How much acid in the gut is too much?

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Summary

Rapid fermentation of carbohydrate in the rumen or in the hind gut (caecum and colon) can lead to the accumulation of acid. While the acute condition of lactic acidosis or grain poisoning is well known there is a wide range of adverse consequences of sub–clinical acid accumulation not commonly associated with diet. This paper outlines the range of ways in which acids produced during fermentation in the gut can affect the health and productivity of animals and investigates the level of acid required to initiate these adverse effects. Absorbed acids from rapid fermentation contribute to imbalance of anions/cations and acids/bases at the tissue level and exacerbate problems associated with bone resorption and oedema. The direct effects of acid on the gut wall and the effect of acid fermentation on bacteria in the gut interact to cause inflammation and tissue damage to the gut wall, affecting its permeability to toxins and causing a range of problems mediated via cytokines and the immune system. Further problems may be caused by exposure of bacteria to acidic conditions in the hind gut, thus increasing their pathogenicity through enhanced resistance to acidic conditions in the stomach. It is suggested that many of the adverse effects may be initiated by levels of acidity commonly experienced in the rumen or hind gut of ruminant and monogastric animals fed 'normal' production diets.

Introduction

The condition of acute acidosis in ruminant animals is well understood (Dirksen 1970; Dunlop 1972) and animals suffering from this condition have invariably been fed large amounts of grain without gradual introduction to that feed, or large amounts of fermentable carbohydrate have been introduced directly into the rumen via fistula or stomach tube. Animals with acute acidosis show clear signs of being unwell and will either die or suffer long–term disabilities. There is increasing recognition of the condition of chronic acidosis, and the studies reported by Reid et al. (1957) and Koers (1976) are cited by Slyter (1976) as examples of this. In chronic acidosis there is clearly an abnormal concentration of acid in the rumen, but the animal shows no sign of being sick. Animal performance under these conditions is, however, sub–optimal due to less efficient fibre degradation and reduced feed intake. A third level of fermentative acidosis, acidic gut syndrome, has been described (Rowe 1997) in which acid concentrations in the gut, although considered to be 'normal' by most veterinarians and nutritionists, may still be at a level capable of causing adverse effects on the health and welfare of the host. It is likely that this condition of acidic gut syndrome is principally a result of acid accumulation in the hind gut, and adverse effects on the animal are mediated via three avenues: mild metabolic acidosis; inflammation of the gut wall; and modification of the gut microbes by the acidic environment.

Is the gut adapted for acidic conditions?

Parts of the digestive tract of all animals have developed into pouches or large diameter tubes that retain large volumes of digesta and slow down the rate of passage in order to allow microbial digestion of fibrous material. In these regions of the gut, adapted for microbial activity, pH is maintained close to neutral which optimises conditions for the fibre–degrading microbes. In cattle and sheep, most fermentation takes place prior to gastric digestion and intestinal absorption and the rumen wall is thick and muscular with a keratinised epithelium, and papillae to increase the absorptive surface. On the other hand the hind gut (caecum and colon) is distal to gastric digestion and the substrates available for fermentation there are normally the fibrous dietary components not digested and absorbed from the small intestine. The wall of the caecum and colon is far thinner than that of the rumen, does not have papillae, and appears to be far less well adapted for rapid fermentation and acid production than is the rumen.

Rapid fermentation and the accumulation of acid in the hind gut are only likely to occur when sugars or starch pass undigested through the small intestine and are available to the microbes in the caecum and colon. The consumption of large quantities of starch in the form of cereal grain or the availability of high concentrations of fructans in lush green pastures are two situations when this can occur. When considered on an evolutionary scale, the dietary conditions likely to produce hind gut acidosis are relatively new developments in animal nutrition. It is therefore unlikely that herbivores are well adapted either for efficient digestion of large quantities of sugars and starches or for acid accumulation in the hind gut. Intensive feeding systems are almost certain to produce acid accumulation in the hind gut at levels for which the epithelium of the caecum and colon are not well prepared.

Poultry, and to a lesser extent pigs, are the only animals intensively selected for performance on diets containing high levels of cereal grain. This indirect genetic selection for efficient intestinal digestion of starch would involve many hundreds of generations in poultry and far fewer in pigs. In the case of all other domestic livestock, companion animals, and even humans, the rate of increase in the level of consumption of starches, gums and sugars has probably been far more rapid than genetic changes in digestive physiology needed to ensure efficient intestinal digestion. Cats and dogs are carnivores, yet in recent years pet food has contained increasing levels of processed cereal grain and it is likely that these animals may have special problems associated with the fermention of undigested carbohydrates in the hind gut. The human diet in Australia, as in many other parts of the world, has changed very rapidly with major trends towards increased intake of cereal products. It is possible that incomplete intestinal carbohydrate digestion may also lead to acid accumulation in the hind gut of humans. This paper discusses the ways in which low pH in the caecum and colon may have adverse effects on the host.

How can acidity of the gut contents affect the host?

Acids produced in the gut can affect the animal in three ways as summarised in Figure 1 and described below:

- absorbed acids can have a direct effect on the systemic acid–base balance and on the balance between anions and cations, producing 'fermentative' metabolic acidosis

- accumulation of acid within the gut may have a direct effect on the gut wall

- acidic conditions can modify the composition of the bacterial population as well as changing characteristics of individual organisms, and this may produce adverse effects for the host animal

Effects of increased absorption of acid from the gut

When carbohydrate is hydrolysed to monosaccharides and absorbed from the small intestine it contributes no hydrogen ions to the host animal, and does not affect the acid–base balance or anion or cation concentrations. On the other hand when carbohydrate is fermented in the rumen (Leng 1970) or hind gut (Bach Knudsen *et al.* 1991), the end products are principally the volatile fatty acids and/or lactic acid and these end products do contribute hydrogen ions as well as adding to the cation load (Figure 2).

The pH of blood affects the extracellular and intracellular pH and this, in turn, has a significant effect on practically all biochemical pathways. While blood is very well buffered at around pH 7.4 by various systems involving bicarbonate, phosphate, proteins and a wide range of ions, in the long term there needs to be a balance between the production/absorption and the excretion/utilisation of acids, bases, anions and cations. Factors that can disrupt the balance of acids and bases include
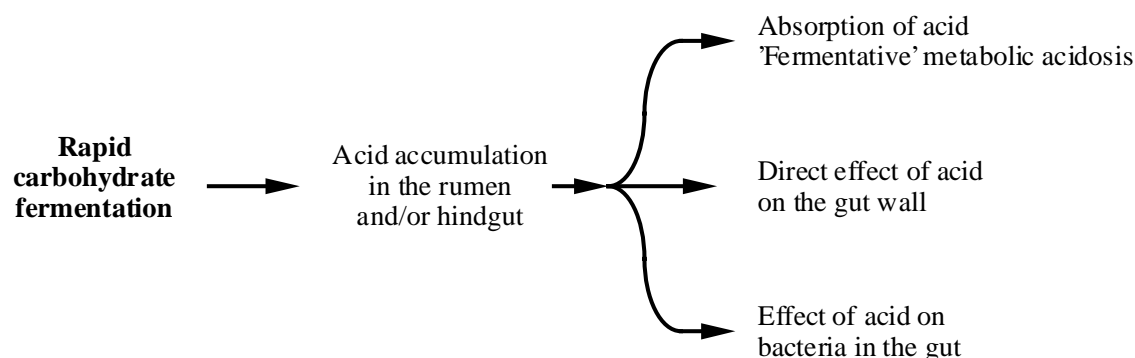
Rapid carbohydrate fermentation → Acid accumulation in the rumen and/or hindgut →

- Absorption of acid 'Fermentative' metabolic acidosis
- Direct effect of acid on the gut wall
- Effect of acid on bacteria in the gut

Figure 1 Summary of the pathways linking intake of starch and sugar with the major ways that acid in the gut can adversely affect the host animal.

respiratory disorders, dietary mineral disturbances, renal failure, lactic acid produced during intense exercise, and acid produced during fermentation of carbohydrate in the digestive tract. Respiration plays a major role in modifying carbon dioxide levels, and hyper– or hypo–ventilation can create respiratory alkalosis or acidosis respectively. The kidneys also play a very important role in maintaining acid–base balance and the pH of urine provides a sensitive indicator of the body's need to excrete acid. Most of the surplus acid excreted by the kidney is in the form of phosphoric acid or ammonia (see Figure 3). Although phosphoric acid can be an important medium for acid excretion in the urine of humans and monogastric animals (see Figure 3a) it is only of minor importance in ruminants where ammonium ions provide the major mechanism for acid excretion (Scott 1975). The loss of ammonium in this way constitutes a loss of protein because the renal tubule cells degrade amino acids to ammonia in order to excrete hydrogen in the form of ammonium ions (Figure 3b)..

The mobilisation of bone following imbalance of the cation/anion balance is the basis of the DCAD program of mineral nutrition for pre–partum cows as a method of reducing milk fever (Beede 1992). In order to reduce the risk of milk fever during onset of lactation a
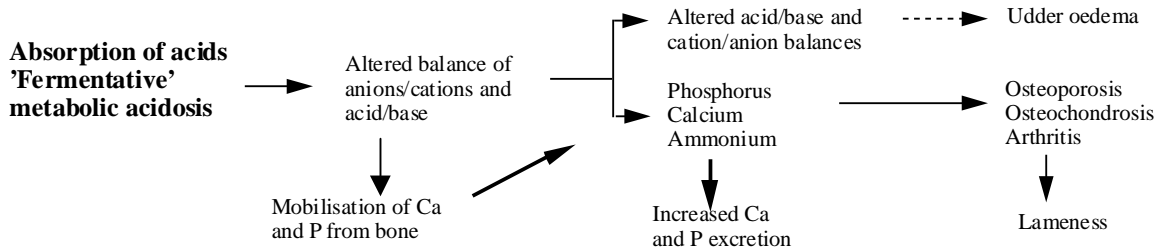

Figure 2 Summary of the ways in which absorbed acids may have adverse effects on anion/cation as well as acid/base balances within the host animal.
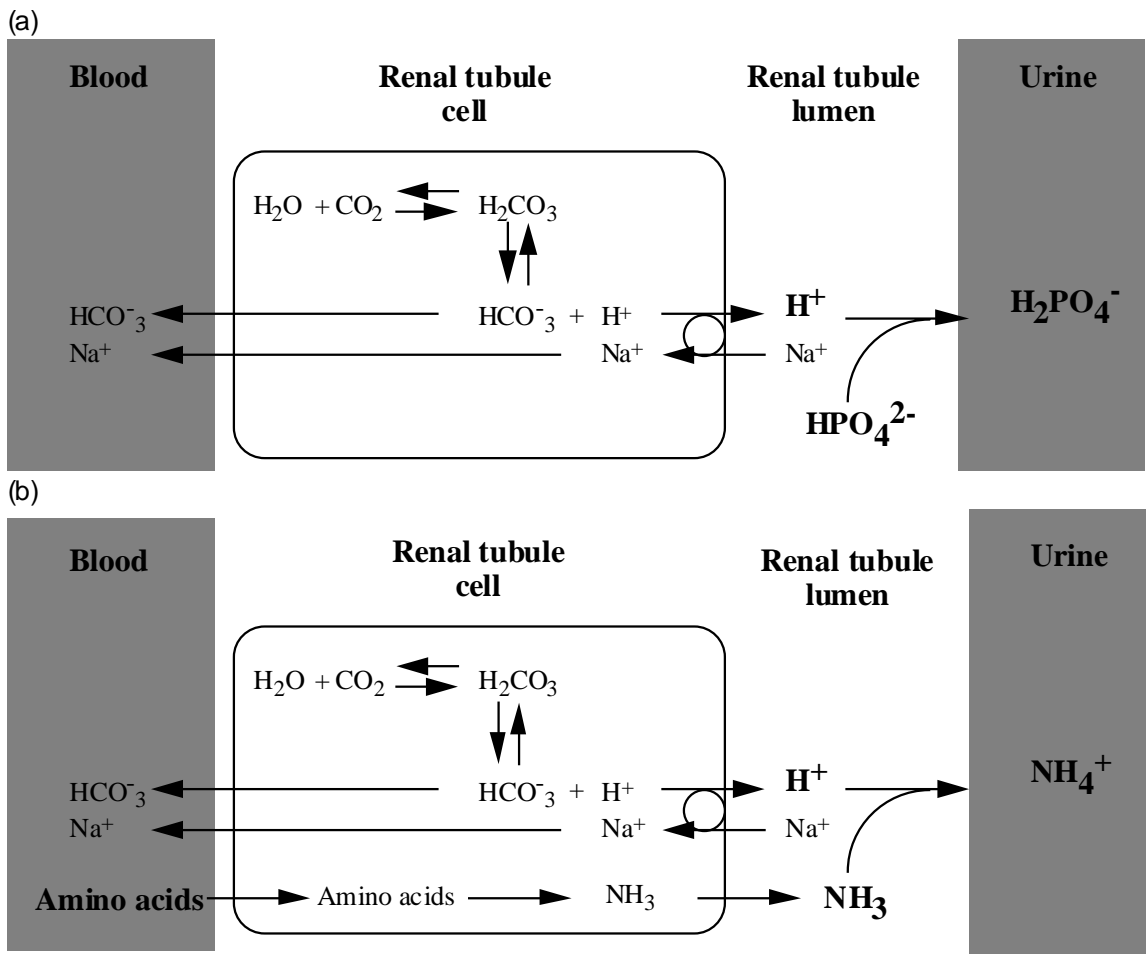

Figure 3 Summary of the pathways in the renal tubule cells responsible for acid excretion in the form of (a) phosphoric acid and (b) ammonium ions (from Baggott 1992).

surplus of the anions Cl$^-$ and S^{2-} are commonly included in the diet for a period before calving in order to create a mild acidosis. In order to correct the acid/base balances CO$_3^{2-}$ is mobilised from bone and in this process calcium is released and can be used during the peak demand in early lactation (Beede 1992).

Rapid fermentation, producing a range of acids (VFA and lactate), is likely to change the acid and cation balance and thus create a situation where bone reserves are mobilised, and changes occur in urinary excretion which are characteristic of metabolic acidosis (Figure 4). Elevated levels of urinary phosphorus have been reported in cattle (Topps 1966) and in sheep (Nokata *et al.* 1977) fed high levels of grain. The loss of phosphorus in urine appears to be a short term adaptive phenomenon following introduction of grain, with nearly all subsequent acid excretion in ruminants being in the form of ammonium ions (Scott 1975). Longer term excretion of phosphorus in the urine has been found in humans and dogs subject to acidosis (Scott 1975) and it is likely that this increase in excretion of phosphorus is due in some part to resorption of bone.
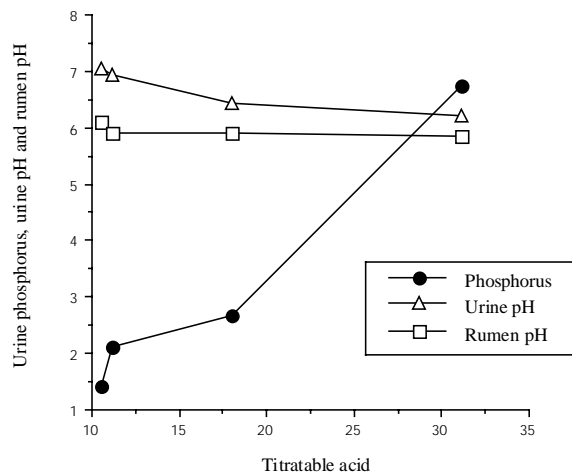
The pH of urine from different classes of animals was recently measured in a commercial piggery in Australia (Rowe 1997). Values of around pH 4.5 were common, levels of titratable acid and ammonium excretion being highest in sows at the end of their first week of lactation. The pH of faeces in grower pigs was also clearly acidic (around 6.2), indicating extensive fermentation in the hind gut. A secondary effect of such a constant acid load may be a change in the requirements for other anions and cations. It is likely that udder oedema, observed in some cows and sows, reflects an ionic imbalance (van der Kolk 1998) and may be a detrimental consequence of an underlying problem of fermentative acid production in the hind gut.

Direct effect of acid on the gut wall

Although the gastric stomach is adapted to withstand the low pH which is associated with acidic enzymatic degradation of proteins and carbohydrates, all other parts of the digestive tract, including the rumen, caecum and colon, normally encounter digesta with a pH of around 6.5 to 7.0. These enlarged compartments within the digestive tract are effective at maintaining a barrier against the entry of bacteria (Sharpe 1975) but are damaged during lactic acidosis (Dunlop 1972).

When rapid fermentation leads to accumulation of acid there are several possible effects on the host and these are summarised in Figure 5. In the rumen there is a thickening of the epithelium and lamina propria resulting in parakeratosis (Kay 1969) and reduced efficiency of VFA absorption (Hinders 1965). Severe cases of acidosis can result in rumenitis and sloughing of epithelium from the rumen wall (Kay 1969). A secondary development resulting from damage to the rumen epithelium is leakage of toxins and bacteria into the portal circulation and the most common indication of this problem is the occurrence of liver abscess in grain fed cattle (Jensen 1954; Nagaraja 998). Damage to the gut wall is not confined to fermentative acidosis in the rumen and major structural changes to the epithelium in the caecum have been reported in horses overfed with grain (Krueger *et al.* 1986). Further evidence for an adverse effect of acid on the gut wall is provided by the fact that acetic acid has been widely used to induce colitis under experimental conditions (Fabia 1992). The
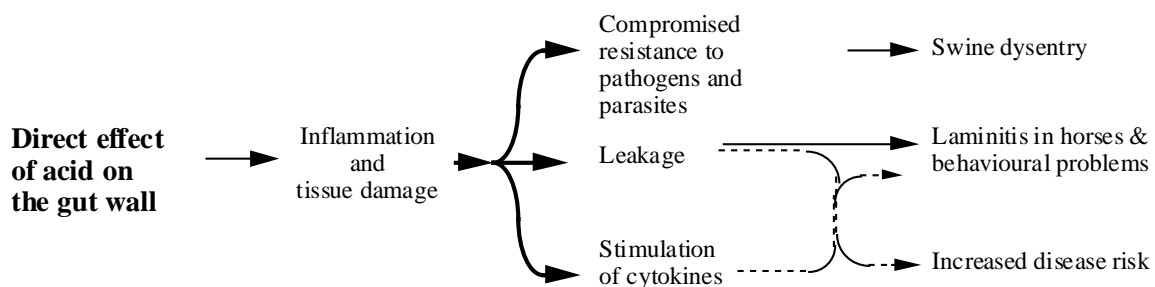
Figure 4 Relationship between the urine titratable acid (ml N NaOH against 24 hour urine excretion) and the excretion of phosphorus (g/d), urine pH and rumen pH in cattle fed high levels of maize grain (Topps *et al.* 1966).

Figure 5 Direct effects of acidic digesta contents on the gut wall and the likely consequences for domestic animals.

standard method for inducing colitis involves the introduction of acetic acid (4% solution) into the caecum and colon for 15 seconds. This exposure to acid results in a 100% incidence of a moderately severe colitis lasting for around 4 days (Fabia 1992). Higher concentrations of acid or longer exposure times have been found to produce deep colitis with a concomitant high mortality rate (Fabia 1992). While the concentration of acid used to induce experimental colitis is five to 10 times higher than would normally be encountered as a result of fermentation in the gut, the time needed to bring about tissue damage is far shorter than the normal residence time of digesta in the fermentation compartments of the gastrointestinal tract. It is highly likely that low levels of acid, say pH 5 to 6, over an extended period, may result in changes to the hind gut or rumen epithelium similar to those observed with more concentrated acids over shorter periods.

Bacterial population

Pluske *et al*. (1997) reported a dietary effect on the susceptibility of pigs to infection with swine dysentery (*Serpulina hyodysentariae*). Pigs fed diets providing high levels of carbohydrate that passes through the intestines and is rapidly fermented in the hind gut are very much more susceptible to infection with *S. hyodysentariae* than animals fed boiled rice, which is almost totally digested in the small intestine. Diets associated with high rates of infection produced more acidic conditions in the hind gut than observed when boiled rice was fed. It is possible that increased susceptibility to infection is a direct effect of acids damaging the epithelium of the hind gut epithelium and making it possible for it to establish in the mucosa. Alternative explanations could be that *S. hyodysentariae* becomes more invasive with the correct substrates for its metabolism in the hind gut, or that the substrates and accumulation of acids allow facilitating bacteria to promote an increased susceptibility of the gut wall to infection.

Although it is very difficult to determine exactly what level of acid in the gut may constitute a risk to the gut wall, it is likely that over a long period any level of acidity will have an adverse effect.

Consequences of acidic damage to the gut wall

In the previous sections the discussion has focussed on the physical damage to the tissue of the gut wall as a result of acid conditions and possible leakage of bacteria through the rumen wall to colonise the liver and cause abscesses. It is also likely that increased acidity within the gut will have secondary systemic effects via the following mechanisms:

- inflammation leading to increased cytokine production

- stimulation of tissue remodeling agents such as matrix metalloproteinases

- leakage of toxins and bacteria from the gut

Cytokines

It is almost certain that inflammation caused by the direct action of acid on the gut wall will lead to the release of cytokines, and that local effects can become systemic problems through activation of gut associated lymphoid tissue (GALT) and the immune system in general. The possible roles of cytokines and the immune system in mediating the adverse effects of acidosis have been discussed previously (Rowe 1997). The release of TNF–α into the gut as a result of colitis can be detected in faeces (Watkins *et al*. 1997) and it is likely that inflammation caused by fermentative acid accumulation will have similar effects. Cytokines are known to have a very wide range of systemic effects including oedema of the mammary gland (Shuster 1993), resorption of bone (Votta 1995), joint diseases (May 1997), respiratory diseases (Horovov *et al*. 1997), and stomach ulcers (Akimoto 1998).

Matrix metalloproteinases

One of the best defined secondary complications associated with excessive fermentable carbohydrate intake in horses is laminitis. It has been suggested (Pollitt 1996) that damage to the basement membrane in the lamellar region of the hoof, responsible for separation of hoof wall and subsequent founder, may be initiated by metalloproteinases. These enzymes are responsible for tissue breakdown and reformation of tissues during growth and are also produced by ecto–parasites as part of the process of penetrating skin. Under normal conditions the levels and activities of metalloproteinases are closely controlled by numerous feedback mechanisms but it is possible that under conditions of significant stimulation, such as might occur during fermentative acidosis, that their overproduction can trigger tissue damage. It is possible that unregulated activity of matrix metalloproteinases are responsible for the damage that occurs to the lining of the rumen (Kay 1969) and caecum (Krueger *et al*. 1986) as a result of acidic conditions within the gut. As in the case of cytokines, it is possible that locally stimulated metalloproteinase production could escalate into a systemic effect, acting on the hoof and other tissues. This could occur by the matrix metalloproteinases produced in the gut wall either being transported or acting as a catalyst for further matrix metalloproteinase production in other tissues. Tissue damage to the gut wall and to the hoof, following acidosis, can be very rapid (Kay 1969; Krueger *et al*. 1986; Pollitt 1996a) and even if levels of matrix metalloproteinases are elevated for only a short time they may have significant detrimental effects. While the above is an attractive hypothesis, the possible role of matrix

metalloproteinases requires further definition in terms of the factors that may trigger their release and their specific connection with acid and/or microbial toxins produced within the gut.

Leakage of toxins and bacteria from the gut

With inflammation and damage to the gut wall resulting from acidic insult, it is possible for bacteria and various toxins to 'leak' into the gut tissue as well as into the visceral blood and lymphatic systems (Nagaraja 1998). This leakage is likely to have direct effects through the release of toxins from pathogenic bacteria into the body. There is also likely to be a range of important indirect effects.

With a 'leaky' gut, full of bacteria, there are many parallels that can be drawn with problems well known in relation to chronic and acute bacterial infections triggered by lipopolysaccharide and mediated through cytokines. The two major routes for the development of laminitis in horses are through excessive carbohydrate intake (fermentative acidosis), and as a result of serious infections such as a retained placenta. In the case of carbohydrate overload it is likely that the gut wall becomes a less effective barrier to microbes and endotoxins and becomes the site of a serious infection similar to that of an infected, retained placenta. Although neither pathway linking laminitis to serious infection or laminitis to fermentative acidosis is well defined, it is likely that some combination of endotoxins, cytokines and metalloproteinases are involved in both.

Effect of acidity on microbes within the gut

There are two major ways in which acid accumulation in the gut has been shown to affect microbes and these are summarised in Figure 6.

- in the presence of high levels of carbohydrate, and an acidic pattern of fermentation, elevated levels of microbial endotoxin have been reported (Dougherty and Cello 1949; Moore *et al*. 1979)

- bacteria exposed to acids in the gut can develop acid resistance to the extent that their pathogenicity is greatly increased (Diez–Gonzalez *et al*. 1998). It is also possible that the acidic

conditions alter the microbial balance and that the fermentation activity of certain bacteria is altered to facilitate pathogenic activity against the host

Endotoxin production

There is evidence that rapid fermentation of starch or sugars leads to the accumulation of lactic acid and that the resultant low pH is associated with an increased concentration of cell wall lipopolysaccharide (endotoxin) derived from Gram negative bacteria (Moore *et al*. 1979). Endotoxins from Gram–negative bacteria or derived from their cell walls are important agents of sepsis and septic shock (Coran 1997). Microbial endotoxins are normally present in the gut without causing apparent harmful effects. Following carbohydrate overload there is, however, evidence of a marked, but transient, increase in intestinal permeability (Weiss 1998). It is possible that this increased permeability contributes to the absorption of endotoxins and their systemic toxic effect. It is interesting that endotoxins injected into the intestines of rabbits caused changes such as reduced appetite and diarrhoea (Siarkas, 1997). These effects are totally consistent with some of the best known signs of grain overload in ruminants and suggest that endotoxins may have adverse effects on the animal even under apparently normal dietary conditions.

Acid–resistant pathogens

Recent studies by Russell and colleagues (Diez–Gonzalez *et al*. 1998) have shown that pathogenic bacteria of the small intestine can develop the level of acid–resistance they require to survive passage through the gastric stomach by being exposed to acid conditions in the rumen and hind gut of grain–fed cattle. This phenomenon has implications for human food safety because there is always a risk of bacterial contamination of the carcase in the abattoir. There is also an increased disease risk for the host animal, particularly where coprophagy is practised. The question as to what level of acid accumulation is sufficient to increase acid tolerance to the point where there is a significant increase in the development of acid resistance in pathogenic bacteria is answered by the data of Diez–Gonzalez *et al*. (1998). Summarised in Figure 7, these data suggest an increased incidence of acid–resistant *E.coli* is directly related to acidity in the colon. It appears that the numbers of acid resistant
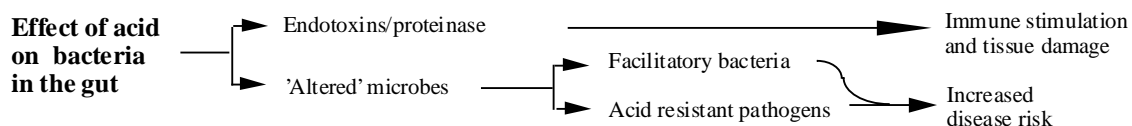

Figure 6   Effects of acid accumulation during anaerobic fermentation on bacteria in the gut.

E. coli start to increase even when the colonic pH is around 6.5.

Facilitating bacteria

There are several situations where the pathogenic effects of one microbe are facilitated by another. For example in the case of swine dysentery the infective organism *S. hyodysentariae* does not establish in germ free animals (Meyer *et al.* 1974; Meyer *et al.* 1975) or in the absence of acidic conditions in the hind gut (Pluske *et al.* 1997). In this example it is not clear whether the acidic conditions associated with carbohydrate fermentation create suitable conditions for the facilitating bacteria, or whether there is a direct effect of acid on the mucosal lining creating the appropriate environment for both *S. hyodosenteriae* and facilitating bacteria to establish in the gut wall. Bacteria growing in a wound can produce toxins that damage host tissue and reduce the effectiveness of the immune system response through killing phagocytes in the vicinity and facilitating growth of the pathogen.

Interaction and synergism of the adverse effects of fermentative acidosis

As shown in Figure 8, and discussed above, there are numerous interactions between the various adverse effects of fermentative acidosis. It is a clear that the direct effect of acid on the gut wall, by causing inflammation and leakage, is likely to exacerbate the toxicity associated with absorption of endotoxins and other large molecules. In the same way endotoxins are likely to have a direct effect on the gut wall and act together with acid to increase the extent of inflammation and leakage. At the secondary level it is well known that cytokines have a direct effect on bone resorption (Votta 1995) and are involved in arthritis and joint disease (May et al. 1997). It is likely that this action of cytokines on bone resorption will exacerbate the effects of systemic acid load, directly associated with rapid carbohydrate fermentation, leading to increased calcium and phosphorus mobilisation (Figure 2).
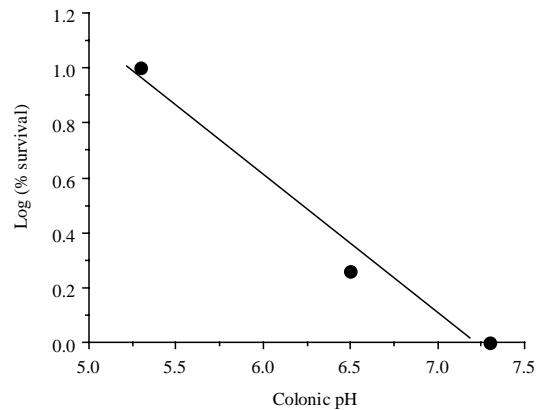

Figure 7 The relationship between pH of digesta in the colon of cattle fed different amounts of rolled maize and the survival of acid–resistant *E. coli* excreted in the faeces (from the data of Diez–Gonzalez *et al.* 1998).
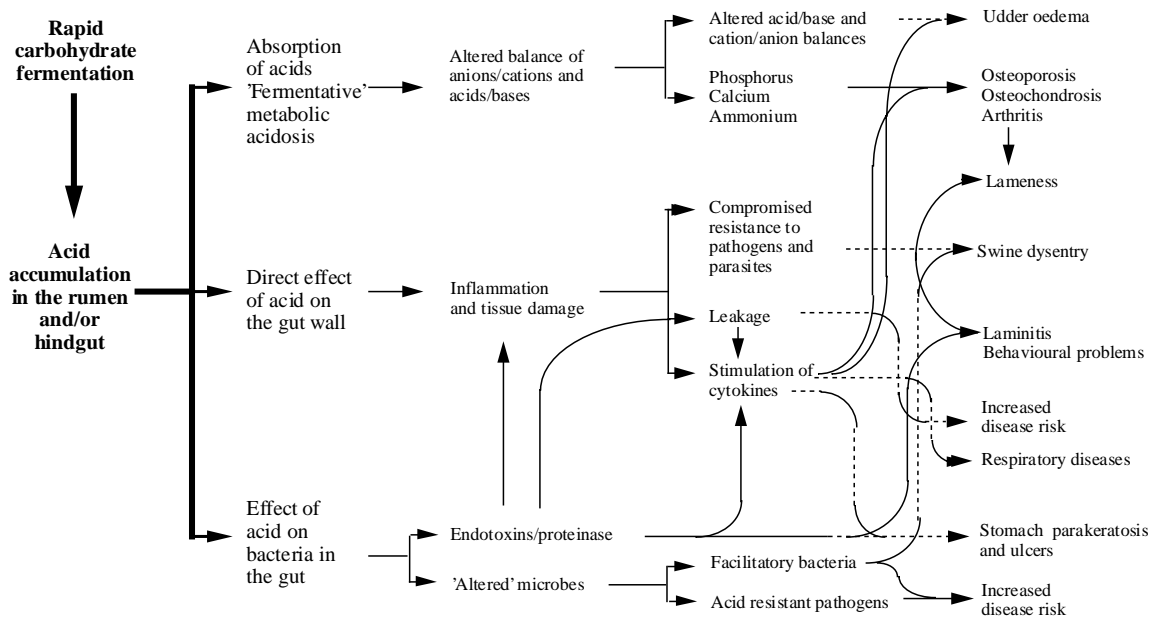

Figure 8 Summary of the direct and indirect effects of acid accumulation in the rumen and/or hind gut. The dotted lines indicates 'likely' interactions and the solid lines those effects already well documented.

The connections between a range of disorders of farm livestock fed high grain diets are summarised in Figure 8. These conditions are commonly associated with high levels of production, intensive management, animals being confined indoors (often on concrete) and diets characterised by high levels of grain inclusion. Disorders such as respiratory problems, lameness and disease have commonly been attributed to stress associated with intensive housing and concrete flooring. Very little attention has so far been directed to the possible effects of diet. This is despite the fact that diets characterised by high levels of cereal grain are common to all the animal management systems where problems of lameness, udder oedema, and elevated incidence of 'production' disease are increasing. It is likely that some of the problems identified in relation to farm livestock may now also be significant issues in the diet of companion animals, and in humans, as raw and modified carbohydrates form an increasing component of their diets.

Conclusion

The three major effects of fermentative acidosis, namely absorption of acid, effect of acid on the gut wall, and effects of acid on bacteria in the gut are able on their own, and more particularly in combination, to explain a number of the common disease problems associated with intensive livestock production. By understanding the importance of these side–effects of grain feeding we will be able to modify our choice of grain and the way in which it is processed to ensure minimum risk of fermentative acidosis.

Acknowledgement

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