increase in amine production namely phenylethylamine and isoamylamine. Absorption of these vasoactive compounds into the circulation system from a carbohydrate overload may be enough to cause peripheral circulatory disturbances (Bailey *et* *al.*, 2002). pH in GIT is affected by both the region of the GIT and the nutrient density of the diet, although difference in nutrient density are more clearly visible in the stomach.

Table 2.2 The *post mortem* pH of different regions of the GIT from horses fed a low, medium or high nutrient-dense diet.

Region	**Nutrient Density Class**	**pH**
Stomach Cardia	Low	5.14^{b}
	Medium	4.66^{b}
	High	4.61^{b}
Stomach Pyloric	Low	3.12^{a}
	Medium	3.28^{a}
	High	5.27^{b}
Duodenum	Low	6.33^{c}
	Medium	6.11^{c}
	High	6.00^{c}
Jejenum	Low	6.50^{cde}
	Medium	6.27^{c}
	High	6.26^{c}
ileum	Low	7.16^{e}
	Medium	7.08^{de}
	High	6.66^{cde}
Caecum	Low	6.38^{cd}
	Medium	6.39^{cd}
	High	6.19^{c}
Colon	Low	6.38^{cd}
	Medium	6.18^{c}
	High	6.16^{c}
Rectum	Low	6.62^{cde}
	Medium	6.47^{cde}
	High	6.57^{cde}
Overall Mean		5.88
SED		0.363
LSD $_{5\%}$		0.715
Fprob Region		<0.001
Fprob Nutrient Density		0.033
Fprob Region*Nutrient Density		0.002

^{a,b,c,d,e,} Values within a column are different if superscript differs (P<0.005)

Region of the GIT had a significant effect on pH with both the cardiac and pyloric stomach having the lowest pH (Table 2.3). This was to be expected, due to the stomach being responsible for chemical digestion. In an adult horse, gastric secretions are produced at the rate of one and a half litres/hour. These secretions contain between 4-60mmol of hydrochloric acid. The pH of the stomach contents is dependent on the type of feed and the region of the stomach (Andrews and Nadeau, 1999). The pH in the pyloric stomach of horses less than 5 years was significantly lower than in horses older than 6 years of age. The pH in the pyloric stomach of horses older than 18 years, was higher ($P<0.05$) from the pH in the pyloric stomach of horses between 6 and 18 years. The pH of the cardiac region of the stomach in horses 11-18 years of age is lower ($P<0.05$) than that of horses younger than 5 years. There was no significant difference between the pH in the duodenum, ileum, jejunum, caecum, colon and rectum over the different age categories. Generally older horses have teeth that are in poor condition that do not allow for sufficient chewing and will thus negatively impact the buffering capacity of the saliva (Pagan, 1997b; Meyer *et al.*, 1985), this was not demonstrated in this study (Table 2.3). There has been some evidence that dentition can cause digestive disturbances and colic (Frape, 2010). Horses with smooth occlusal surfaces are unable to chew correctly (Ashley *et al.,* 2005). This leads to a decrease in apparent digestibility and increases the fibre length in the faeces (Frape, 2010), which in turn can lead to impaction colic in the flexures. This causes a loss in peristaltic movement, which affects digestibility (Mair & Hillyer, 1997). Digestibility can also be impacted by the presence of worms. Worms in the digestive tract can cause extreme damage to blood vessel lining (Cohen *et al*., 1999; Frape, 2010) thus negatively impacting absorption.

Table 2.3 The *post mortem* pH of different regions of the horse GIT

Region	Age Class (years)	pH
Stomach Cardia	< 5	5.14^{d}
	5-11	4.86^{cd}
	11-18	4.29^{bc}
	> 18	4.80^{cd}
Stomach Pyloric	< 5	2.68^{a}
	5-11	3.83^{b}
	11-18	4.22^{bc}
	> 18	4.59^{cd}
Duodenum	< 5	6.35^{efgh}
	5-11	5.96^{e}
	11-18	5.91^{e}
	> 18	6.33^{efgh}
Jejenum	< 5	6.53^{efgh}
	5-11	6.14^{ef}
	11-18	6.18^{ef}
	> 18	6.47^{efgh}
ileum	< 5	7.34^{i}
	5-11	6.92^{ghi}
	11-18	6.68^{fghi}
	> 18	6.97^{hi}
Caecum	< 5	6.53^{efgh}
	5-11	6.20^{ef}
	11-18	6.25^{efg}
	> 18	6.32^{efgh}
Colon	< 5	6.43^{efgh}
	5-11	6.11^{ef}
	11-18	6.14^{ef}
	> 18	6.24^{efg}
Rectum	< 5	6.72^{fghi}
	5-11	6.46^{efgh}
	11-18	6.38^{efgh}
	> 18	6.59^{efgh}
Overall Mean		5.879
SED		0.357
LSD $_{5\%}$		0.702
Fprob Region		< 0.001
Fprob Age		0.105
Fprob Region*Age		0.001

^{a,b,c,d,e,} Values within a column are different if superscript differs (P<0.005)

Horses on a high nutrient dense diet, greater than 18years old had a higher GIT pH (P=0.006) than all other interactions. Possible reasoning for this is that horses of different ages respond differently to nutrient density, this could be due to the microbes being able to adapt over a long time period. (Table2.4)

Table 2.4 The *post mortem* GIT pH of different age categories of horses on a low, medium and high nutrient dense diet.

Age Class	**Nutrient Density Class**	**pH**
	Low	5.92[a]
< 5 years	Medium	5.91[a]
	High	6.08[a]
	Low	6.16[a]
5-11 years	Medium	5.77[a]
	High	5.52[a]
	Low	5.85[a]
11-18 years	Medium	5.73[a]
	High	5.70[a]
	Low	5.88[a]
> 18 years	Medium	5.80[a]
	High	6.55[b]
Overall Mean		5.88
SED		0.335
$LSD_{5\%}$		0.659
Fprob Age		0.105
Fprob Nutrient Density		0.033
Fprob Age*Nutrient Density		0.006

[a,b,c,d,e,] Values within a column are different if superscript differs (P<0.005)

A relationship was discovered between nutrient density and ulceration. Nutrient density increased (P<0.05) the probability of ulcers occurring, with older horses being more prone than younger horses (Table 2.5). Horses had a 99.99% chance of having ulcers when they received more than 50% concentrate (high nutrient dense diet) in their ration. Horses that were on a medium nutrient diet had a 69.6% chance of ulcers occurring when being compared to a low nutrient diet (Table 2.5). The duration of the nutritional insult in horses has a significant impact on ulceration. In this study, age is significantly related to ulceration: the older the horse, the higher the prevalence of ulceration. Horses between the ages of 6 and 18 years (class 2 and 3) have a 55-60% chance of obtaining ulcers in comparison to a young horse less than 5 years of age (class 1). A horse that is above 18 years of age has a 23-27% higher chance of obtaining ulcers than horses between the ages of 6 and 18 years (Table2.5). Orsini and Pipers (1997; cited in Bell et al., 2007) obtained similar results, but these results can be influenced by other factors or an interaction of factors. Young race horses can develop ulcers after three months of being in intense training (Murray *et al.*, 1994; Vasistas et al., 1999a) and Murray *et al.* (1989) found that even after intense training had ceased, 52% of race horses still had ulcers.

Table 2.5 Transformed logit probabilities of ulcers occurring in the stomach of horses following *post mortem* inbestigation on a high, medium or low nutrient diet with varying levels of age, sex, breed and body weight.

Treatment	Level	Chi pr	α	β		p(1)
Nutrient Density	Medium	0.007	0.405	0.421	p(x)	0.696
	High		0.405	8.800	p(x)	0.999
Age class	2	0.011	9.200	-8.800	p(x)	0.599
	3		9.200	-9.000	p(x)	0.550
	4		9.200	-7.600	p(x)	0.832
Sex	2	ns	0.944	0.154	p(x)	0.750
Breed	2	ns	1.288	0.320	p(x)	0.833
	3		1.288	-1.980	p(x)	0.334
	4		1.288	-1.000	p(x)	0.572
Body Weight	2	ns	-0.288	1.600	p(x)	0.788
	3		-0.288	1.490	p(x)	0.769

[a, b,c,d,e,] Values within a column are different if superscript differs ($P<0.05$) ns not significant where p(x) when x =1 is the probability of the horse having ulcers, p(x) when x = 0 is not having ulcers and α&β are parameters of the model

In this study 76% of horses displayed discolouration. Sex, breed, age, body weight and nutrient density did not influence discolouration (Table 2.6). This result was unexpected. Discolouration is the deviation from a normal colour. The squamous mucosa of the stomach is very sensitive to acid secretions. Any damage that is done to the mucosa is displayed in the form of discolouration or ulceration (Widenhouse *et al.*, 2002) and can be seen from images taken during this study (Figure 2.2 and Figure 2.3). The stomach mucosa is a soft fleshy pink colour. When damage has been done, the mucosa begins to turn to a darker pink almost red, and progresses to a dark purple brown colour depending on severity of the insult (Figure 2.3)

Figure 2.2 Stomach lining of a two-year-old colt off the racetrack, fed a high nutrient dense diet displaying ulceration along the *margo plicas.*

Figure 2.3 The stomach lining of a two year old filly off the racetrack fed a high nutrient dense diet, displaying abnormal purple discolouration in both the pyloric and cardiac region.

Table 2.6 Transformed logit probabilities of discolouration occurring in the stomach of horses investigated *post mortem* on a high, medium or low nutrient diet with varying levels of age, sex, breed and body weight.

Treatment	Level	Chi pr	α	β		p(1)
Nutrient Density	Medium	ns	1.386	0.172	p(x)	0.826
	High		1.386	-0.087	p(x)	0.786
Age class	2	ns	1.099	1.390	p(x)	0.923
	3		1.099	0.000	p(x)	0.750
	4		1.099	0.510	p(x)	0.833
Sex	2	ns	1.153	0.639	p(x)	0.857
Breed	2	ns	1.455	-0.762	p(x)	0.667
	3		1.455	6.800	p(x)	1.000
	4		1.455	0.340	p(x)	0.858
Body Weight	2	ns	1.79	-0.290	p(x)	0.818
	3		1.79	-0.590	p(x)	0.769

[a, b,c,d,e,] Values within a column are different if superscript differs (P<0.05) ns not significant where p(x) when x =1 is the probability of the horse having discolouration, p(x) when x = 0 is not having discolouration and α&β are parameters of the model

There was a 76% and 65% mean incidence of horses with discolouration and ulceration respectively.

In this study majority of horses on a high nutrient diet were either race or show horses that were doing high intensity exercise and therefore receiving large amounts of bucket concentrate feed. High grain concentrates, drops the pH and causes ulcers in most instances (Murray *et al.*, 1989; Lorenzo *et al.*, 2002; Dionne *et al.*, 2003; Andrews *et al.*, 2005). This was clearly be seen in this study and others, where horses receiving a medium to high nutrient dense diet had a 70% more likely chance of obtaining ulcers. Horses that were fed a mixed feed of 128g DM/kg protein and 175g DM/kg crude fibre (low to medium nutrient dense diet) had a higher prevalence of ulcers in the cardiac region of the stomach along the *margo plicatus*, as compared to horses on a hay diet (Coenen, 1990). Diets that are high in carbohydrates and protein increase the risk of ulceration (Andrews *et al.*, 2005). It has been determined that a high energy feed plays a role in gastric ulceration (Goodson *et al.,* 1988; Clarke *et al.,* 1990; de Fombelle *et al.,* 2001; Drogoul *et al.,* 2001; Julliand *et al.,* 2001; Hussein *et al.,* 2004; Lopes *et al.,* 2004). Ulceration can be seen clearly in Figure 2.5 and Figure 2.6.

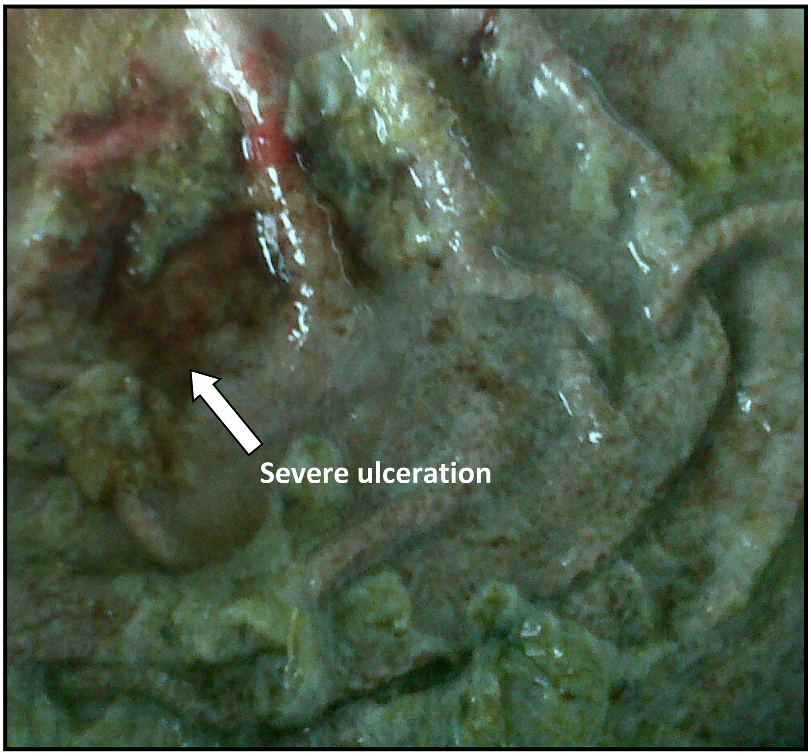

Figure 2.5 Stomach lining of a retired partbred Arab displaying ulceration occurring along the margo plicatus

Figure 2.6 Stomach lining of a retired partbred Arab with severe ulceration that has occurred in the cardiac stomach.

The ulcers that were seen in performance horses were due to the incorrect feeding and management (Pagan, 1997b). It is common for concentrate to make up 50% or more of the diet. This causes a poorly buffered and acidic environment in the stomach (Davidson & Harris, 2002; Andrews *et al.*, 2006). Horses that were on a high nutrient diet were high performance horses confined to small areas for long periods of time. Behavioural changes such as crib biting, wind sucking and ill temperament are all consequences of the feeding management and confinement (Frape, 2010). Although not seen in this study, poor body and coat condition (Vasistas *et al.* 1999a; Dionne *et al.*, 2003) can be signs of ulceration. Horses that are in high intensity training generally have a degree of ulceration as there is a strong correlation between exercise and ulceration (Pagan, 1997b). This is due to the admixture of stomach contents, which allows the gastric acid to wash up against the stomach mucosa (Luthersson *et al.*, 2009). Common practice in the racing industry is for horses to train early morning prior to the morning meal. The stomach pH of horses that have been fasted for several hours is either less than or equal to two. Fasted horses have a lower pH than horses that receive timothy hay *ad libitum* (Murray & Schusser, 1989). Fasting even for a short period has been linked to ulceration, due to the stomach lining having been exposed to stomach acid, this may the reason horse on a high nutrient dense diet have a higher ($P<0.005$) occurrence of ulceration. In the current study, horses that are on a low nutrient diet have fewer ulcers than horses on a high nutrient dense diet. Horses on a medium nutrient dense diet had 69% more ulcers than horses that are on a low nutrient diet, Pagan (1997b) saw similar results.

There has been some controversy with regard to breed having an impact on ulcer occurrence. In this study there was no significant difference of ulcer occurrence between Thoroughbreds and Arabians. However, Furr *et al.* (1994) showed, an increase (P 0.035) in post-prandial concentrations of gastrin serum in Arabians that had high exercise levels thus leading to the possibility of increasing the occurrence of ulceration and colic (Cohen *et al.*, 1999). The results that were obtained may not be linked to breed but rather the interaction between exercise intensity and breed. In this study, horses on a high nutrient diet generally had a higher ($P = 0.007$) than average exercise level and showed a 99.99% chance of obtaining ulcers. These results are similar to those found by Vasistas *et al.* (1999b) and Murray *et al.* (1996). Murray *et al.* (1996) investigated the occurrence and severity of ulcers in 2-9 year old race horses that had either been raced, in the previous two months prior to endoscopic examination or not. In a study done by Orsini and Pipers (1997) it was found that the duration that horses were in training impacted the amount and severity of ulcers. In the same study 28% of the horses had ulcers prior to training, with ulceration increasing to 63% in training. The results found in this study and by others shows that although there is a strong correlation between exercise and ulceration, exercise is not the only factor impacting ulceration.

Conclusion

There is a consequence of nutrient intake and pH along the GIT. Investigations of horses *post mortem* indicate that horses are being fed more than 50% bucket concentrate feed, of a high nutrient density, which causes detrimental effects along the GIT, observed through ulceration and discolouration.

CHAPTER 3

IN VIVO NUTRIENT DIGESTIBILITY

Abstract

There has been a change in feeding management with the expectation that by increasing bucket concentrate feeds there will be an improvement on performance. The ability of the digestive tract to sufficiently digest the additional nutrients has not been taken fully into consideration. The aim of this study was to determine the impact that nutrient intake, age and mucosal damage had on digestibility in the horse. Novel use was made, *post mortem*, of a sample of 53 sport and leisure horses shot for mechanical failure and financial reasons for an *in silico* analysis of nutrient intake and digestibility. The horses were segregated according to three nutrient categories, with the stomach ingesta categorising either a high, medium or low nutrient intake. Stomach, ileal, caecal, colon and rectal content samples were collected and analysed for standard nutrient parameters. Laboratory analysis of the stomach and rectal samples were used to calculate digestibilities of several nutritional variables. Nutrient synchrony was investigated as the relationship between certain nutrients in the fore- and hindgut. Low and high nutrient intakes had lower ($P<0.01$) protein and NDF ($P<0.05$) digestibilities while NSC digestibility was significantly lower ($P<0.05$) in horses on a low nutrient diet. It was found that ulceration also had a negative impact on digestibility. These results show that increasing bucket concentrate feed not only increases the occurrence of ulceration, but also decreases the digestibility of nutrients.

Keywords: Equine ulcers, nutrient digestibility, nutrient synchrony

Introduction

There has been a large amount of research performed on improving digestibility of feeds (Meyer *et al.*, 1993; Kohnke, 1998; Jackson, 2000; Julliand *et al.,* 2006). Different raw materials have varying degrees of digestibility which are further altered by feed processing. This is mainly due to the gelatinization of starch and protein denaturation (Rosenfeld & Austbo, 2009). The degree to which gelatinization of starch occurs is dependent on the type of processing that has occurred. Micronization and extrusion have been the most common feed processing methods to improve the digestibility of raw materials. Raw materials that are more digestible are more easily utilized by the horse. Mean retention time (MRT) in the gastrointestinal tract (GIT) is correlated to digestibility, water balance, exercise performance and digestive disorders (van

Weyenberg *et al.*, 2006). Factors that have an impact on MRT are; breed, exercise intensity and feed composition. The amount of fibre slows passage rate. If the feed passes through the digestive tract at a rapid rate, absorption of nutrients is not maximised and there is thus a negative impact on digestibility. Longer retention times are positively correlated with improved digestibility, increased microbial activity and increased water absorption (Rosenfeld & Austbo, 2009). Diets with high percentage of low quality roughage have a negative impact on digestibility due to higher proportions of Lignin (Abdullah *et al.*, 2012). Factors that can cause variation in digestibility are; feeding level, diet composition, physical form, body size, exercise and nutrient synchrony (Huhtanen & Kukkonen, 1995; Pagan *et al.*, 1998; Volden, 1999; Drogoul *et al.*, 2000; NRC, 2007; Hersom, 2008). If nutrient synchrony may be obtained then microbial activity is optimised in the caecum thus improving digestibility (Hersom, 2008).

The function of the small intestine is for the absorption of non-structural carbohydrates, protein and fat while that of the large intestine is digestion of fibre. When an excess amount of nutrients such as rapidly CHO_f, is fed, the absorptive capacity of the small intestine is exceeded. Nutrients that were meant to be absorbed in the small intestine then pass into the hindgut; this increases VFA production which drops the pH and negatively impactsdigestibility (Julliand *et al.*, 2006).

There is a positive correlation between light exercise, DM intake and digestibility (Orton *et al.,* 1985a; 1985b). Pagan *et al.* (1998) found a slight decrease in DM digestibility with an increase in exercise intensity, which was due to a slight decrease in ADF digestibility. Increasing the percentage of fat in the concentrate feed to a level in excess of nine percent is becoming increasingly popular especially in the endurance fraternity (Treiber *et al.*, 2006; Frape, 2010). However, there has been concern on how additional fat impacts fibre digestibility, as microbial function is sub-optimal with high fat diets (Jansen *et al.,* 2007a, 2007b).

There are many factors that have an impact on digestion. However, there is limited information available on how digestion is influenced in the different regions of the gastrointestinal tract and how digestion in one region affects digestion in the next region (Julliand *et al.*, 2008). Kriebel *et al.* (1995) found that acute short-term nutritional insults can have long term implications with regard to organic acid absorption in ruminants. It has been found that an increase in concentrate feed can have a negative impact on gut mucosa (Vasistas *et al.*, 1999a) and feeding as little as 1g/kg BW starch has a negative impact on the digestive capacity of the small intestine (Potter *et al.*, 1992). Therefore, pathology in one section of the tract is likely to influence digestibility. Poor appetite, dullness, attitude changes, decreased performance, poor BCS, rough hair and coat, weight loss and colic are all symptoms of gastric ulceration (Vasistas *et al.*, 1999b).

Hence, the objective of this research was to determine if there is a link between nutrient digestibility in the sections of the GIT with mucosal integrity observed in the previous chapter (2). Higher nutrient densities and intakes may be less well utilised in the horse, and the pathologies that are evident (ulceration/discolouration) may influence digestion coefficients of key nutrients.

Materials and Methods

Samples were collected *post mortem* from 27 horses in the KZN Midlands that were shot for mechanical injuries or financial reasons. Samples of 500ml each were collected from the stomach and rectum for chemical analysis. The samples were placed into sample packets and frozen until analysis could be performed. The samples were defrosted, dried and milled through a 1mm sieve. Dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent Fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and ash content were determined. Hemi-cellulose (HC), cellulose, non-structural carbohydrates (NSC) on an as fed basis (Pagan, 1997a) and digestible energy (DE) in kCal/kg DM (Pagan, 1998) were calculated using the equations below. ADL was used as the marker for the digestibility calculations.

$$\text{HC} = \text{NDF} - \text{ADF} \tag{3.1}$$

$$\text{Cellulose} = \text{ADF} - \text{ADL} \tag{3.2}$$

$$\text{NSC \%} = 100 - \text{Moisture (\%)} - \text{CP (\%)} - \text{EE (\%)} - \text{NDF (\%)} - \text{ash (\%)} \tag{3.3}$$

$$\text{DE} = 2118 + 12.18\text{CP\%} - 9.37\text{ADF\%} - 3.83\text{HC\%} + 47.18\text{EE\%} + 20.35\text{NSC\%} - 26.3\text{Ash \%} \tag{3.4}$$

$$\text{Digestibility (nutrient) \%} = 100 - \text{ADL}_{feed}/\text{ADL}_{faeces} \times \text{Nutrient}_{faeces}/\text{Nutrient}_{feed} \times 100 \tag{3.5}$$

Age class had four levels, with five year intervals, less than or equal to 5 years (1), 6-11 years (2), 11-18 years (3) and greater than 18 years (4). Each age class comprised of a minimum of 6 replicates, with the average being 8 per group. Ulcer severity was classified as follows: no lesions (0) ; one or two localized lesions (1); three to five localized lesions (2); five to ten localized lesions (3); and greater than 10 lesions or a large area of diffuse loss of surface epithelium(4) (Andrews *et al.*, 2002). Each age class comprised of an average of 6 per group with the exception of class 4, that comprised of 2 replicates. Discolouration was classified as follows: none (1); less than

30% surface area (2); 30–50% surface area (3); 50-70% surface area (4); greater than 70% surface area (5). Each age class comprised of an average of 6 per group with the exception of class 4, that comprised of 2 replicates

Nutrient density class were assigned using the NIR values of the analysis of the stomach content of 27 horses. A hierarchical cluster analysis (Genstat® version 14, 2014) using the following classifying variables (CP, EE, DE, Ash, NDF, ADF, ADL and NSC) was done. Three clusters each with 9 replicates, were determined that were described as being of a high, medium and low nutrient density. This was on account of the fact that CP, DE and NSC were from highest for high nutrient density classes and lowest for the low nutrient density classes (table 2.1 in chapter 2). Given that stomach samples were homogenous and stomach transit time is 6 hours (van Weyenberg, 2006) one can safely assume that reflects intake over foraging and meal time.

Unbalanced ANOVA using Genstat 14th edition was performed on each of EE, NDF, ADF, Ash, HC, Cellulose, NSC, DE and CP digestibility values with nutrient density, age, ulceration presence and discolouration presence as treatment factors. Unbalanced ANOVA using Genstat 14th edition was performed on the EE, NDF, ADF, Ash, HC, Cellulose, NSC, DE, CP and CHL digestibility values with nutrient density, age, ulceration severity and discolouration severity as treatment factors.

Results and Discussion

The digestibility of nutrients is significantly impacted ($P<0.001$) by nutrient density (Table 3.2). There was a higher ($P<0.05$) cellulose digestibility in horses fed a low nutrient diet than horses on a medium and high nutrient diet. DE, CP and fat digestibility was higher ($P<0.001$) for the low nutrient dense diet while a high nutrient dense diet decreased ($P<0.05$) the ADF and NDF digestibility. In general, horses that were on a high nutrient dense diet were unable to digest nutrients, mainly fibre as efficiently as horses that were on a lower nutrient dense diet.

Table 3.2a The effect of age, nutrient density, discolouration and ulceration presence on the digestibility of CP, ADF, NDF, EE, Ash, CHL, Cellulose, DE, HC and NSC in the digestive system of the horse.

Treatment	Level	CP (%)	ADF (%)	Ash (%)	EE (%)	NDF (%)	CHL	Cellulose	DE	HC	NSC
Age	< 5 years	49.79[ab]	32.82	20.49	-25.67	48.42	73.56	45.03	43.66[ab]	69.63	37.98[b]
	5-11 years	49.8[ab]	31.1	22.23	1.855	51.43	75.9	42.79	32.2[a]	73.72	1.95[a]
	11-18 years	40.38[a]	25.77	4.345	-56.72	41.27	72.19	42.5	32.67[a]	74.96	24.39[ab]
	> 18 years	61.48[b]	17.01	18.58	-32.64	45.93	72.33	38.04	50.16[b]	79.16	49.41[b]
Fprob		0.01	ns	ns	ns	ns	ns	ns	0.023	ns	0.035
SED		6.88	8.573	15.39	30.86	10.72	9.89	7.641	6.171	10.448	15.81
LSD $_{(5\%)}$		14.66	19.1	34.3	65.77	23.89	22.39	17.62	13.75	23.63	35.23
Nutrient density	Low	64.81[b]	43.47[b]	30.61	51,13[b]	40.95[a]	88.28[b]	57.41[c]	61.4[b]	83.76	52.55[b]
	Med	42.52[a]	31.48[b]	14.38	-54,59[a]	64.44[b]	71.8a[b]	42.82[b]	25.91[a]	73.39	-2.38[a]
	High	45.5[a]	11.85[a]	8.016	-56,86[a]	48.91[ab]	63.53[a]	28.11[a]	36.74[a]	65.44	40.95[b]
Fprob		<0.001	0.004	ns	<0,001	0.008	0.022	0.002	<0.001	ns	0.009
SED		4.363	7.207	12.94	22,16	9.012	7.966	5.879	5.187	8.41	13.29
LSD $_{(5\%)}$		9.721	16.06	28.83	49,38	20.08	18.02	13.56	11.56	19.03	29.61
Ulceration Presence	No	43.39	25.58	14.21	-106,6	47.49	68.88	49.76	31.26	80.14	-1.187
	Yes	50.63	28.25	16.86	-11,21	46.78	74.73	41.63	40.55	72.26	32.96
Fprob		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
SED		5.73	10.14	14.38	25,70	10.32	8.944	13.51	7.256	10.28	15.34
LSD $_{(5\%)}$		12.22	21.61	30.64	54,81	22.00	19.18	29.2	15.47	22.06	32.7
Discolouration Presence	No	43.8	15.96	5.65	-18.41	35.35	61.72	16.41[a]	36.98	58.24	37.19
	Yes	49.9	28.91	17.42	-28.69	48.01	74.86	36.98[b]	39.12	75.23	26.05
Fprob		ns	ns	ns	ns	ns	ns	0.011	ns	ns	ns
SED		5.859	9.678	17.38	29.76	12.1	10.43	7.235	6.966	11.02	17.85
LSD $_{(5\%)}$		13.05	21.57	38.72	66.32	26.97	23.6	16.68	15.52	24.92	39.77

[a,b,c,d,e,] Values within a column are different if superscript differs, ns not significant, CP Crude Protein, ADF acid detergent fibre, NDF neutral detergent fibre, EE ether extract

Table 3.2b The effect of ulceration and discolouration sevrity on the digestibility of CP, ADF, NDF, EE, Ash, CHL, Cellulose, DE, HC and NSC in the digestive system of the horse.

Treatment	Level	CP (%)	ADF (%)	Ash (%)	EE (%)	NDF (%)	CHL	Cellulose	DE	HC	NSC
Ulceration Severity	1	43.39	25.58	14.21	-106.6[b]	47.49	68.88	49.76	20.22	80.14	-1.187
	2	51.09	26.45	15.01	-4.695[a]	46.2	74.66	39.93	37.73	72.59	34.6
	3	48.83	30.04	21.11	-1.853[a]	45.00	72.38	40.63	33.73	63.1	39.24
	4	51.27	36.34	20.35	-72.3[ab]	54.13	79.83	53.76	23.37	88.54	9.743
Fprob		ns	ns	ns	0.025	ns	ns	ns	ns	ns	ns
SED		7.07	11.68	20.97	35.92	14.6	12.83	8.968	8.406	13.54	21.54
LSD $_{(5\%)}$		15.75	26.02	46.73	80.03	32.54	29.01	20.68	18.73	30.63	47.99
Discolouration Severity	1	43.8	15.96[a]	5.65	-18.41	35.35	61.72	24.14[a]	36.98[ab]	58.24	37.19
	2	48.41	26.61[ab]	17.99	-43.74	46.33	70.27	41.78[abc]	33.09[a]	72.2	12.88
	3	52.23	25.27[ab]	9.203	-5.398	47.75	81.49	42.84[abc]	47.59[b]	83.81	45.12
	4	52.29	47.8[b]	32.24	-35.02	60.72	85.45	62.62[c]	43.45[b]	81.74	29.08
	5	45.18	19.34[a]	16.04	16.03	29.89	60.5	30.44[ab]	41.53[ab]	46.25	47.48
Fprob		ns	0.029	ns	ns	ns	ns	0.004	0.036	ns	ns
SED		9.372	11.27	27.8	47.61	19.36	16.64	11.51	11.143	17.57	28.55
LSD $_{(5\%)}$		20.88	25.11	61.94	106.08	43.14	37.64	26.54	10.07	39.74	63.61
Overall Mean		49.37	27.78	16.4	-27.8	46.91	73,66	42.79	38.93	73.69	27.02

[a,b,c,d,e,] Values within a column are different if superscript differs, ns not significant, CP Crude Protein, ADF acid detergent fibre, NDF neutral detergent fibre, EE ether extract

A balance of good quality nutrients is important for optimal utilisation as depicted in this study. Potter *et al.* (1992) found feeding as little as 1g/kg BW starch impacts the digestive capacity of the small intestine, this is even more so if the starch is of a low quality (Hussein *et al.,* 2004). Horses in this study that were on a medium nutrient dense diet had a better overall digestibility than horses on either a low or high nutrient dense diet. These results are more likely due to the fibre quality for the low nutrient diets or an overload of rapidly fermentable substrate for the high nutrient diets. Fibre digestibility is reduced with the incorporation of concentrate into the diet (Pagan *et al.*, 1998), the effect being more severe with intense exercise. These results were not mirrored in this study as horses on the medium nutrient diet had better ADF and NDF digestibility than the low or high nutrient diets. This could be due to the quality of the fibre that was being fed in either of the nutrient density categories. It was expected that horses on the high nutrient diet would have a lower fibre digestibility and that horses on a low nutrient diet would in turn have a higher fibre digestibility. These results could be due to the acclimatisation of the microbes or quality of roughage. Grass fed horses generally fell into the low nutrient density category. Grass can provide a source of highly digestible nutrients, dependent on the quality, which is influenced by type, maturity, soil quality, rainfall and season (Pagan, 2000). The forage quality may be responsible for causing the low digestibilities of horses on a low nutrient diet. Race horses are fed between 3.8 – 13.2kg of concentrate a day and 27% of racehorses have a faecal pH less than 6.2. This has an adverse effect on the microbial population and mucosa present in the intestine (Richards *et al.*, 2006). This explains the digestibility of rapidly fermentable substrate being lower for horses on a high nutrient diet in comparison to a medium nutrient diet.

The digestibility of fat is perplexing as there were incidences of negative digestibility across all treatments factors. Fat digestibility was higher ($P<0.001$) for horses on a low nutrient dense diet. Although ideal fat inclusion levels are yet to be determined, it can still be seen that as fat intake increases there is a negative impact on fibre digestibility. Jansen *et al.* (2007a, 2007b) found similar results of fat intake as low as 5% inclusion, having a negative impact on fermentative capacity and fibre digestibility. Different types of fats have varying degrees of digestibility within the gastrointestinal tract (Flothow, 1994; cited in Zeyner, 2008). Adding fats into the diets of horses increases the amount of fat present in the faeces (Zeyner, 2002). Endogenous fats are present with 130mg/kg BW/day at the end of the ileum and 60 mg/kg BW/day in the faeces. In this study higher levels of fat were found in the rectal region. However, the amount exceeds that reported by Meyer and Sallmann (1996; cited in Zeyner, 2008). The excess fat may be due to the breakdown of the phospholipid membrane from a combination between nutrient density, ulceration and discolouration. Nutritional disorders such as

Zollinger- Ellison syndrome, coeliac sprue and hyperparathyroidism may also be contributing factors and need to be taken into consideration.

Zollinger- Ellison syndrome (ZES) is caused by the non-beta islet cells that are present in the gastrin secreting tumour of the pancreas. These cells stimulate the acid secreting cells that are found in the stomach to maximal capacity therefore leading to ulceration of the gastrointestinal tract. These tumours have also been found in the duodenum and the abdominal lymph nodes. Common signs of this syndrome are diarrhoea and steatorrhea and can be treated with a proton pump inhibitor such as omeprazole, the same drug used to treat gastric ulcers.

Coeliac disease also known as coeliac sprue, an auto immune disease of the small intestine is a common disease that occurs in 1:100 people and is caused by the intake of gluten products. The inflammation that occurs with coeliac sprue leads to the malabsorption syndrome with diarrhoea, steatorrhea, weight loss and failure to thrive as well as deficiencies of the fat soluble vitamins, folic acid, calcium and iron (Woodward *et al.,* 2010).

There are other diseases that are linked to coeliac sprue, of particular interest, are diseases that relate to the thyroid having 6-8% prevalence (Ciclitira *et al.*, 2005). It has been found in human studies that there is a 10-15% association between coeliac sprue and distal ulcerative colitis (Kiln *et al.*, 1980; cited by Ciclitira *et al.,* 2005). The proximal small intestine is the most affected. In severe cases the lesions can extend into the ileum (Ruben *et al.*, 1962; cited by Ciclitira *et al.,* 2005) and abnormalities of the mucosa in the rectum have also been found. A difference in histological appearance of the intestinal mucosa can be seen where coeliac disease is present. The intestinal villi structure and height is affected, with the mucosa appearing flattened, due to the partial mild villi atrophy where length is affected or severe atrophy where the villi are non-existent (Ciclitira *et al.*, 2005).

Hypoparathyroidism, a decreased function of the parathyroid hormone, leads to low plasma calcium levels. Hypoparathyroidism can be caused by a number of factors but of particular interest is from an auto immune disease such as coeliac sprue (Wortsman *et al.*, 1994; Frysak *et al.*, 2000; Gelfand & DiMeglio 2007). The treatment of steatorrhea in hypothyroidism can be done with the use of medium chain triglycerides (Lorenz and Burr, 1974), administration of vitamin D (Corazza *et al.*, 1995) and the correction of hypocalcaemia (Peracchi *et al.*, 1998). Calcium levels can appear normal in secondary hypoparathyroidism that is caused by malabsorption and steatorrhea. High calcium diets reduced serum triglycerides by 23% this is due to the reduction in saturated fatty acid absorption (Brink *et al.,* 1995). Increasing calcium leads to the formation of non-digestible calcium soaps which in turn reduces the fatty acid absorption within the digestive tract (Denke *et al.*, 1993; Papakonstantinou *et al.*, 2003). Steatorrhea is the main digestive manifestation of hypoparathyroidism, which can be linked to an autoimmune process (Maeda *et al.*, 2006). A duodenal biopsy can be done to determine for the presence of coeliac sprue. A gluten free diet needs to be considered if coeliac sprue is present (Matsueda & Rosenberg, 1982; cited in Abboud *et al.* 2011; Isaia *et al.*, 2004; cited in Abboud *et al.* 2011). In the presence of coeliac sprue the level of parathyroid hormone may not deviate from normal due to parathyroid atrophy or secondary hypoparathyroidism (Jorde *et al.*, 2005). The relationship between primary hypoparathyroidism and peptic ulceration has no conclusive results as the research that was performed was done so 50 years ago and had a great degree of controversy (Barreras & Donaldson, 1967; Ellis, 1968). Peptic ulcers had a 5% occurrence while hypoparathyroidism had a 30% occurrence in the general population. Other studies have seen a 10-fold increase of hypoparathyroidism in patients that had duodenal ulcers (Frame & Haubrich, 1960; cited in Abboud *et al.,* 2011). Majority of the symptoms that are present in Zollinger-Ellison syndrome, coeliac sprue and hypoparathyroidism are similar to the results of the current study. The above-mentioned nutritional orders are more prominent in human studies, and although data available in this study is not sufficient to conclude that these disorders can be present in horses, the possibility cannot be discounted.

Ulceration severity had an effect on fat digestibility, while discolouration severity affected cellulose and digestible energy significantly ($P<0.05$). A discolouration severity of one had the lowest digestibility while a severity level of five and four had the highest digestibility for cellulose and digestible energy respectively. It was expected that either the presence or severity of discolouration and ulceration would have an impact on digestibility. However this was not seen in this study, in some instances digestibility was higher when ulceration and discolouration was present.

Age significantly ($P<0.05$) influenced the digestibility of CP (Table 3.2). Horses that were over 18 years digested protein better than horses between 11 and 18 years. It is interesting that an older horse has a better crude protein digestibility. A possible explanation for this is that older horses are generally retired and face less insults to the digestive system or they have recovered from nutritional insults.

Conclusion

Feeding management is imperative for the overall GIT functioning of the horse. It can be concluded that a high nutrient dense diet that is commonly being fed to the average performance horse does not only decreases the digestibility of nutrients within the feed, but also negatively impacts the mucosal integrity of the GIT, which has a compounding effect on digestibility.

CHAPTER 4

THE EFFECT OF BUFFER pH ON GAS PRODUCTION FERMENTATION PROFILES OF HIGH STARCH HORSE FEEDS *IN VITRO*

Abstract

Certain microclimates within the hindgut need to be maintained for optimal functioning of the gastrointestinal tract (GIT). The objectives of this study were to investigate the effect of current feeding management on the caecal environment, and how to adjust fermentation protocols to accurately mimic that of the equine digestive tract. Treatments consisted of 2 faecal inoculum levels, 3 buffer pH levels and 2 substrates. Treatments were incubated for 24 hours and gas profiles fitted to the model as derived by Campos *et al.* (2004) to determine gas production kinetics. The results indicate that the standard IVGPT protocol can be influenced significantly ($P<0.001$) by buffer pH and while IVGPT methods promote a buffer of pH 7.2, degradation parameters and change in pH indicates at buffer of pH 6.5 may provide a better representation of degradability *in vivo.*

Keywords: Faecal inoculum, gas production, microbial activity

Introduction

A horse is a hindgut fermenter that requires large quantities of good quality roughage within the diet for optimal gut function (Ellis & Hill, 2005), which is often overlooked in feeding management (Geor, 2010). Many changes in the management systems of the horse have been related to an increase in the provision of high starch diets. There have also been marked increases in the incidence of metabolic diseases (Kalck, 2009), which may well be related to feeding frequency, the amount of forage and grazing available and mostly to the composition of the diet the horses receive. At the one extreme are race horses that have been shown to receive close to 80% of their daily intake as bucket concentrate feed (Hackland, 2007). Horses leave their racing careers for several unidentified reasons (Hayek, 2004; Parkin *et al*., 2004; Velie *et al*., 2013) one of which could be related to the increased incidence of nutritional issues.

The IVGPT method has already been adapted and refined (Tilley and Terry, 1963; Minson and McLeod, 1972; Murray *et al.*, 2003; Hussein *et al.*, 2004; Lindsay, 2005; Hackland, 2007) to mimic the digestive system of the horse, but with current feeding regimes changing to incorporate increased proportions of bucket concentrate, it is worth considering whether the method needs to be re-evaluated for accurate prediction of degradability. The method of McDougall (1948) is still followed for the preparation of buffer solution in IVGPT. The buffer pH that is obtained in conventional horse assays is 7.2 (Bailey *et al*, 2002; Young, 2011). The function of the buffer is to resist minimal changes in pH (van Slyke, 1922). This is necessary for optimal microbial activity. With an increase in bucket concentrate feeding there is an increase in the production of VFA's which decreases the pH (Hoffman, 2003). A decrease in pH will lead to a change in the microbial population (Ellis and Hill, 2005) hence a change in the degradability of feedstuffs. It is important that the standard buffer pH that is currently stipulated in degradability research is re-evaluated to mimic these changes.

Numerous types of grasses and cereals are incorporated into a horse's diet. Lucerne, grass legume, maize and cereal are common raw materials that are used in horse feeds. Maize grain has 8-10% CP, 65% starch and 9.5% NDF (NRC, 2007; Frape, 2010). Lucerne has 15-20% CP and 32-50% NDF depending on maturity (NRC, 2007; Frape 2010). Horses are grazing animals with a gastrointestinal tract that is adapted to the assimilation of energy and nutrients from plant fibre (Geor, 2010). Excessive amounts of rapidly fermentable substrates that enter the large intestine can destabilise the microbial environment (Milinovich *et al*., 2006, 2010). Horses receiving large amounts of bucket concentrate feeds will therefore have a different microbial population to horses that predominantly graze. Faecal inoculum that is obtained from race horses will have a microbial population that is better adapted to a high starch diet in comparison to horses having roughage as the main part of their diet. Buffers by definition are there to resist small changes in pH. If caecal pH is lower due to a higher starch content then for accurate prediction of degradability, buffer pH used in IVGPT should be similar to that found in the caecum.

Materials and Methods

The *in vitro* gas production technique (IVGPT) as described by Pell and Schofield was used following the modification of Tilley and Terry (1963) for correct hindgut representation.

Buffer pH consisted of 3 levels: 7.2, 6.5 and 5.5. Buffer 7.2 was used as the standard, with buffer pH 6.5 and 5.5 having been determined from pH results obtained along the GIT in the previous chapter (2). Inoculum consisted of 2 treatments; race inoculum and idle inoculum and substrate consisted of 2 treatments; maize and lucerne.

Buffer solution was prepared using the method of McDougall (1948) where 4ml of Solution B was added to 3.996L of Solution A. Buffer solution was adjusted using a 10% solution of citric acid solution to create the 3 treatment levels and then warmed to 37°C.

Innoculum was prepared from faeces collected either from race horses (race inoculum) being fed a diet consisting of a 70:30 ratio of concentrate to roughage or from horses not in work (idle inoculum) fed a diet that had a 30:70 ratio of concentrate to roughage. Faeces were collected 15 minutes prior to inoculum preparation. Upon collection, faeces were placed in a sealed packet in an infused CO_2 flask as they were produced. The temperature was maintained at 37°C and transported to the laboratory. The faeces (as-is basis) were mixed 50:50 (w/w) with either one of the three buffer solution treatments and strained through 4 layers of cheese cloth, under a stream of CO_2.

1g of substrate was added to each bottle with 67ml of a treatment buffer then followed by 33ml of faecal inncoulum prepared as per method above. Each bottle was then filled with CO_2, tightly closed and fitted to the pressure sensor. Each bottle was allocated to 24 pressure transducer channels in the *in vitro* incubator (Pienaar, 1994). The pressure recordings were set at ten second intervals for 20 minutes before pressure logging started. This was done so that samples could settle and any problems could be identified. The recordings were then set at 20 minute intervals for the duration of 24 hours. The pressure data were converted to gas volume (ml) using a predetermined calibration equation where 1kPa = 2.2489ml gas (Nsahlai & Umunna, 1996). The gas profiles were corrected for control fermentation profiles in the absence of substrate. The profiles were then fitted to the model as described by Campos *et al.* (2004) to derive GP kinetics as follows.

$$Y= A/ [1+\exp (2+4a1(C-t))] + B/ [1+\exp (2+4b1(C-t))] \qquad 4.1$$

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, a1 and b1 are the degradation rates (h-1) for the fast and the slowly degradable fractions and C is the colonization or lag time (h).

Blummel *et al.* (1997) describes the efficiency of microbial protein synthesis in the form of a partitioning factor (degraded substrate: gas volume mg.ml-1). The partitioning factor indicates the relationship between substrate entering the hindgut and the fermentation that occurs. The degradation efficiency factor is calculated from the partitioning factor as follows:

$PF = \frac{Deg}{V}$ 4.2

And

$DEF = \frac{Deg}{\text{T½ x V½}} = \frac{2PF}{\text{T½}}$ 4.3

Where deg = true degradability (mg), V = total gas volume (ml), V½ = half of V (ml) and T½ = time taken to produce V½.

Upon completion of 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 JLA-16.250. The supernatant was discarded and the pellet residue (R) was dried in a fanned oven at 60˚C for 72 hours. The apparent degraded fraction (APD) was calculated as the difference in weight between R and 1gDM. The pellet residue was refluxed with neutral detergent solution (NDS) and the residue (NDF) was dried. The weight of the NDF was subtracted from the 1g DM which gave the truly degraded fraction (TD). The microbial yield (MM) was calculated as the difference between the true and the apparent degradability. Absolute ΔpH was calculated as the difference between initial pH and terminal pH.

SASv9.3 software was used to fit the gas production responses over the control *in vitro* treatments to the model by Campos *et al.* (2004). ANOVA using Genstat 14th edition was performed to determine if there were differences in gas production kinetics (Max GP, A, B, a1, b1, C) and PF, DEF, APD, TD, MM, Absolute ΔpH and Terminal pH, with buffer pH, inoculum and substrate as factors. Means for the individual, two and three way interactions were compared by least significant differences and were considered different at P <0.05.

Results and Discussion

Following *in vitro* fermentation, gas production kinetics and degradation parameters were determined over the varying treatment levels (Table 4.1 and Table 4.2). There was a difference (P<0.001) for max GP in the individual, two and three way interactions. Lucerne with a buffer pH of 6.5 achieved lower max GP than with a buffer pH of 5.5, while for the most part buffers at pH 7.2 significantly dropped the max GP. Max GP of lucerne has been reported by Murray *et al*. (2006) to be 157.06ml.g^{-1} while Ouda *et al*. (2006, 2007) also observed similar results. Max GP however was slightly lower at 119ml.g^{-1} in this study. It was expected that race horse inoculum with maize substrate would have the highest max GP, as the microbial population would be adapted to a high CHO diet, however the results of this study did not reflect that.

Time taken to reach half maximum gas production ($T_{1/2}$) was different over all three buffer pH (P< 0.001) levels with buffer pH of 6.5 reaching $T_{1/2}$ in the shortest amount of time followed by buffer pH 7.2 and pH 5.5. The substrate treatments produced significant (P<0.001) results with lucerne reaching $T_{1/2}$ in a shorter time period than that of maize. There were no significant two and three way interactions for $T_{1/2}$. The shorter time period taken to $T_{1/2}$ indicates that using a buffer pH level of 6.5 allows fermentation to begin sooner than at other buffer treatments. Using a buffer pH of 6.5 with lucerne resulted in the shortest $T_{1/2}$, immaterial of inoculum used. This suggests that micro-organisms found in both inocula had adapted to a pH of 6.5 and lucerne as a substrate. Time taken to half max GP can be used to determine nutritive value (Murray *et al*., 2006) as microbial activity is close to maximum at half max GP (France *et al*., 1993; Dhanoa *et al*., 2000; Makkar, 2004; cited in Ouda, 2007). Feeds that take longer to reach half max GP are the feeds that have been formulated to contain a higher proportion of unprocessed ingredients or lucerne. It is known that the ratio of amylose: amylopectin as well as the quality has an effect on the speed and extent of the CHO digestion which in turn can influence microbial development and activity (de Fombelle *et al.*, 2003). It is thus particularly interesting that lucerne reached $T_{1/2}$ in a shorter time than that of maize. A possible explanation could be that microbes have become accustomed to the substrate as lucerne is the base of numerous horse feeds and more often than not the forage of choice for roughage intake (Crozier *et al*., 1997). Microorganisms in race horse inoculum should ferment CHO better and the microorganisms in idle horse inoculum should use forage better. A decrease in pH is anticipated with an increase in concentrate in a diet, which would account for the buffer pH of 6.5 reaching $T_{1/2}$ in a shorter time period. The buffer pH that is most appropriate for fermentation will have the least amount of variation between inoculum and substrate.

There was no significant three way interaction in amount of gas produced from rapidly fermentable fractions. However, significant (P<0.05) two way interactions of inoculum*buffer and substrate*buffer were observed. The combination of idle horse inoculum at buffer pH of 7.2 had a higher rate of degradation than all other treatment combinations. The use of inoculum from race horses on lucerne over all three buffer pH treatments resulted in a lowergas production from slowly degradable fractions. The treatment interaction of idle horse inoculum*Lucerne*5.5 buffer pH did not fit the Campos model over a 24-hour fermentation period.

A significantly (P<0.05) shorter lag time with the three-way interaction of buffer pH* inoculum*substrate can be seen. Substrate and Inoculum treatment with a buffer pH of 7.2 had more than double the colonization time. The rapidly degradable fraction was important in identifying that the response to the substrate and the inoculum was not constant at the varying buffer pH levels. This could imply that differences in GP can be attributed to the amount of rapid fermentation that can occur at buffer pH of 7.2. Idle inoculum producing more gas from the slowly degradable fraction is most likely due to the microbes being better adapted to a higher roughage diet. The degradation rate from rapidly degradable substrate not being constant over all treatment structures could be an indication that the micro-organisms from race inoculum are more adapted to a lower pH than those in idle inoculum due to the type of diet that they are normally subjected to. A decrease in pH is seen with the addition of concentrate feeds (Austbo, 2005; Noesset & Austbo, 2010; Young, 2011). The degradation rate of slowly degradable substrate showed that the combination of idle inoculum with lucerne at a pH of 5.5 does not conform to the Campos model in a 24-hour fermentation period. This result could be due to a reduction in roughage degradation from a decrease in cellulolytic activity and an increase in amylolytic activity from the change in pH (Austbo, 2005; Noesset & Austbo, 2010).

Table 4.1a Mean gas production kinetics for lucerne and maize fermentation over 24 hours using race and idle faecal inoculum at three buffer pH levels of 5.5, 6.5 and 7.2.

Treatment									
Buffer pH	**Inoculum**	**Substrate**	**Max GP ml.gDM^{-1}**	**$T_{1/2}$**	**Gas vol (ml) for rapidly degradable fraction**	**Gas vol (ml) for slowly degradable fraction**	**Degradation rate of rapidly degradable fraction (h^{-1})**	**Degradation rate of slowly degradable fraction (h^{-1})**	**Lag/ colonization time (h)**
5.5	Race	Maize	199.70^{d}	16.98	0.06	58.30^{abc}	0.044	0.037	10.97^{e}
		Lucerne	221.40^{e}	14.03	0.06	3.90^{ab}	0.30	0.058	9.56^{de}
	Idle	Maize	100.00^{b}	16.58	0.10	144.20^{cd}	0.14	0.082	8.40^{cd}
		Lucerne	159.70^{c}	14.31	0.10	97.00^{bc}	0.04	0.046	4.49^{ab}
6.5	Race	Maize	185.50^{d}	12.47	0.08	33.20^{ab}	0.70	0.081	6.51^{bc}
		Lucerne	107.30^{b}	11.08	0.07	13.20^{ab}	0.25	0.067	3.47^{a}
	Idle	Maize	205.40^{de}	14.10	0.06	226.80^{d}	0.05	0.079	3.94^{a}
		Lucerne	97.90^{b}	10.64	0.07	20.30^{ab}	0.26	0.068	4.84^{ab}
7.2	Race	Maize	193.00^{d}	14.39	0.12	21.80^{ab}	0.57	0.110	10.01^{de}
		Lucerne	65.40^{a}	12.53	0.09	2.40^{a}	0.08	0.080	8.39^{cd}
	Idle	Maize	78.20^{a}	16.00	0.14	57.10^{abc}	0.13	0.656	11.05^{e}
		Lucerne	62.40^{a}	13.21	0.10	67.40^{abc}	0.10	0.274	8.85^{de}
Buffer Means	5.5		170.20	15.47	0.08	75.80	0.13	0.058	8.43
	6.5		149.00	12.07	0.07	73.40	0.32	0.073	4.69
	7.2		99.80	14.03	0.11	37.20	0.22	0.237	9.57
Inoculum	Idle		117.30	14.14	0.09	102.10	0.12	0.157	6.98
Means	Race		162.10	13.58	0.08	22.10	0.32	0.070	8.15
Substrate	Lucerne		119.00	12.63	0.08	34.00	0.17	0.088	6.65
Means	Maize		160.30	15.08	0.09	90.20	0.27	0.133	8.48
Overall Mean			139.70	13.86	0.09	62.10	0.22	0.11	7.56
SED			8.53	1.16	0.01	45.08	0.24	0.010	1.08
LSD			17.61	2.39	0.03	93.74	0.51	0.045	2.24
Fprob: Buffer pH			<0.001	<0.001	<0.001	0.183	0.331	<0.001	<0.001
Fprob: Inoculum			<0.001	0.250	0.011	<0.001	0.056	0.005	0.014
Fprob: Substrate			<0.001	<0.001	0.038	0.006	0.320	0.117	<0.001
Fprob: Buffer pH*Substrate			<0.001	0.969	0.022	0.075	0.401	0.254	0.420
Fprob: Buffer pH*Inoculum			<0.001	0.587	0.003	0.515	0.623	0.003	0.001
Fprob: Inoculum*Substrate			<0.001	0.420	0.486	0.190	0.214	0.175	0.666
Fprob: Inoculum*Substrate*Buffer pH			<0.001	0.499	0.283	0.015	0.109	0.500	0.025

^{a,b,c,d,e,f,g} Values within a row are different if superscript differs (P<0.005). $T_{1/2}$ is time in hours to half maximum gas production, max GP = maximum gas production

The three way interactions ($P<0.001$) as well as the two way interactions; buffer pH*substrate ($P<0.001$), buffer pH*inoculum ($P<0.05$) and substrate*inoculum ($P<0.05$) for PF were significant. A lower PF is obtained with lucerne as a substrate at buffer pH of 5.5 or 6.5 using either a race or idle horse inoculum. The PF is an accurate way of comparing treatments over a short incubation period (Blummel *et al.*, 2005). The PF is defined as the degradation per unit volume and is a measure of the efficiency of rumen microbial production (Dijkstra *et al*., 2005; Ouda, 2007). In the horse, PF shows the relationship between substrate entering the hindgut and the fermentation that occurs (Young, 2011). The PF indicates that there is generally a better degradation rate with a buffer pH of 7.2. The degradation Efficiency Factor (DEF) is a function of both the PF, $T_{1/2}$ and total gas volume.

The three-way interaction ($P<0.001$) and the two-way interaction of buffer pH/ substrate ($P<0.001$), buffer pH/inoculum ($P<0.05$) and substrate/inoculum ($P<0.05$) for DEF were significant. In general, the DEF was lower for the treatments with a buffer pH of 5.5 as opposed to a pH of 7.2, immaterial of inoculum or substrate that was used. Apparent digestibility in the three-way interaction is significant ($P<0.001$), lucerne using idle inoculum at a buffer pH of 5.5 has a lower APD than maize using race inoculum at a pH of 5.5, 6.5 and 7.2. This shows that the results are not consistent over the varying buffer pH levels. The three way interactions for TD were significant ($P<0.05$), race inoculum using maize as a substrate with a buffer pH of 6.5 or 7.2 resulted in a higher TD than lucerne at a pH of 5.5 using either race or idle inoculum. Combinations with lucerne tended to result in a lower TD than with maize. Race inoculum showed a higher TD than idle inoculum even with Lucerne as a substrate. An average gas production is seen with feeds that have a large proportion of lucerne (Young, 2011). Feeds with higher fibre content will have a lower TD and APD (Ellis & Hill, 2005; Young, 2011). Race inoculum was also expected to yield a higher TD, as the microbes that are present in the inoculum should be better adapted to a high CHO substrate. It was expected that a buffer pH of 5.5 will have negative impact on degradability, while buffer pH of 6.5 has a higher degradability value than 7.2. However, this did not occur, this is most likely attributed to the microbial population having acclimitised to the change in GIT Ph. MM was lower when idle inoculum was used with lucerne at buffer pH 7.2 and 6.5 in comparison to idle inoculum with lucerne at buffer pH 5.5 and with idle inoculum with maize at a pH of 6.5 and 7.2. Microbial mass is a significant ($P<0.05$) in the three-way and two-way interactions. The result of maize having a higher TD was expected as maize in general has a higher degradability than that of lucerne. Higher gas productions are seen in feeds that have more fermentable substrate (Young, 2011).

Absolute change is significant when a buffer with a pH of 6.5 is used. As expected with absolute change in pH, there is a significance in treatments that the microbial population was not accustomed to. Treatments with Buffer pH of 7.2 and 5.5 had a mean absolute change in pH of 0.67 and 0.7 pH units respectively, in comparison to buffer pH of 6,5 changing by 0.26 pH units. These results suggest that the buffer pH of 6.5 buffered the treatments most effectively.

Table 4.1b Degradation paramaters calculated from gas production kinetics for lucerne and maize fermentation over 24 hours using race and idle faecal inoculum at three buffer pH levels of 5.5, 6.5 and 7.2.

Treatment			PF	DEF	APD $g.kg^{-1}DM$	TD $g.kg^{-1}DM$	Microbial Mass $g.kg^{-1}DM$	Terminal pH	Absolute ΔpH
Buffer pH	**Inoculum**	**Substrate**							
5.5	Race	Maize	5.79^{d}	0.682^{d}	478.0^{d}	726.0^{fg}	248.0^{bcd}	5.28^{a}	0.22^{a}
		Lucerne	2.94^{ab}	0.426^{abc}	203.0^{ab}	447.6^{ab}	244.0^{bcd}	6.20^{bc}	0.70^{d}
	Idle	Maize	4.76^{cd}	0.574^{bcd}	277.0^{bc}	508.5^{bc}	232.0^{abc}	5.50^{a}	0.45^{b}
		Lucerne	2.50^{a}	0.349^{a}	106.0^{a}	381.3^{a}	353.0^{de}	6.21^{bc}	0.70^{d}
6.5	Race	Maize	4.77^{cd}	0.768^{d}	513.0^{de}	798.7^{gh}	285.0^{cde}	6.01^{b}	0.50^{bc}
		Lucerne	3.80^{abc}	0.687^{d}	338.0^{c}	597.7^{de}	259.0^{cd}	6.54^{d}	0.03^{a}
	Idle	Maize	4.23^{bcd}	0.606^{cd}	357.0^{c}	733.2^{fg}	377.0^{e}	6.07^{b}	0.45^{b}
		Lucerne	4.15^{abcd}	0.780^{d}	351.0^{c}	487.2^{bc}	136.0^{ab}	6.55^{de}	0.04^{a}
7.2	Race	Maize	4.27^{bcd}	0.604^{cd}	655.0^{e}	878.2^{h}	223.0^{abc}	6.44^{cd}	0.61^{bcd}
		Lucerne	9.18^{ef}	1.450^{f}	307.0^{bc}	540.3^{cd}	234.0^{abc}	6.83^{f}	0.22^{a}
	Idle	Maize	9.91^{f}	1.241^{ef}	324.0^{c}	676.9^{ef}	353.0^{de}	6.50^{d}	1.06^{e}
		Lucerne	7.87^{e}	1.194^{e}	394.0^{cd}	512.0^{bc}	118.0^{a}	6.79^{ef}	0.77^{d}
Buffer Means	5.5		4.00	0.51	266.00	515.90	269.00	5.80	0.52
	6.5		4.24	0.71	390.00	651.90	264.00	6.29	0.26
	7.2		7.81	1.12	420.00	654.20	232.00	6.64	0.67
Inoculum Means	Idle		5.57	0.79	301.00	549.90	261.00	6.27	0.58
	Race		5.13	0.77	416.00	664.80	249.00	6.22	0.38
Substrate Means	Lucerne		5.07	0.81	283.00	494.40	224.00	6.52	0.41
	Maize		5.62	0.75	434.00	720.30	286.00	5.97	0.55
Overall Mean			5.35	0.78	359.00	607.30	255.00	6.24	0.48
SED			0.82	0.11	57.40	40.05	56.40	0.12	0.09
LSD			1.69	0.22	118.80	82.67	116.40	0.24	0.19
Fprob: Buffer			<0.001	<0.001	<0.001	<0.001	0.370	<0.001	<0.001
Fprob: Inoculum			0.199	0.627	<0.001	<0.001	0.593	0.285	<0.001
Fprob: Substrate			0.115	0.126	<0.001	<0.001	0.013	<0.001	<0.001
Fprob: Buffer*Substrate			<0.001	<0.001	0.087	0.486	0.004	<0.001	<0.001
Fprob: Buffer*Inoculum			0.004	0.032	0.409	0.418	0.543	0.656	<0.001
Fprob: Inoculum*Substrate			0.012	0.028	<0.001	0.009	0.023	0.234	0.762
Fprob: Inoculum*Substrate*Buffer			<0.001	<0.001	0.029	0.022	0.005	0.793	0.172

$^{a,\ b,c,d,e,f,g}$Values within a row are different if superscript differs (P<0.005) where PF = partitioning factor, DEF = degradation efficiency factor, APD = apparent degradability, TD = True degardability

Conclusion

Microclimates within the equine GIT have changed concurrently with feeding management. In order to accurately predict degradability in the horse, buffer pH in standard IVGPT protocols needs to be adjusted to 6.5 for equine studies.

DISCUSSION

The intention of this thesis was two-fold. Firstly to see if a relationship existed between gastrointestinal tract pH, ulceration, nutrient density and digestibility and secondly to use IVGPT to get a better understanding of gastro-intestinal microbial fermentation potential as well as how gastro-intestinal pH affects degradability. Demographical, pH and ulceration data along with digesta samples were collected from horses *post mortem.* Chemical analysis of the digesta provided data for hierarchical cluster analysis for the determination of nutrient density classes. Predisposing factors were then analysed with ANOVA.

Different segments of the digestive system of the horse are closely linked. Factors that directly impact one section can have indirect ramifications further in the digestive tract. This is seen in chapter two where older horses have a lower pH than younger horses which could be due to the dentition. The poor dentition causing these results will not only decrease pH in the digestive tract but could also be the cause of impaction colic (Mair & Hillyer, 1997) and fibre length which will in turn affect fibre digestibility (Frape, 2010). Common causes of ulceration are fasting, quantity and type of feed, amount of exercise and medication. Excess starch provision increases fermentation and the production of VFA's, which makes the environment more acidic which negatively affects mucosal integrity. This is common problem in race horses. Concentrate feeds are fed as 80% of the diet (Hackland, 2007) and ulcer occurrence ranging from 80% (Acland *et al.*, 1983) to 91% (Murray *et al.,* 1996). In this study similar results were seen in chapter two. Horses on a high nutrient diet (averaging 50% and above of concentrate) had a 99% probability of developing ulcers and horses on a medium nutrient diet (averaging up to 30% concentrate), having a probability of 69% incidence of ulcers in comparison to horses on a low nutrient diet (0% concentrate). There has been disagreement on high concentrate bucket feed being the reason for gastric ulcer development as there has been a high prevalence of ulceration in grass fed horses (le Jeune *et al.,* 2006), but this study confirms the effect of nutrient density on incidence of ulcers.

There has been a large amount of research performed on improving digestibility of feeds. Raw materials, feed processing, feed quality and rates of passage are just a few influencing factors. Pagan (2000) reported that fibre quality is directly linked to digestibility. Similar results can be seen in this study. Horses on a high or low nutrient diet had a lower digestibility in general. A low nutrient diet is a good indication of grass fed horses. Digestibility for this category would normally be attributable to a low forage quality. This can be influenced by stage of maturity, location of growth and inhibitory substances (Pagan, 2000). High nutrient diets are normally linked to race horses. The reason for poor digestibility in this category will be due to an oversupply of nutrients. The effort of improving the digestibility of feed is all fair and well, provided that the administration of the feed is also focused on maximizing digestibility for optimal health and performance. However, the main focus of feeding management has not been in this area. Horses are being fed two to three large bucket concentrate meals a day and the small intestine of the horse is unable to deal with these meal sizes. Pagan (2000) found that when the capacity of the upper digestive tract is overwhelmed the excess substrate passes into the hindgut where rapid fermentation occurs. This has led to metabolic disorders and impact digestibility especially that of fibre as seen in chapter three of this study. A negative digestibility of fat was found in chapter three, an anomaly. Endogenous losses can account for some of the fat loss but the amount obtained in this study far exceeds amounts that have been reported (Zeyner, 2002). The symptoms of steatorrhea can be linked to metabolic problems such as coeliac sprue, hypoparathyroidism and Zollinger-Ellison syndrome. However, further research needs to be performed to ascertain this.

During data collection, it was seen that the pH along the digestive tract, especially in the caecum averaged at 6.3. This is far lower than the buffer pH that is used in standard IVGPT protocols. IVGPT trials were run, using a buffer pH of 5.5, 6.5, and 7.2 to determine if substrate degradability is affected. It was determined that buffering at pH 6.5 elucidates differences in *in vitro* behaviour on roughage/CHO substrates. For accurate gas production parameters to be attained the pH of the buffer standard cannot remain at 7.2, but needs to be reduced to 6.5.

The outcome of this dissertation adds to the knowledge and understanding of equine nutrition. This will allow informed decisions to be made regarding feeding management.

OVERVIEW AND FUTURE RESEARCH DIRECTIVES

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

1) To determine if pH along the digestive tract is altered and the probability of ulceration and discolouration with nutrient density, age, breed and sex as treatment factors.
2) To determine if nutrient digestibility is affected by feeding level, age, ulceration or discolouration.
3) To determine optimal buffer pH to be used in *in vitro* studies for accurate predictions of digestion in the horse's gastrointestinal tract.

The literature review dealt with the digestive tract of the horse, which included nutritional modelling, *in vivo* and *in vitro* methods, digestibility and flow rate. Feed and feeding management along with the impact of nutritional insults to the horse's epistemic imperative.

The pH in the digestive tract did vary between regions as expected. A decrease in pH increases the incidence of foregut ulceration. A higher nutrient diet had more of a negative impact on digestibility than a medium nutrient diet in comparison to grass fed horses. This confirms the strong correlation between higher concentrate fed horses having a higher ulcer incidence. There was a stronger correlation between older horses and ulceration than younger horses. This is most likely due to the time period that the digestive system is under nutritional insult.

It is seen in chapter three that there is no relationship between nutrient digestibility and ulceration. Discolouration of the stomach mucosa had no impact on digestibility and no strong correlation could be found between discolouration and ulceration presence or severity. A high and low nutrient diet had a much lower digestibility than horses with a medium nutrient diet. This shows that an oversupply of nutrients will impact digestibility negatively. Fat digestibility in this chapter has become an anomaly. Negative digestibilities were found, that exceeded amounts reported from endogenous losses (Zeyner, 2002). Further research needs to be done on the gastrointestinal tract histopathology to determine if coeliac sprue, hypoparathyroidism and Zollinger-Ellison syndrome is a possibility in. If these metabolic problems, do present themselves in horses, then conventional feeding management will have to be revised. Gluten will have to be removed from the diet and alternative feed sources high in protein and energy will need to be found while optimal performance is maintained.

To obtain accurate gas production parameters the pH of the buffer standard cannot remain at 7.2. The results obtained at the three-buffer pH levels used in this study varied greatly between the race and idle inoculum and the maize and lucerne treatments. The influence of buffer pH on fermentation characteristics cannot be discounted. The evidence in this study suggests that buffer conditions for *in vitro* fermentation techniques for evaluation of high CHO diets in horses need to be adjusted to 6.5.

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