Excessive grain feeding; acid–resistant bacteria and their impact on cattle

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Summary

Grain can enhance the growth of cattle, but excessive grain utilization causes a variety of problems (low ruminal pH, ruminitis, founder, liver abscesses, etc.). These problems are caused by the overgrowth of pH–resistant, starch–fermenting bacteria, and the inability of grain–fed cattle to absorb fermentation acids as rapidly as they are produced. *Escherichia coli* is never a predominant ruminal bacterium, but it can be found at high numbers in the colon if starch bypasses the rumen. *Escherichia coli* has a life cycle that involves growth in fecal material as well as the intestinal tract, and to complete this cycle *E. coli* must be able to survive the low pH of the gastric stomach (extreme acid resistance). Extreme acid resistance of *E. coli* can be induced by grain–dependent increases in colonic volatile fatty acids (VFA).

Introduction

Ruminants evolved as grazing herbivores and their capacity to harvest the photosynthetic potential of grasslands was facilitated by their ability to harbor and exploit cellulolytic bacteria (Hungate 1975). Ruminants can utilize fiber that would otherwise be indigestible, but fiber digestion is never complete. When the plant cell wall matures and becomes lignified, the rate of digestion decreases, and the fraction escaping ruminal fermentation increases. Highly lignified grasses can maintain cattle but cannot be fermented fast enough to allow rapid growth or high levels of milk production (Kotarski *et al*. 1990).

Grains are fermented faster than fiber, and grain can be an extremely valuable supplement for domestic ruminants. Starch and cellulose are both glucose polymers, but the glucose polymers of starch (α 1,4 and 1,6 linked) do not have as much inter–chain hydrogen bonding as cellulose (French 1973; Tomme *et al*. 1995). The β 1,4 linked glucose polymers of cellulose are strongly hydrogen bonded, and this association decreases the surface area that is exposed to degradative enzymes (Tomme *et al*. 1995).

The rumen is well buffered by the bicarbonate of salivary secretions, but this buffering capacity can be exceeded if the fermentation rate is very rapid (Slyter 1976). Declines in ruminal pH can have a deleterious effect on animal performance and health. Cattle suffering from ruminal acidosis eat less and can develop a variety of abnormalities that include sore feet (founder), ulcerated rumen walls, and abscessed livers. If the acidosis is severe, animals can die. Any rapidly fermented carbohydrate can cause ruminal acidosis, but low ruminal pH is usually caused by excessive starch fermentation.

Starch is inherently digestible, but the starch granules of cereal grains are encased in protein (French 1973). Ruminal bacteria can quickly bore through the protein coat of some cereal grains (e.g. barley), but the starch granules of corn are coated by zein, a slowly degraded protein (McAllister *et al*. 1990). When cattle are fed raw corn, relatively large amounts can escape ruminal fermentation and pass into the intestines (Waldo 1973). Because ruminants do not secrete as much pancreatic amylase as simple–stomached species, starch passes to the colon where it is subjected to yet another fermentation (Harmon 1991; Diez–Gonzalez *et al*. 1998).

Lactic acidosis

Streptococcus bovis is found at low numbers in the rumen of forage fed cattle (Hungate *et al*. 1952), but it has a very active amylase (Cotta 1988). If cattle are abruptly shifted from forage to grain, *S. bovis* can outgrow other ruminal bacteria and become the dominant species (Hungate *et al*. 1952). When *S. bovis* grows slowly, it produces acetate, formate and ethanol, but it is homolactic if the fermentation rate is rapid (Russell and Baldwin 1979). Lactate is a stronger acid than the normal end–products of ruminal fermentation (VFA), and lactate accumulation in the rumen can cause a sudden decrease in ruminal pH (Hungate *et al*. 1952). Most ruminal bacteria are more pH–sensitive than *S. bovis*, and declines in ruminal pH lead to an even

greater shift towards increased *S. bovis* numbers. Hungate (1966) noted that lactobacilli replaced *S. bovis* when the ruminal pH was very low, and he reasoned that this shift was due to differences in pH resistance. However, recent work indicated that this shift could occur even if the rumen pH was not very low (Wells *et al*. 1997). *S. bovis* and lactobacilli numbers were inversely correlated (Table 1), and subsequent experiments indicated that the lactobacilli were producing a bacteriocin (Wells *et al*. 1997). This bacteriocin killed *S. bovis*, but its effect on other ruminal bacteria was not determined.

Non–lactic acidosis

Some ruminal bacteria can utilize lactate and counteract lactic acidosis if the rate of lactate production is not too fast and the ruminal pH is not too low (Russell and Allen 1984). Ruminal lactate conversion to VFA is facilitated by gradual diet shifts, but grain–fed cattle can be chronically acidotic even if lactate is not detected (Britton 1989). Chronic acidosis is caused by elevated VFA concentrations in the rumen (Figure 1). Because ruminal VFA absorption from the rumen is a passive process and undissociated species are absorbed at a faster rate than dissociated forms (Ash and Dobson 1963), the question then arises, why doesn't the rate of VFA absorption simply increase when pH declines?

Davis *et al*. (1964) noted that grain–fed cattle did not produce as much saliva as those consuming forage, and he reasoned that the low pH of grain fed cattle could be at least partially explained by low ruminal buffering (less dissolved bicarbonate). However, the rumen represents a significant fraction of the total body fluid. If changes in bicarbonate flow into the rumen (via saliva or diet) cause an increase in ruminal ion concentration, water will pass from the blood into the rumen or the animal will drink more water. The propensity of water flux to or from the rumen to stabilize ionic concentrations prevents significant changes in bicarbonate or ruminal buffering (Russell and Chow 1993).

Grain–dependent increases in ruminal VFA concentration could be aggravated by decreased mixing motions. Grain–fed cattle ruminate for shorter periods of time than cattle consuming forage, and rumination is coordinated with ruminal mixing motions (Van Soest 1982). The impact of ruminal mixing on VFA absorption has not been studied in a systematic fashion, but it should be noted that the diffusion rate of substances through liquids is inversely proportional to the diffusion distance (Fick's first law of diffusion). Since the rumen is a very large compartment, mixing would enhance VFA absorption. If saliva flow is severely depressed, ruminal viscosity would increase, and effective mixing would be further impaired.

When cattle consume food, ruminal VFA concentrations increase, and blood flow to the mucosa is enhanced (Barnes *et al*. 1986). Increased blood flow decreases the VFA concentration in portal blood, and this removal would have a positive effect on VFA diffusion from the rumen. When animals are fed large amounts of grain and ruminal pH is chronically depressed, the rumen wall becomes inflamed. Ruminal inflammation would be expected to have a negative effect on blood flow, and hence VFA absorption.

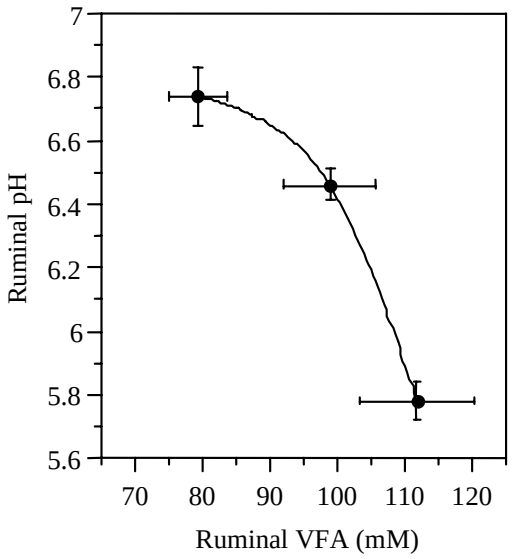

Figure 1 The relationship between ruminal VFA concentration and ruminal pH. Cattle (four animals, 3 observations per animal) were fed three ratios of timothy hay to grain (10:90, 55:45, 100:0). Figure redrawn from data of Lana *et al.* (1998).

Table 1 Effect of diet and ruminal pH on the most probable number (MPN) of S. bovis and lactobacilli. The MPN were based on the duplicate samples from two animals (n=4). Taken from Wells et al. (1997).

Diet	Ruminal pH	*S. bovis* (per ml ruminal fluid)	Lactobacilli (per ml ruminal fluid)
100% Forage	6.7-6.8	3×10^7	4×10^3
80% Cereal grain & 20% Forage	5.6-6.0	2×10^3	5×10^7

Some VFA are washed from the rumen via the fluid dilution, and this passage can have a significant impact on the amount of VFA that must be absorbed from the rumen (Allen 1997). This point is illustrated by simple calculations (Table 2). When cattle are fed hay, ruminal carbohydrate digestion, VFA production rates and ruminal VFA concentrations are low, but the fluid dilution rate is relatively fast. Under these conditions, a large fraction of total VFA is washed from the rumen by fluid dilution, and less needs to be absorbed across the rumen wall. When cattle are fed large amounts of grain, ruminal carbohydrate digestion, VFA production rates and ruminal VFA concentrations are much higher, but the fluid dilution rate is relatively slow. Under these conditions, a small fraction of total VFA is washed from the rumen by fluid dilution, and a large fraction of the VFA must be absorbed across the rumen wall.

Liver abscesses

Fusobacterium necrophorum is the primary etiologic agent of liver abscesses in cattle, and numbers of this lactate–utilizing ruminal bacterium increase when cattle are fed grain. *F. necrophorum* has been isolated from lesions in the ruminal wall, and it migrates from the rumen to the blood and finally to the liver where it causes abscesses. As stated by Nagaraja (1996): "Evidence in support of this relationship is based on the high statistical correlation for the occurrence of liver abscesses and ruminal pathology (ruminitis, hyperkeratosis, ulcerative lesions)".

Intestinal starch digestion

Starch that escapes ruminal digestion can be hydrolyzed by pancreatic amylase, but ruminant animals do not secrete as much amylase as simple stomached animals (Harmon 1991). Starch that is not hydrolyzed in the small intestine passes into the large intestine and colon. Starch fermentation can also cause a decrease in colonic pH, and VFA concentrations can be as high as those observed in the rumen. When cattle were fed 90% grain rations (chiefly rolled corn), the ruminal pH was always greater than 6.3, but the colonic pH was 5.3 (Diez–Gonzalez *et al*. 1998). Cattle fed hay had a colonic VFA concentration of 25 mM, but the concentration in cattle fed 90% grain was 80 mM.

Intestinal microflora

Starch reaching the lower gut is fermented by an anaerobic microflora, but this microflora has generally has higher numbers of facultative anaerobes than the rumen. The intestinal tract is a long tube with a high surface to volume ratio, and this configuration allows greater oxygen flux from the blood to the microflora.

Early work indicated that the rumen had low numbers of *Escherichia coli*, and this effect was explained by the toxic effect of volatile fatty acids (Rasmussen *et al*. 1993). These experiments were, however, conducted with laboratory strains had been kept in the lab for long periods of time. *E. coli* strains obtained directly from the rumen could grow at pH

Table 2 Hypothetical calculations showing ruminal fermentation and VFA clearance from the rumen of cattle fed hay or grain.

	Hay	Grain
Ruminal Carbohydrate Digestion (g/day)[a]	5,000	20,000
VFA Production Rate (mol/liter/h)[b]	0.02	0.08
Dilution Rate (per h)[c]	0.15	0.08
VFA Concentration (M)[d]	0.080	0.120
VFA Passage (mol/liter/h)[e]	0.012	0.010
Passage Rate/Production Rate (%)[f]	60	12.5

[a] Assumes that cattle were fed 10 kg of timothy hay or 25 kg grain per day. Assumes ruminal digestion coefficients for hay and grain of 50 and 80%, respectively.

[b] Assumes 33% of the hexose would be used as carbon for microbial growth, 67% of the hexose fermented, 180 g carbohydrate/mol hexose, 1.8 moles of VFA per mol hexose, 70 liter rumen, and 24 h per day.

[c] Assumes fluid dilution rate of cattle grain 50% lower than cattle fed hay (Geotsch *et al.* 1987).

[d] Assumes ruminal VFA concentrations of 80 and 120 mM for cattle fed hay and cattle fed grain, respectively (Lana *et al.* 1998; Russell and Chow 1993).

[e] Concentration multiplied by dilution rate.

[f] Passage rate divided by production rate times 100.

values less than 6.0 even if VFAs were present, and *E. coli* O157:H7, a highly pathogenic strain, was also able to tolerate acetate concentrations greater than 100 mM at pH 5.9 (Diez–Gonzalez and Russell 1997). Based on these results, low *E. coli* numbers in the rumen cannot be explained solely by VFA toxicity.

Escherichia coli cannot use starch or other plant polymers and this characteristic restricts its niche within the GI tract. *Escherichia coli* has, however, well developed systems for utilizing maltodextrins and maltose, extracellular intermediates of starch digestion. When cattle were fed increasing amounts of grain, *E. coli* numbers in the rumen remained more or less constant, but colonic numbers increased more than 1000–fold (Diez–Gonzalez *et al*. 1998). These results indicated that starch–digesting colonic bacteria were able to 'cross–feed' *E. coli* with starch degradation products.

Bacterial growth at low pH

Many lactic acid bacteria can grow at acidic pH values even if VFA are present, and experiments with *Streptococcus bovis* indicated that this pH resistance is related to intracellular pH (Russell 1992). If bacteria maintain a neutral intracellular pH, the pH gradient increases at acidic pH (inside alkaline). Because undissociated VFA migrate across the cell membrane by simple diffusion and dissociate in the more alkaline interior, VFA anion accumulation is a logarithmic function of the pH gradient. By letting intracellular pH decrease, the pH gradient stays small, and potentially toxic accumulations of VFA anions are prevented (Russell 1992). The ability of bacteria to decrease intracellular pH has metabolic constraints. Pathways leading to acetate are often pH sensitive, and *S. bovis* shifts from acetate, formate and ethanol production to lactate production even if the rate of glucose catabolism is slow.

Continuous culture experiments (pH 5.9) indicated that *E. coli* O157:H7 had a similar strategy of tolerating VFAs at low pH (Diez–Gonzalez and Russell 1997). When increasing concentrations of acetate were added to the medium reservoir, the intracellular pH decreased, intracellular anion accumulation was never greater than 350 mM, and the fermentation shifted from acetate to lactate. *Escherichia coli* K–12 was twice as sensitive to acetate, and it was unable to decrease its intracellular pH as much. Acetate anions accumulated in response to the large pH gradient, and the cells accumulated large amounts of potassium as a counter–cation. When the acetate anion and potassium cation concentrations were greater than 500 mM, growth was no longer possible.

It has long been recognized that cellulolytic bacteria cannot grow at low pH values in the rumen, and this defect appears to be a general impairment of growth rather than an inhibition of cellulases *per se* (Russell and Wilson 1996). When *Fibrobacter succinogenes* was grown on cellobiose at acidic pH values, the pH gradient across the cell membrane initially increased. The pH gradient eventually declined, but cells lost their ability to take up cellobiose or grow. *Ruminococcus albus* lets its intracellular pH decrease as a function of extracellular pH, but it was unable to tolerate this decrease (Thurston *et al.* 1993). Ruminal bacteria that grow well at low pH and can let intracellular pH decline must have a metabolism adapted to tolerate low intracellular pH. *Streptococcus bovis* and *Selenomonas ruminantium* can produce lactate, but *Megasphaera elsdenii* and some stains of *Prevotella*, and *Clostridium aminophilum* grew well at low pH values even though lactate was not an end–product (Russell 1992).

Bacterial survival at very low pH

Because most ruminal and gastrointestinal bacteria occupy niches that are only moderately acidic, there has been little selection pressure for extreme acid tolerance. *Escherichia coli* is, however, a bacterium with a more complicated life cycle. *Escherichia coli's* primary niche is the colon, but it is never a predominant part of the population. When *E. coli* passes from the colon with the feces, it is able to grow aerobically and utilize the fermentation acids of the fecal material. This source of food is, however, short lived, and it must be able to re–enter the gastrointestinal tract to complete its life cycle. *E. coli* can pass into the gastrointestinal tract with contaminated food, but it must be able to survive the low pH of the gastric stomach before it passes into the intestines. "Gastric acidity is recognized as the first line of defense against food–borne pathogens, and the ability of pathogens to resist this pH corresponds to their oral infective dose" (Waterman and Small 1998).

The ability of *E. coli* to colonize or infect animals is facilitated by an extreme acid resistance that is distinct from its strategy of growing at moderately acidic pH in the presence of VFA. Naturally occurring *E. coli* can develop the ability to tolerate HCl shock, but this adaptation is an inducible characteristic. When *E. coli* O157:H7 was grown either aerobically or anaerobically with low concentrations of glucose and acetate, the cells were sensitive to extreme acid shock (Diez–Gonzalez and Russell, 1999). If VFA were added to the growth medium, extreme acid–resistance increased, and the viability was as much as 10,000–fold greater. The concentration of VFA needed to induce acid resistance was lower at low pH values, and viability after acid shock was highly correlated with the undissociated VFA concentration.

When cattle were fed increasing amounts of grain, colonic *E. coli* numbers increased (Figure 2a), colonic VFA concentrations increased, pH declined, and the concentration of undissociated VFA increased from 0.2 to 16 mM (Diez–Gonzalez *et al*. 1998), an amount more than sufficient to trigger the extreme acid resistance of *E. coli* (Figure 2b) (Diez–Gonzalez and Russell, 1999). When fecal samples were subjected to an acid shock

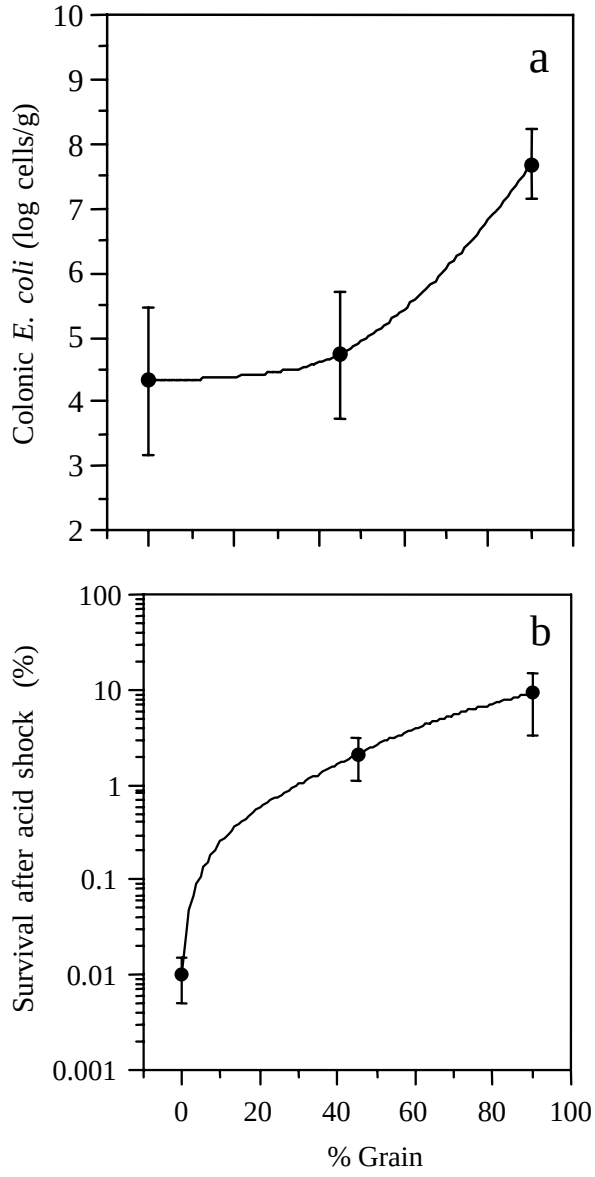

Figure 2 (a) Effect of grain-feeding on the numbers of *E. coli* in the colons of cattle, and (b) their ability to resist and acid shock that mimicked the gastric stomach (pH 2.0, 1 h). Cattle (three animals, 3 observations per animal) were fed three ratios of timothy hay to grain (10:90, 55:45, 100:0). Figure redrawn from the data of Diez–Gonzalez *et al.* (1998).

that mimicked the gastric stomach, the number of acid–resistant *E. coli* of grain–fed cattle was more than 100,000–fold greater than the acid–resistant count from hay–fed cattle (Diez–Gonzalez *et al*. 1998; Figure 3). The acid–resistant *E. coli* count decreased after cattle were switched from grain–based diets to hay, and within 5 days acid–resistant *E. coli* could no longer be detected.

Escherichia coli strains that were isolated from cattle fed hay became highly acid–resistant when they were grown in a medium containing high concentrations of glucose (high VFA production), and *E. coli* isolated from cattle fed grain became sensitive if they were grown with low concentrations of glucose (low VFA production) and vice versa. These results indicated that the diet–dependent changes in acid resistance were due to an induction of traits in existing strains rather than a selection of strains that were acid–resistant (Diez–Gonzalez *et al*. 1998). *Escherichia coli* O157:H7 acquired the same pattern of acid–resistance as the non–pathogenic strains that were isolated, and this comparison supports the idea that acid–resistance is an inducible and ubiquitous feature of naturally occurring *E. coli*.

Pathogenic *E. coli* and food safety

Escherichia coli is a normal inhabitant of the gastrointestinal tract and most strains are harmless. In the 1980's, a strain designated as O157:H7 was isolated from the feces of patients with bloody diarrhea, and this strain causes approximately 250 deaths and 20,000 infections each year in the United States (Diez–Gonzalez and Russell, 1999). The emergence of *E. coli* O157:H7 has not been precisely defined, but it appears to have resulted from interspecies gene transfer (Armstrong *et al.* 1996). *E. coli* O157:H7 has shiga–toxins that are similar to those found in *Shigella*, a related bacterium that is only rarely found in the gastrointestinal tract.

Mature cattle are unaffected by *E. coli* O157:H7, and only small numbers of cattle (approximately 1 to 3%) carry this bacterium (Armstrong *et al*. 1996). The epidemiology has been complicated by the fact that animals rarely, if ever, shed *E. coli* O157:H7 for long periods. Some workers have monitored the 'shedding' of *E. coli* O157:H7 after experimental inoculation of cattle, but the validity of these models to study natural shedding of *E. coli* O157:H7 has not been firmly established. Doyle and his colleagues (Zhao *et al*. 1998) noted that shedding of *E. coli* O157:H7 was decreased after animals were inoculated with nonpathogenic strains. This latter result indicates that *E. coli* O157:H7 is not as well adapted to the gastrointestinal tract as nonpathogenic strains, but the reason why some animals carry *E. coli* O157:H7 has not been defined.

The observation that feeding of hay can decrease the number of *E. coli* and the acid resistance of *E. coli* has food safety implications, but further work is clearly needed. How important is acid–resistance in the infectivity of *E. coli*? How long do diet–dependent changes in acid–resistance persist in feces, manure and foods? Can other dietary changes (e.g., the replacement of raw corn starch with cooked corn) be substituted for hay feeding? It should be stressed that 'hay feeding' is an indirect method of fighting *E. coli* O157:H7 infection that is analogous to modern warfare which seeks to kill all people (*E. coli)* rather than just military combatants (*E. coli* 0157:H7).

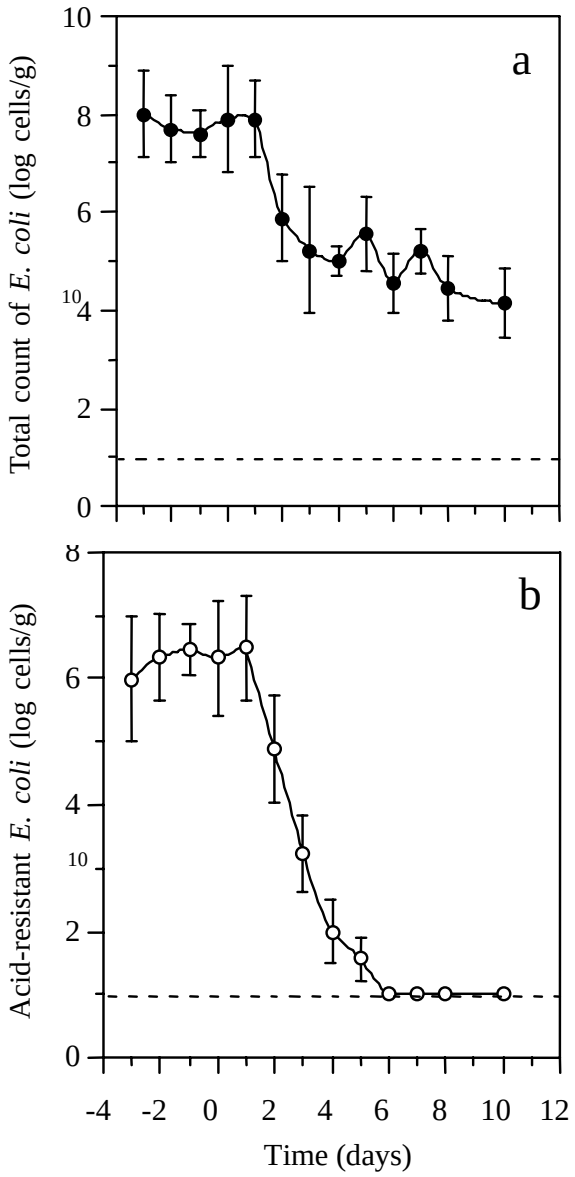

Figure 3 (a) The effect of hay on the total numbers of colonic *E. coli* in cattle that had been consuming the 90% grain diet. Cattle were switched from 90% grain to hay on day zero. (b) The numbers of *E. coli.* that were able to survive acid shock (pH 2.0, Luria broth, 1 h). The bars indicate standard deviations of the mean (3 animals, 1 replicate per animal, 2 independent experiments). The dotted lines show the detection limit of our enumerations. Figure redrawn from the data of Diez–Gonzalez *et al.* (1998).

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References

Allen, M. S. (1997). Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. *Journal of Dairy Science* **80**, 1447–1462.

Armstrong, G. L., Hollingsworth, J. and Morris Jr., J. G. (1996). Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. *Epidemiology Reviews* **18**, 29–51.

Ash, R. W. and Dobson, A. (1963). The effect of absorption on the acidity of rumen contents. *Journal of Physiology* **169**, 39–61.

Barnes, R. J., Comline, R. S. and Dobson, A. (1986). The control of splanchnic blood flow. In: *Control of Digestion and Metabolism in Ruminants*, pp. 41–59. (eds. L. P. Milligan, W. L. Grovum, and A. Dobson). Prentice Hall, Englewood Cliffs, NJ.

Britton, R. (1989). Acidosis: A continual problem in cattle fed high grain diets. *Cornell Nutrition Conference for Feed Manufacturers*, pp. 8–15.

Cotta, M. A. (l988). Amylolytic activity of selected species of ruminal bacteria. *Applied and Environmental Microbiology* **54**, 772–776.

Davis, C. L., Brown, R. E. and Beitz, D. C. (1964). Effect of feeding high–grain restricted–roughage rations with and without bicarbonates on the fat content of milk produced and proportions of volatile fatty acids. *Journal of Dairy Science* **47**, 1217–1226.

Diez–Gonzalez, F., Callaway, T. R., Kizoulis, M. G. and Russell, J. B. (1998). Grain feeding and the dissemination of acid–resistant *Escherichia coli* from cattle. *Science* **281**, 1666–1668.

Diez–Gonzalez, F. and Russell, J. B. (1997). The ability of *Escherichia coli* O157:H7 to decrease its intracellular pH and resist the toxicity of acetic acid. *Microbiology* **143**, 1175–1180.

Diez–Gonzalez, F. and Russell, J. B. (1999). Factors affecting the extreme acid resistance of *Escherichia coli* O157:H7. *Food Microbiology* (in press).

French, D. (1973). Chemical and physical properties of starch. *Journal of Animal Science* **37**, 1048–1061.

Geotsch, A. L., Owens, F. N., Funk, M. A. and B. E. Doran. (1987). Effects of whole or ground corn with different forms of hay in 85% concentrate diets on digestion and passage rate in beef heifers. *Animal Feed Science and Technology* **18**, 151–155.

Harmon, D. L. (1991). Dietary influences on carbohydrases and small intestinal starch hydrolysis in ruminants. *Journal of Nutrition* **122**, 203–210.

Hungate, R. E. (1966). *The Rumen and Its Microbes*. Academic Press, New York. 1966.

Hungate, R. E. (1975). The rumen microbial ecosystem. *Annual Reviews of Ecology and Systematics* **6**, 39–66.

Hungate, R. E., Dougherty, R. W., Bryant, M. P. and Cello, R. M. (1952). Microbiological and physiological changes associated with acute indigestion in sheep. *Cornell Veterinarian* **42**, 423–449.

Kotarski, S., Waniska, R. D. and Thurn, K. K. (1990). Starch hydrolysis by the ruminal microflora. In: *31st Annual Ruminant Nutrition Conference*, pp. 178–190. (ed. C. B. Theurer). American Institute of Nutrition, Washington, DC.

Lana, R. P., Russell, J. B. and Amburgh, M. E. V. (1998). The role of pH in regulating ruminal methane and ammonia production. *Journal of Animal Science* **76**, 2190–2196.

McAllister, T. A., Cheng, K.–J., Rohde, L. M.., Forsberg, C. W. and Costerton, J. W. (1990). Digestion of barely, maize and wheat by selected species of ruminal bacteria. *Applied and Environmental Microbiology* **56**, 3146–3153.

Nagaraja, T. G. (1996). Liver abscesses and Tylan. In: *Scientific Update on Tylan and Micotil for the Professional Feedlot Consultant I. Elanco Animal Health*, pp. C1–C13. Indianapolis, IN.

Rasmussen, M. A., Cray, Jr. , W. C., Casey, T. A. and Whipp, S. C. (1993). Rumen contents as a reservoir of enterohemorrhagic *Escherichia coli*. *FEMS Microbiology Letters* **114**, 79–84.

Russell, J. B. (1992). Another explanation for the toxicity of fermentation acids at low pH: anion accumulation versus uncoupling. *Journal of Applied Bacteriology* **73**, 363–370.

Russell, J. B. and Allen, M. S. (1984). Physiological basis for interactions among rumen bacteria using *Streptococcus bovis* and *Megasphaera elsdenii* as a model, In: *Current Perspectives in Microbial Ecology*. pp. 239–247. (eds. M. J. Klug, C. A. Reddy), American Society for Microbiology, Washington, DC.

Russell, J. B. and Baldwin, R. L. (1979). Comparison of maintenance energy expenditures and growth yields among several rumen bacteria grown on continuous culture. *Applied and Enviromental Microbiology* **37**, 537–543.

Russell, J. B. and Chow, J. M. (1993). Another theory for the action of ruminal buffer salts: Decreased starch fermentation and propionate production. *Journal of Dairy Science* **76**, 826–830.

Russell, J. B. and Wilson, D. B. (1996). Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? *Journal of Dairy Science* **79**, 1503–1509.

Slyter, L. L. (1976). Influence of acidosis on rumen function. *Journal of Animal Science* **43**, 910–929.

Thurston, B., Dawson, K. A. and Strobel, H. J. (1993). Cellobiose versus glucose utilization by the ruminal bacterium *Ruminococcus albus*. *Applied Environmental Microbiology* **59**, 2631–2637.

Tomme, P., Warren, R. A. J. and Gilkes, N. R. (1995). Cellulose hydrolysis by bacteria and fungi. In: *Advances in Microbial Physiology*, pp. 1–81. (eds. R. K. Poole) Harcourt Brace & Co., New York, NY.

Van Soest, P. J. (1982). *Nutritional Ecology of the Ruminant*, pp. 182–188. O & B Books, Corvallis, OR.

Waldo, D. R. (1973). Extent and partition of cereal grain starch digestion in ruminants. *Journal of Animal Science* **37**, 1062–1074.

Wells, J. E., Krause, D. O., Callaway, T. R. and Russell, J. B. (1997). A bacteriocin–mediated antagonism by ruminal lactobacilli against *Streptococcus bovis*. *FEMS Microbial Ecology* **22**, 237–243.

Wolin, M. J. (1969). Volatile fatty acids and the inhibition of *Escherichia coli* growth by rumen fluid. *Applied Microbiology* **17**, 83–87.

Waterman, S. R. and Small, P. L. C. (1998). Acid–sensitive enteric pathogens are protected from killing under extremely acidic conditions of pH 2.5 when they are inoculated onto certain food sources. *Applied and Environmental Microbiology* **64**, 3882–3886.

Zhao, T., Doyle, M. P., Harmon, B. G., Brown, C. A., Mueller, P. O. E. and Parks, A. H. (1998). Reduction of carriage of enterohemorrhagic *Escherichia coli* O157:H7 in cattle by inoculation with probiotic bacteria. *Journal of Clinical Microbiology* **36**, 641–647.