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Review Articles

Molecular insights into dietary induced colic in the horse

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Summary

Equine colic, a disorder manifested in abdominal pain, is the most frequent cause of emergency treatment and death in horses. Colic often requires intestinal surgery, subsequent hospitalisation and post operative care, with a strong risk of complications arising from surgery. Therefore strategies that explore approaches for preventing the condition are essential. To this end, a better understanding of the factors and mechanisms that lead to the development of colic and related intestinal diseases in the horse allows the design of preventive procedures.

Colic is a multifactorial disorder that appears to be induced by environmental factors and possibly a genetic predisposition. One factor that seems to influence the risk of developing colic is the excessive consumption of diets containing high levels of carbohydrates. Therefore, major efforts have been made by various laboratories and institutions across the world to study the type and digestibility of various feed in order to formulate accurate and safe feed components and proportions. However, relatively little work has been carried out to characterise, in detail, the carbohydrate digestive and absorptive capacity and mechanisms underlying the potential adaptive response of equine gut epithelium to a changing diet.

This review focuses on advances made towards understanding the molecular and cellular mechanisms involved in digestion and absorption of dietary carbohydrates in the equine gastrointestinal tract and the implication of these processes for the whole body physiology. It addresses the underlying mechanisms that may govern the adaptive response of equine small intestine to increased dietary hydrolysable carbohydrates. Furthermore, it describes changes that occur in the equine large intestinal microbiology and host tissue biology brought about by alterations in diet and in colic. It is hoped that a better understanding of the molecular and cellular processes that play important roles in the physiology and pathology of the equine gastrointestinal tract will assist the development of effective strategies to prevent equine colic.

Introduction

Horses are trickle feeders whose natural diet is grass. They possess a voluminous and elaborate large intestine endowed with a microbial population uniquely adapted to ferment dietary plant fibre. The microbial hydrolysis of grass leads to the release of soluble sugars, which are subsequently fermented to monocarboxylates (commonly referred to as short chain fatty acids [SCFA] or volatile fatty acids) acetate, propionate and butyrate. A significant proportion of the horse's body energy is provided by SCFA absorbed from the caecum and the colon (Bergman 1990). However, to provide enough energy for the demands of work and performance, today's horse is fed high energy diets containing a large proportion of hydrolysable carbohydrates, hCHO (grains). These diets are hydrolysed in the small intestine by pancreatic α-amylase and brush border membrane disaccharidases to monosaccharides such as glucose, which, when absorbed in the small intestine, provides an important source of energy for the horse in intense work (Argenzio and Hintz 1972).

Diet, especially grain feeding and recent dietary change, has been identified by epidemiological and clinical studies as an important risk factor for the development of colic and laminitis, both major causes of equine mortality (Clarke *et al*. 1990; Hintz and Cymbaluk 1994; Hillyer and Mair 1997; Mair and Hillyer 1997; Tinker *et al*. 1997; Hudson *et al*. 2001). It is proposed that when horses are introduced suddenly to diets containing high levels of hCHO (>0.4% of bodyweight), a substantial proportion of starch reaches the large bowel (Potter *et al*. 1992; Lopes *et al*. 2004). It is then rapidly fermented to excess metabolites, such as lactic acid, that cause drastic alterations in the caecal/colonic pH, perturb the microbial populations and predispose the horse to intestinal dysfunction, such as colic (Goodson *et al*. 1988; de Fombelle *et al*. 2001; Julliand *et al*. 2001). It is not apparent whether the limiting step(s) is in the small intestinal capacity to digest hCHO and/or to absorb monosaccharides or even if horses are capable of increasing their intestinal digestive and absorptive capacity in response to increased carbohydrate load.

It is important to note that consumption of pasture containing high levels of rapidly fermentable carbohydrates (F_R-CHO) have also been implicated in the development of colic and laminitis (Hoffman *et al*. 2001). The seasonal variation observed in colic development may be a reflection of seasonal changes in the hCHO and F_R-CHO content of the pasture (Cohen *et al*. 1999; Longland *et al*. 1999).

Recognition that a high proportion of concentrates in the feed is a strong risk factor and a prequel to small intestinal overload

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(Proudman 1991; Reeves *et al*. 1996; Tinker *et al*. 1997; Hudson *et al*. 2001), has attracted major attention to feed composition, digestibility and feeding management in order to avert deleterious effect of diet and to enhance health and performance. These approaches, while credible, are intuitive and empirical. Little attention has been given to the molecular mechanisms underlying small intestinal starch digestion and glucose absorption or their response to increased dietary carbohydrate. The opportunity to maximise rationally the capacity of the intestine to absorb dietary carbohydrates should provide a scientifically-based approach to the maintenance of health and enhanced performance.

This review first describes the nature of proteins and mechanisms involved in the absorption of monosaccharides in the equine small intestine, their expression along the longitudinal and radial axes, and changes in their function and expression in response to increased hydrolysable carbohydrate content of the diet. Next, these findings are related to the underlying mechanisms that may lead to small intestinal carbohydrate overload and the implication that this has for feeding practices. The nature of the microflora that populate the equine large intestine, their digestive activity and fermentation products are then considered following a discussion of the mechanisms involved in the absorption of short chain fatty acids across the equine colonic epithelium.

Since the target tissue in colic development is the equine large intestine, different forms of colic are discussed and the changes observed in the colonic microenvironment, microbiology and tissue physiology brought about by alterations in diet type and in colic are considered. Finally, an attempt is made to relate how these changes associate with colic development.

Digestion and absorption of hCHO in the equine small intestine

Starch digestion

The small intestinal enzymes responsible for the hydrolysis of dietary carbohydrates can only break down carbohydrates with the monomer units linked by α-linkages and not β-links. The latter are broken down by the microflora of the large intestine (see below). The α-linked polysaccharides, such as starch, constitute the hydrolysable carbohydrates. Starch is mainly hydrolysed in the small intestine by pancreatic α-amylase to oligosaccharides, which are further hydrolysed by the intestinal brush border membrane dissacharidase, maltase, to glucose (Roberts 1975; Shirazi-Beechey 1995; Dyer *et al*. 2002). There are reports that the activity and concentration of α-amylase in the equine intestine are low, compared to other species, but highly variable between horses (Roberts 1974; Kienzle *et al*. 1994). It is thought, however, that the activity of this enzyme is adequate under normal circumstances and only becomes problematic when horses are fed high grain diets (Roberts 1974). Work in our laboratory (Dyer *et al*. 2002) has shown that sucrase (the disaccharidase that hydrolyses sucrose to glucose and fructose) activity is highest in the proximal small intestine of the horse and the levels are similar to those reported in the intestine of other nonruminant species (Roberts 1974; Dyer *et al*. 2002). Maltase activity is similar in proximal, mid and distal regions of equine small intestine and it is extremely high compared with that in other species (Dyer *et al*. 2002). Therefore, it appears that horses possess high activity of brush border disaccharidases to digest large quantities of disaccharides that may be present in their natural grass diet (Kienzle and Radicke 1993); and it is unlikely that there is a deficiency in brush border disaccharidase activity limiting starch digestion in the horse (Roberts *et al*. 1974). It has been proposed that it is likely that the breakdown of starch into maltose, maltotriose and α-dextrin may limit starch digestion in horses given high grain diets (Richards *et al*. 2004).

Studies carried out in other species have indicated that there is an adaptive response in amylase concentration and synthesis in response to the levels of the hydrolysable dietary carbohydrate; amylase synthesis increases by 15% in 24 h in response to high carbohydrate feeding and continues to increase by 200% in 5–7 days when new steady state levels are reached (Brannon 1990). In lambs, feeding a high energy/high starch diet resulted in enhanced levels of pancreatic α-amylase protein abundance and activity (Swanson *et al*. 2000). Although the mediators of pancreatic adaptation to dietary carbohydrate are not known precisely, plasma glucose and insulin levels appear to be involved in regulating acinar amylase synthesis and mRNA levels (Brannon 1990).

Richards *et al*. (2004) have shown that the addition of exogenous α-amylase to equine diets containing a digestible source of starch can enhance starch digestion in the small intestine of a small number of horses. They concluded that further research needs to be conducted to determine whether exogenous α-amylase is needed for horses well adapted to grain based diets. It is not known if, in the horse, a longer term adaptation period is needed for the enhancement in the endogenous α-amylase activity in response to increased dietary starch (see below).

Glucose absorption

In the majority of species, absorption of glucose (and galactose) from the lumen of the intestine into enterocytes is accomplished by sodium/glucose cotransporter isoform 1 (SGLT1). SGLT1, as the rate limiting step for entry of glucose into the body, underlines its importance in glucose homeostasis (Shirazi-Beechey 1995, 1996). Fructose is transported across the brush border membrane specifically by a Na^+-independent transporter, GLUT5. These monosaccharides, once accumulated in the enterocytes (reaching concentrations above that in the blood) exit the cell across the basolateral membrane down their concentration gradient by another Na^+-independent monosaccharide transporter, GLUT2. These monosaccharide transporters are structurally and functionally distinct and together provide the transcellular route for the absorption of monosaccharides from the lumen of the intestine into the systemic system (Shirazi-Beechey 1995).

It has been shown that absorption of glucose takes place in the small intestine of the horse and can fulfil some of the basal energy requirements (Argenzio and Hintz 1972; Roberts 1975). However, until recently, there was very little information on mechanisms and intestinal sites of monosaccharide absorption in the equine small intestine. Cloning and sequencing the cDNA encoding equine SGLT1 and the determination of SGLT1 amino acid sequence allowed: 1) the production of specific antibodies to equine SGLT1 protein; and 2) employment of equine SGLT1 cDNA for the determination of SGLT1 expression (at the protein and mRNA levels respectively) in the equine small intestine (Dyer *et al*. 2002). In the horse, glucose is transported mainly across the brush border membrane of enterocytes by a Na^+-glucose co-transport mechanism, SGLT, with the highest rate of transport in proximal>mid>distal part of the small intestine (see Fig 1).

There is a good correlation between levels of functional SGLT1 protein and SGLT1 mRNA abundance along the length of the small intestine (Dyer *et al*. 2002). This indicates that the major site of glucose absorption in horses maintained on conventional pasture forage diets is in the proximal to mid small intestine and that the expression of equine intestinal SGLT1 along the proximal to distal axis of the intestine is regulated at the level of mRNA abundance (Dyer *et al*. 2002) (Fig 1).

Adaptation in intestinal glucose absorption

In many herbivorous and omnivorous species (but not in carnivores) there is an enhancement in their intestinal capacity to absorb monosaccharides in response to increased dietary carbohydrate levels (Ferraris and Diamond 1989; Buddington *et al*. 1991; Shirazi-Beechey *et al*. 1991; Dyer *et al*. 2003). This increase is also observed in response to introduction of glucose and a range of monosaccharides into the small intestinal lumen (Solberg and Diamond 1987; Shirazi-Beechey *et al*. 1991; Dyer *et al*. 2003). The horses' natural diet, grass, undergoes seasonal variation in carbohydrate and protein content. However, the variation in hCHO is much less than in the diet of omnivores and indeed is less than the difference between grass and the artificial diets high in hCHO fed to horses in intense work. It has been proposed that the equine small intestine may have a slower or blunted adaptive response to dietary change (Buddington and Rashmir-Raven 2002), which may be an important consideration for the development of dietary-induced intestinal dysfunction in horses.

The activity of disaccharidases and the activity/expression of SGLT1 along the length of the small intestine in a number of horses maintained long-term on a concentrate (oats, corn + hay) based diet, with those of horses maintained on pasture forage were determined. It was demonstrated that in horses on a concentrate diet there were 2- and 4 to 5- fold increases in SGLT1 protein expression in the mid and distal small intestine, respectively, compared to horses on pasture (Dyer *et al*. 2002). This increase was reflected in a concomitant enhancement in the rates of Na^+-dependent glucose transport and SGLT1 mRNA abundance (Fig 1). A similar pattern of increase in mRNA, protein abundance and glucose transport function was observed for the basolateral membrane glucose transporter, GLUT2 (Salmon *et al*. 2002). This indicates that there is a coordinated increase in the rates of glucose transport across the luminal and basolateral membrane of enterocytes resulting in enhanced transcellular transport of glucose from the lumen of the intestine into the blood.

There was a wide variation in SGLT1 expression between individual horses. This may be significant in terms of their responsiveness to the diet type. The activity of disaccharidases in the intestine of both groups of horses either maintained on a pasture or concentrate diet was similar, supporting the proposition that horses already possess sufficient disaccharidase activity, and it is unlikely that there is a deficiency in disaccharidase activity limiting starch digestion in the horse.

To determine the effects of the level of hCHO feeding on the magnitude and time-course of SGLT1 upregulation, the author inaugurated a collaborative programme with Ray Geor and his associates in Canada. Six mature (age 4–6 years) sound, Standardbred horses were fed a controlled all-hay diet (timothy hay supplemented with protein, vitamins and minerals) for 3 months (*phase 1*) before harvest of duodenal and ileal mucosal biopsies via laparoscopic technique (Bracamonte *et al*. 2008). Horses were then introduced to a diet consisting of 60% hay, 40% grain (2/3 oats, 1/3 corn, 3.3 g starch/kg bwt/day). Subsequently, biopsies (duodenal and ileal) were removed after one week (*phase 2*) and one month (*phase 3*). At this time horses were switched to 40% hay, 60% grain (6.0 g starch/kg bwt/day) for a further month before duodenal and ileal biopsies were removed (*phase 4*). During all diet phases horses were fed at least 1% bwt as hay.

SGLT1 expression measured quantitatively, by real time PCR (Fig 2), indicated that when horses were on a forage diet only (*phase 1*), SGLT1 mRNA abundance was higher in the duodenum (1.28 ± 0.16 units; Fig 2a) than the ileum (0.57 ± 0.14 units;

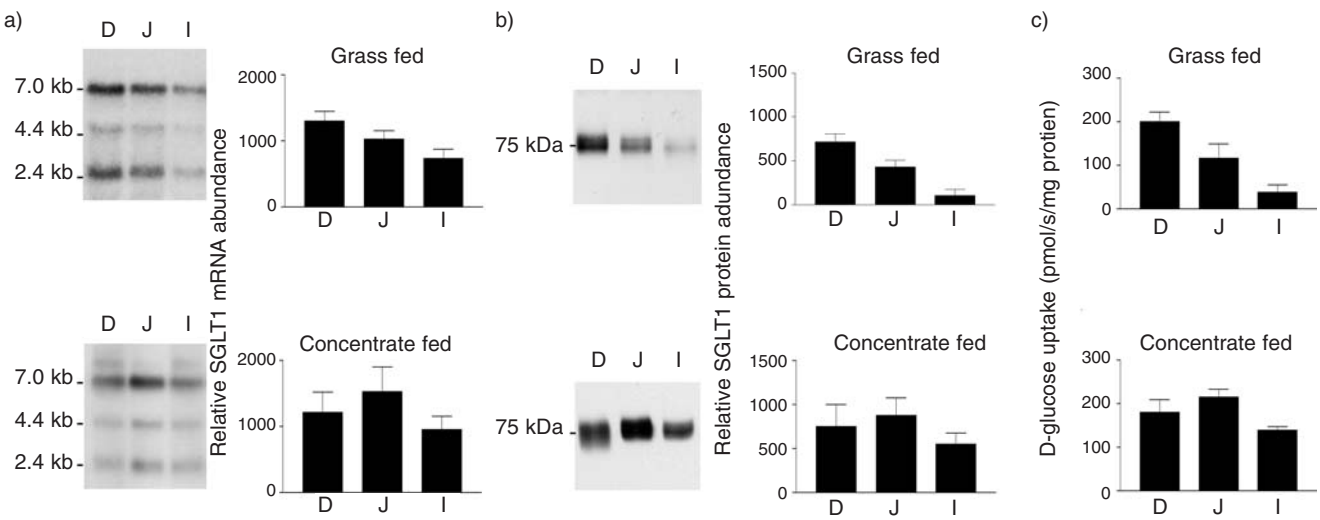

Fig 1: SGLT1 expression and Na^+/glucose co-transport in the small intestine of horses maintained at pasture compared with concentrate-fed horses. Intestinal tissue was removed from 6 horses that had been maintained on forage at pasture (grass) (upper panel) and from 6 horses that had received concentrates (grain) (lower panel). a) A typical northern blot showing equine SGLT1 mRNA transcripts in duodenum (D), jejunum (J) and ileum (I) along with combined densitometric analyses demonstrating SGLT1 mRNA abundance. b) A typical western blot showing the presence of a 75 kDa equine SGLT1 protein, along with densitometric analysis. c) The initial (3 s) rate of Na^+-dependent D-glucose transport into intestinal brush border membrane vesicles (Dyer et al. *2002). All uptakes were measured in triplicate. All data are expressed as a mean ± s.e. (n = 6) in arbitrary units.*

Fig 2b) in agreement with previous results (Dyer *et al*. 2002, Fig 1). After one week of grain feeding (*phase 2*), there was a modest increase in SGLT1 mRNA expression in the duodenum (1.71 ± 0.17 units) and a more significant 2-fold increase in SGLT1 expression in the ileum (1.15 ± 0.11 units). After a further month on grain (*phase 3*), SGLT1 expression in the duodenum was increased further to nearly twice the original level (2.31 ± 0.39 units), with no further change in ileal SGLT1 expression. However when the hCHO content of the diet was increased to 6.0 g/kg bwt/day for a further month (*phase 4*) there was a further increase in ileal SGLT1 expression (1.68 ± 0.38 units) to around 3 times the level observed in *phase 1* (J. Dyer, L. Waterfall, R. Geor and S.P. Shirazi-Beechey, unpublished data). The data clearly show that with time (one month on 40% grain followed by a further month on 60% grain) there is a significant 3-fold increase in the levels of the SGLT1 mRNA in the ileum (the region that expressed very low levels of SGLT1 in the intestine of horses on pasture forage, see Fig 1). As shown previously, there is a good correlation between changes at the level of SGLT1 mRNA to that observed in SGLT1 protein abundance and function. Therefore, it appears that the equine intestinal glucose transporter expression is enhanced, with time, in response to increased dietary hCHO. This is accomplished through enhancement of SGLT1 expression not only in the proximal but predominantly in the distal small intestine.

SGLT1 expression is only upregulated by monosaccharides and not by starch (Wood 1995). Our observation that SGLT1 is upregulated, with time, infers that starch hydrolysis must increase

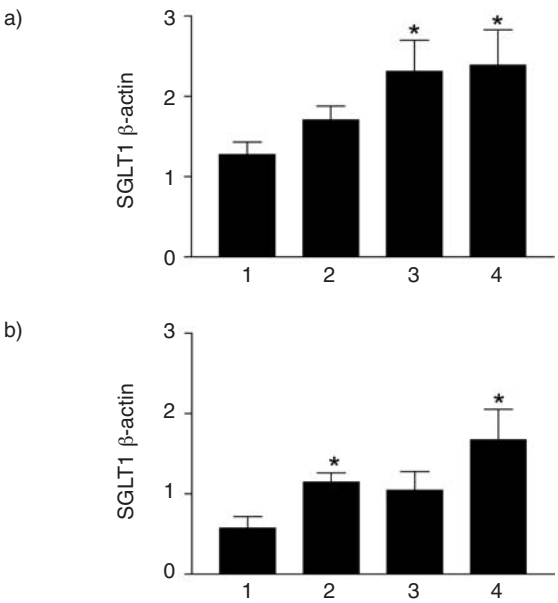

*Fig 2: Effect of dietary hCHO on SGLT1 expression. Steady-state levels of SGLT1 mRNA determined by QPCR in RNA isolated from duodenal (a) and ileal (b) biopsies. Horses on hay only diets (1) were switched to 60% hay, 40% oats (3.3 g starch/kg bwt/day). Biopsies (duodenal and ileal) were removed after one week (2) and one month (3). At this time horses were switched to 45% hay, 55% oats (6.0 g starch/kg bwt/day) for a further month before duodenal and ileal biopsies were removed (4). Data are normalised to β-actin, the structural protein expression that remained constant. All values are expressed relative to SGLT1 in the duodenum of horses on hay only diets (1) as means ± s.e. Data were generated in triplicate, with n = 6 animals in each group. Statistically significant results are indicated by * (P<0.05), ANOVA and Student's unpaired 2-tailed* t *test.*

over time, to release glucose into the lumen of the intestine of horses during the dietary change period. The glucose then enhances the expression of SGLT1 in the proximal and the distal small intestine. This indicates, indirectly, that α-amylase activity in horse can be enhanced but requires a longer term adaptation. The data suggest that the primary rate limiting step in the increased glucose absorptive capacity of equine small intestine, via SGLT1, is likely to be the inability of the horse to hydrolyse starch to glucose rapidly.

Intestinal glucose sensor and regulation of intestinal glucose absorption

Recent work has identified the underlying mechanisms involved in enhancement of intestinal glucose uptake in response to increased dietary sugars. It has been shown that the sweet taste receptor, T1R2 +T1R3, and its partner, the α-subunit of the guanine nucleotide binding protein (G protein) gustducin, are expressed and associated with the luminal membrane of the enteroendocrine cells of the small intestine in mice and man (Dyer *et al*. 2007; Margolskee *et al*. 2007). Knocking out either α-gustducin or the taste receptor subunit, T1R3, abolishes the ability of mouse intestine to upregulate SGLT1 expression in response to increased dietary carbohydrate. The data indicate that changes in lumenal sugars are detected by the gut sweet taste receptor (sensor). This stimulates a signalling pathway resulting in secretion of gut hormones, GLP-1 (glucagon-like peptide 1) and GIP (glucagon-dependent insulinotrophic peptide), known to enhance insulin secretion, from the enteroendocrine cells. One or both hormones, through a paracrine mechanism, enhance the expression of SGLT1 in the absorptive enterocytes (Margolskee *et al*. 2007). Further, recent work has demonstrated that the sweet taste receptor subunits are expressed in the equine small intestine (J. Dyer and S. P. Shirazi-Beechey, unpublished data).

There is evidence that co-transport of water along with sodium and glucose accounts for about 50% of the total water transport across the human intestinal brush border membrane (Loo *et al*. 1996). The links between sodium, glucose and water transport form the basis for the use of salt solutions containing glucose for oral rehydration therapy (Hirschorn and Greenough 1991). In horses, the small intestine is an important site for water absorption associated with nutrient (glucose) uptake. Thus manipulation of SGLT1 expression, via the activation of the equine intestinal sugar sensor, has the potential to not only enhance the intestinal glucose absorption, but also that of water and electrolyte. This provides a physiological rationale for suggesting nutritional manipulations that can be used to enhance performance and overcome the detrimental effects of post exercise dehydration.

Digestion and absorption of dietary plant fibre in equine large intestine

In the horse the caecum and colon account for two-thirds of the volume of the digestive tract. They contain a diverse community of anaerobic bacteria, protozoa and fungi. These anaerobes initially depolymerise dietary pectin, starch, cellulose and hemicellulose to their component monosaccharides. Metabolism of these monomeric sugars in bacterial cells, through the Embden-Meyerhoff pathway, leads to the formation of pyruvate and subsequently to SCFA (mainly acetate, propionate and butyrate) and gases, namely carbon dioxide (CO_2) hydrogen (H_2) and

methane (CH_4). In normal circumstances, very little lactate is present. The amount and relative proportion of products formed depend upon i) the nature of the dietary polysaccharides fed to the horse and ii) the degradative activity, fermentation pathways and the interspecies interactions of the major anaerobic species present in the equine caecum and colon.

Microbiology of the equine large intestine

The molecular diversity of the microflora within the large intestine of horses maintained on grass pasture was investigated through the analysis of PCR-amplified 16S ribosomal gene sequences (Daly *et al*. 2001; Daly and Shirazi-Beechey 2003). It was found that the bacteria present belonged predominantly to the low %G+C Gram-positive phyla, including the *Clostridiaceae*, the *Cytophaga-Flexibacter-Bacteroides* (CFB) assemblage and the *Eubacterium rectale-Clostridium coccoides* group. Also the *Spirochaetaceae, Fibrobacter* and the *Bacillus-Lactobacillus-Streptococcus* (BLS) group were found to be important components (Daly *et al*. 2001; Daly and Shirazi-Beechey 2003). These studies also revealed that the *Spirochaetaceae*, the CFB, the *E. rectale-C. coccoides* group and a group of bacteria phylogenetically related to the *Clostridiaceae* make up the largest proportion of bacteria in the equine hindgut. Among these are many important cellulolytic and fibrolytic species, with the *Spirochaetaceae* being especially important in terms of acetate production. Figure 3 depicts the microbial population of the equine large intestine and fermentation products.

Using the same approach, we further demonstrated that a change in diet from grass to grain results in a significant 3-fold increase in the relative abundance of the *Bacillus-Lactobacillus-Streptococcus* group, comprising mainly saccharolytic species responsible for the production of lactate from the hydrolysis and fermentation of polysaccharides (K. Daly and S. P. Shirazi-Beechey, unpublished data). However, with the change from pasture to grain, there was a concomitant 4-fold decrease in the relative population abundance of *Fibrobacter* spp., acid-intolerant cellulolytic bacteria, whose growth is greatly suppressed at pH 6.0–6.1 and completely inhibited at pH <5.9 (Miwa *et al*. 1997). This decline is probably a consequence of the reduction in colonic pH due to increased lactic acid production. These findings confirm that changing the diet from grass to grain favours certain populations of bacteria resulting in altered fermentation products.

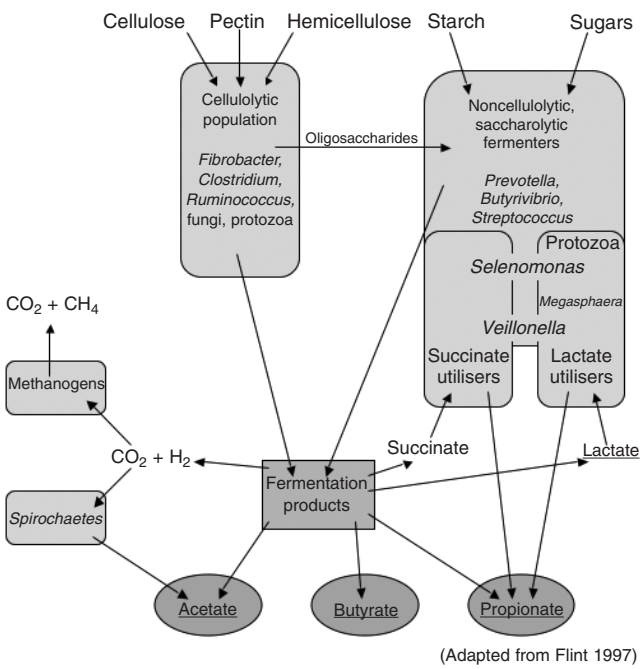

Fig 3: Pathways of fermentation of dietary carbohydrates in equine large intestine. Fermentation of dietary structural and soluble carbohydrates by equine large intestinal microflora results in production of acetate, propionate, butyrate and gases: carbon dioxide (CO_2) hydrogen (H_2) and methane (CH_4).

Absorption of SCFA in the equine large intestine

Monocarboxylates or SCFAs (acetate, propionate and butyrate) are absorbed in the large intestine and provide a significant proportion of horse's body energy. The mechanism for the transport of SCFA in human colon has been fully characterised and it has been shown that SCFA anions are transported across the luminal membrane of human colonocytes by the moncarboxylate transporter isoform 1, MCT1 (Ritzhaupt *et al*. 1998). Butyrate serves as the major respiratory fuel for colonic epithelial cells, plays a role in suppressing mucosal inflammation, and is essential for the maintenance of the health of the colonic epithelium by regulating the expression of genes associated with proliferation, differentiation and apoptosis (Cuff *et al*. 2005; Daly and Shirazi-Beechey 2006). Propionate is utilised in the liver as the precursor of gluconeogenesis and acetate is used as a source of energy by the peripheral tissues (Macfarlane and Cummings 1991).

Work in our laboratory has determined the mechanism for the uptake of SCFA across the equine colonic luminal membrane (T. Nedjadi, K. Daly and S. P. Shirazi-Beechey, unpublished data). The absorption of monocarboxylates, acetate, propionate and butyrate, across the equine colonic luminal membrane into colonocytes is accomplished by a moncarboxylate/H^+ symporter according to the scheme in Figure 4. This activates the absorption of Na^+ and Cl- in exchange for hydrogen and bicarbonate ions,

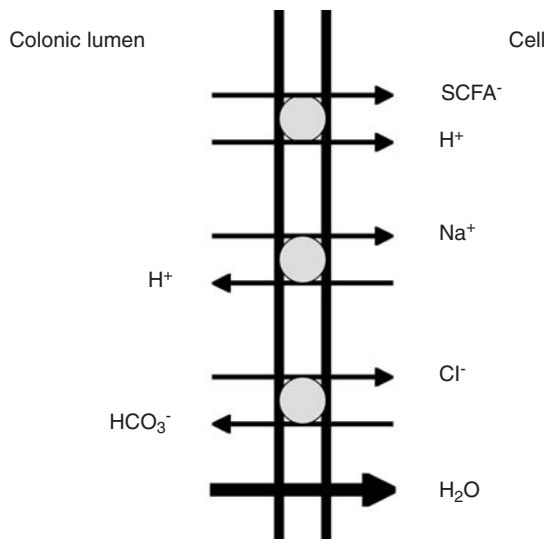

Fig 4: Schematic diagram for SCFA absorption across the luminal membrane of equine colonocytes. SCFA absorption activates salt, water absorption and bicarbonate secretion.

respectively. Absorption of water then accompanies electrolyte absorption in equine large intestine (Frape 1998).

We have shown that the equine colonic monocarboxylate transport is inhibited by various pharmacological agents, and by the monocarboxylates lactate and pyruvate. It is also inhibited by amiloride (the inhibitor of Na^+/H^+ exchanger) and the stilbene derivatives (SITS and DIDS, inhibitors of Cl^-/HCO_3^- exchanger) indicating that the transport of the monocarboxylates from the lumen of the equine colon into the colonocytes is accompanied by NaCl absorption and HCO_3^- secretion.

Forms of colic

Volvulus is defined as a twisting of the intestine around its mesenteric axis. It commonly involves the caecum and proximal colon or left colon. It causes entrapment of digesta and gas in the twisted loop of the bowel and leads to infarction of mesenteric blood supply, causing ischaemia and necrosis (McGavin *et al*. 2002).

In colon displacement, abnormal amounts of gas in the large intestine can cause parts of the colon to become motile within the abdominal cavity; rather than twist, as in volvulus and torsion (see below), these large sections simply move to an unusual site. The left ventral and dorsal colons are more loosely attached to the mesentery and are not held to any other organs via ligaments. Therefore, these sections are susceptible to displacement, which can occur to the left or the right (left or right dorsal displacement).

Other forms of colic include torsion and impaction. In torsion there is a twisting of the intestine around its own long axis. It occurs most commonly in the caecum (McGavin *et al*. 2002) and, like volvulus, it leads to entrapment and infarction, and the excess gas is thought to be responsible.

Excess lactic acid production in the large intestine is accompanied by a significant decrease in the pH of the luminal content and a decrease in fluid content. This leads to a change in the consistency of the digesta, becoming firmer and more solid. There are several normal narrowings in the equine large intestine, which concur with a change in direction of the intestine. These flexures, the pelvic flexure in particular, are vulnerable sites for this solid gut content to become impacted. The blockage forms a plug, causing a build up of gas behind it, leading to distension and pain.

Changes in equine large intestinal microbiology and tissue biology in colic

It has been demonstrated that there is a significant increase in lactic acid concentration when horses are fed high grain (starch) diets (Medina *et al*. 2002). This is accompanied by a marked decrease in caecal/colonic pH (from 7.5 to <6.2) (Julliand *et al*. 2001; Medina *et al*. 2002), which has a profound effect on the microbial population and their fermentation products. These changes, in turn, influence the large intestinal and the whole body physiology of the horse. Using molecular microbiological approaches, we have shown that there is a significant increase in the relative abundance of the *Bacillus-Lactobacillus-Streptococcus* group and a remarkable decrease in the relative population of *Fibrobacter* spp., in the large intestinal content of horses with colic (K. Daly, C. J. Proudman and S. P. Shirazi-Beechey, unpublished data). The *Bacillus-Lactobacillus-Streptococcus* group, Gram-positive, lactate producing species, favour starch as a substrate for fermentation. They proliferate in a starch rich environment producing excess amounts of lactic acid which creates an acidic environment. *Fibrobacter* spp. and other cellulolytics are predominantly acid-intolerant bacteria whose growth is greatly suppressed at acidic pH (Miwa *et al*. 1997).

This alteration in both the microbial community and their metabolites result in a significant reduction in the fermentation of structural carbohydrates and hence SCFA production. SCFAs are an important source of energy for the horse. In addition, butyrate plays an essential role in regulating the expression of genes controlling colonic tissue homeostasis and hence the maintenance of gut health (Cuff *et al*. 2005; Daly and Shirazi-Beechey 2006). Therefore, any reduction in SCFA production will have a deleterious effect on the intestinal tissue and whole body physiology.

A decrease in the lumenal pH has other consequences for the microorganisms of the equine large intestine. Some strains of bacteria express decarboxylase enzymes, which decarboxylate amino acids to amines (Gale 1953; Rice and Koehler 1976). Amine production by these strains of bacteria is a compensatory mechanism to diminish the fall in lumenal pH. However, amines have been implicated in the development of laminitis by acting as a vasoconstrictor of digital blood vessels (Bailey *et al*. 2002). For amines to have such a systemic effect they must be transported across the colonic epithelium. Nothing is known about the membrane proteins involved in the absorption of amines across the equine large intestinal epithelial cells.

Species that readily ferment starch, in preference to structural carbohydrates, will proliferate in a starch rich environment producing not only excess lactate but also large amounts of CO_2, which can cause distension and pain. This excess gas production can lead to different forms of colic (see above).

It has been suggested that grain feeding also affects gastrointestinal function, such as salt, water and SCFA absorption. It has been shown that feeding large grain diets results in dehydration and changes in the concentration of electrolytes (Argenzio and Stevens 1975; Clarke *et al*. 1990; Lopes *et al*. 2004). It was proposed that large grain meals could cause post prandial dehydration of colonic contents and lead to impaction of the large colon, which could initiate some forms of colic such as large colon displacement and volvulus (Clarke *et al*. 1990). It is suggested that lactic acid may be the agent responsible for these changes.

Lactic acid/lactate can enter the cell resulting in an intracellular acidification. A reduction in the intracellular pH, brought about by lactic acid, has been shown to affect the expression of genes controlling apoptosis of the colonic epithelial cells (Mathupala *et al*. 2004), thereby affecting colonic tissue homeostasis. In addition, alterations in the intracellular pH modulate the activity of the Na^+/H^+ antiporter and, in turn, influence salt, water and nutrient absorption.

A better knowledge of the equine digestive system, at the molecular and cellular level, and the recognition of both constitutive (i.e. preprogrammed) and adaptive processes should assist the development of rational strategies to modify the capacity of the intestine to digest and absorb dietary carbohydrates. This will also facilitate a more scientifically-based approach to designing feed formulation and management with the ultimate aim of enhancing the health of the horse and reducing the incidence of intestinal disorders such as colic and laminitis.

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