Physiological Achilles’ Heels of Enteropathogenic Bacteria in Livestock

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Abstract

An elaborate feeding regimen of animals, which takes advantage of the Achilles’ heels of enteropathogenic bacteria, can possibly enable prophylaxis in the intestinal tract, attenuate actual disease symptoms, accelerate recovery from a bacterial gastroenteritis or ensure food safety. There is a wide spectrum of conceivable weak spots in bacteria. Some pathogenic bacteria cannot use certain compounds, or use them less efficient than beneficial bacteria. By addition of such substances to animal feed, non-pathogenic bacteria can grow better than pathogens and competitively exclude the latter ones. Other compounds even have an inhibitory effect on pathogens. Calcium phosphate for example protects against Salmonella, Zn has a prophylactic effect against Brachyspira, and Fe has an inhibiting effect on the enterotoxin synthesis of Yersinia enterocolitica. Besides, there are antimicrobial substances as plant extracts, essential oils, organic acids and other compounds, which inhibit pathogens more than other bacteria. A simultaneous application of several anti-pathogen agents suggest an enhanced effect. Some countermeasures aim at a distinct group of bacteria, while others are more universal. General strategies to repel different pathogenic bacteria are the supply of health-stimulating milk components, antagonistic bacteria for competitive exclusion, and mucus-related attractants for misguidance of adhering and invasive bacteria. This paper gives an overview of Achilles’ heels of enteropathogenic bacteria that can be exploited to develop strategies for keeping control over these pathogens in the gastrointestinal tract of livestock.

Introduction

Pro- and prebiotics, organic acids, and plant extracts are currently in the centre of attention in European research as replacements for antibiotics to enhance the gut health of animals. Up to now, nutritional effects have mainly been studied for prebiotics, i.e. substances that promote probiotic micro-organisms. By application of prebiotics and organic acids, an environment favouring lactic acid bacteria is created. However, in addition to stimulating beneficial bacteria, weak spots of pathogenic bacteria can be attacked.

This review gives an overview of nutrients, minerals, and other substances that have a weakening effect on pathogenic bacteria. The following pathogenic bacteria were taken into account: Brachyspira (= Serpulina) pilosicoli, Brachyspira hydysenteriae, Campylobacter, Helicobacter, Escherichia coli, Salmonella, Shigella, Plesiomonas shigelloides, Yersinia enterocolitica, Aeromonas, Vibrio cholerae, and Clostridium perfringens. These bacteria were chosen because they are known as causative agents of either animal diseases or food-related infections in humans. The fact that animals can carry bacteria, which represent a potential risk for man, sometimes even without causing symptoms in animals, is a serious public health issue. In addition, animals with gastrointestinal problems cause considerable economic losses in livestock farming. The application of sensitive, modern detection methods like PCR keep steadily broadening our knowledge about enteric pathogens. Some well-known bacteria like Vibrio cholerae attain additional attention as possibly important participants in enteric infections of farm animals (Visser et al., 1999). In addition, new causative agents are described for familiar disease symptoms, like the intracellular bacterium Lawsonia intracellularis (McOrist et al., 1995; Van der Heijden, 2004), the physiology of which is still barely known (Lawson and Gebhart, 2000).

The bacteria referred to in this overview are grouped according to the classification of Holt et al. (1994), with the exception of the genus Plesiomonas that has been moved to the family Enterobacteriaceae (Garrity et al., 2001; Ruimy et al., 1994). Salmonella species are named according to the nomenclature given by Tindall et al. (2005). Together with specific properties of pathogens, suggestions are given for making use of their Achilles’ heels (Fig. 1). Most bacteria do not survive high temperatures, a low pH, or dryness. In feed production, measures are taken to keep pathogens out of the production line by taking advantage of these damaging conditions. However, these conditions are no more applicable once bacteria have entered living
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beings. Because lower and higher organisms have a lot in common, it is quite difficult to harm the one without affecting the other. In addition, the nutritional requirements of animals impose some restrictions to feed modifications due to the need of balanced diets. Moreover, side effects of countermeasures are undesirable. A combination and simultaneous application of several anti-pathogen factors will probably make the fight against pathogens more effective. Besides, dosage of individual substances can possibly be kept lower in a combination than in a one-substance approach.

Gut bacterial communities are adaptive, dynamic systems. The basis for their adaptive potential lies in the metabolic versatility of bacteria. Even within a given group, e.g. the genus Lactobacillus (Bateup et al., 1995) or the species E. coli (Oluokoya, 1986), differences in biochemical abilities and proliferation rates may be considerable. Bacteria that metabolise nutrients faster, adapt better, or have specific self-defensive strategies can establish or even outgrow others. Some bacteria benefit from the leaking of degradation products from other bacteria. Protein-degrading species, for example, enable weakly or even non-proteolytic organisms to exist on degradation products of primary nitrogen sources (Mossel, 1971). The same is the case with carbohydrates. Campylobacters, for example, cannot make use of carbohydrates; they need metabolites of carbohydrate breakdown for growth. In this complex way, community structures form that finally comprise up to 10¹⁰ bacterial cells/g faeces, with anaerobes predominating (Harmsen et al., 2002; Zhu et al., 2002, Ziemer et al., 2004).

Bacterial strains differ in their metabolic profiles and virulence, but an animal’s susceptibility to an infection can also vary. Its resistance depends on its immunobiological status and on other biological factors, like a competitive gut microflora for example (Koopman, 1984; Van den Broek et al., 1992). When the sum of the epidemiological factors controlling the presence of a disease exceed a critical level, clinical signs develop.

The knowledge compiled in this review may help to obstruct spreading and establishing of pathogens by means of an elaborate feeding regimen. By impeding colonisation of the intestine by disadvantageous bacteria, animal-pathogens can be warded off, or the risk of transmission of human-pathogens via the food-chain can be reduced. Hence, taking advantage of the Achilles' heels of pathogens (Fig. 1) can possibly enable prophylaxis, attenuate actual disease symptoms, accelerate recovery from a bacterial gastro-enteritis or ensure food safety.

**Brachyspira pilosicoli, Brachyspira hyodysenteriae**

**Occurrence**

*Brachyspira* (= *Serpulina*) has been isolated from intestinal contents and faeces of birds (Jansson et al., 2001; Stephens and Hampson, 2001), dogs (Duhamel et al., 1998; Fellström et al., 2001), swine (Barcellos et al., 2000; Fellström et al., 1996; Møller et al., 1998; Tasu et al., 2004; Thomson et al., 1998), and other mammals with diarrhoea. *Brachyspira hyodysenteriae* is the causative agent of swine dysentery, a severe inflammation of the large intestine with bloody mucous diarrhoea. The disease is common in pigs from 12 to 75 kg, but occasionally severe cases also occur in sows and their sucking piglets. *Brachyspira pilosicoli* is also associated with a diarrhoecal disease of grower-finisher swine, called porcine intestinal spirchoetosis (Taylor et al., 1980; Trott et al., 1996a,b). Apart from diarrhoea, it causes reduced feed efficiency in swine. Another causative agent of porcine spirchoetosis is *Brachyspira intermedia*. In chicken, intestinal spirchotes are associated with wet litter problems and reduced egg production (Stephens and Hampson, 2001).

**Attractants**

The porcine isolate *Brachyspira pilosicoli* showed a chemotactic response towards 10 mM D-/L-serine (Witters and Duhamel, 1999). *Brachyspira hyodysenteriae* showed chemotactic responses towards L-fucose and L-serine in concentrations of 10 – 100 mM (Kennedy and Yancey, 1996). Serine and fucose are constituents of porcine mucin; serine is a polypeptide backbone amino acid and fucose the terminal sugar of the O-linked oligosaccharide side chain (Kennedy and Yancey, 1996; Milner and Sellwood, 1994). Possibly, supplying such attractants as feed additives in an encapsulated or bound form can misguide and trap *Brachyspira*.

**Haemolysis-inhibiting substances**

Zhang et al. (2001) showed that ZnO fed at 2 g/kg had a prophylactic effect against *Brachyspira hyodysenteriae* (isolate B204) infection without affecting the body weight gain of mice. According to Dupont et al. (1994), the adverse effect of ZnSO₄ is due to a direct action of ZnSO₄ on the spirochetes, inhibiting the synthesis of haemolysin. Not only ZnSO₄, but also CuSO₄, in concentrations above 0.1 mM (about 16 mg/l ZnSO₄ or CuSO₄) decreased the haemolysin activity of *Brachyspira hyodysenteriae* isolate B204. The viability of isolate B204 was not affected by this concentration. Transmission electron microscopy revealed that addition of 0.1 mM ZnSO₄ caused morphological changes in the bacterial cells, namely the clumping of ribosomes and clearing of the cell cytoplasm (Dupont et al., 1994).

**Infection-preventive feed**

Baumann and Bilkei (2002) reported that before the development of efficient medicines, swine dysentery was routinely treated by administering oats that were soaked in salt water. Kirkwood et al. (2000) stated that according to “anecdotal evidence”, spirchetoal diarrhoea can be controlled by switching from a pelleted to a meal diet.

Prohászka and Lukács (1984) observed that swine dysentery did not occur anymore in a pig unit after a new feed storage technology was introduced. Instead of drying corn-cob-mix maize by heat as was done before, it was ensiled in maize silos after grinding. The pH of 6.0 in the large intestine of silage-fed pigs was shown to cause a loss of motility in *Brachyspira*, isolated from dysenteric pigs, within 6 hours of incubation in faecal contents. This bacteriocidal effect completely ceased at pH values higher than 6.8.

Hampson et al. (1997), Pluske et al. (1996), and Siba et al. (1996) re-examined whether fermentable material can cause inhibition of the growth of *Brachyspira*.
hyodysenteriae (strains 155/23 and WA 15; both serogroup A). To this end, they compared effects of grain processing (Pluske et al., 1996) and of different low- and high-fibre diets (Hampson et al. 1997; Siba et al., 1996) on the incidence of swine dysentery. Unexpectedly, none of the pigs fed a low-fibre diet consisting of 77% cooked white rice and 18% animal protein developed swine dysentery, while others did. The absence of fermentable fibre, i.e. soluble NSP and resistant starch, evidently was protective. Hampson et al. (1997), Pluske et al. (1996), and Siba et al. (1996) suggested that reduced fermentation in the large intestine may prevent colonization of Brachyspira hyodysenteriae. In another trial, consumption of the rice-based diet, but not vaccination with a formalised B. pilosicoli bacterin, delayed and significantly reduced the onset of faecal excretion of Brachyspira pilosicoli strain 95/1000 after experimental challenge (Hampson et al., 2000). Pluske et al. (1998) confirmed that soluble non-starch polysaccharides and resistant starch can play a role in the pathogenesis of swine dysentery (Brachyspira hyodysenteriae strain 155/23). Durmic et al. (2000) observed that after experimental infection with Brachyspira hyodysenteriae strain WA15 (serogroup A), the incidence of swine dysentery was slightly lower amongst pigs that were fed heat- and enzyme-treated wheat diets, and hence received less fermentable substrate than the control group fed an untreated wheat diet. With sorghum-based diets, however, the opposite was found (Durmic et al., 2000). Baumann and Bilkei (2002) studied the effect of highly vs. low fermentable fibre in the diet on the development of swine dysentery in fattening pigs. They observed milder clinical symptoms, higher daily weight gain, higher feed conversion efficiency and feed consumption in pigs fed diets containing 9.6% highly fermentable neutral detergent fibre compared to pigs fed diets with 6.1% low fermentable neutral detergent fibre after challenge with Brachyspira hyodysenteriae serotype Ack 300/8. Kirkwood et al. (2000), in contrast to Baumann and Bilkei (2002), Hampson et al. (1997, 2000), Pluske et al. (1996), and Siba et al. (1996), found no differences in clinical expression of swine dysentery (Brachyspira hyodysenteriae B204) with diets containing either high proportions of fermentable fibre (30% wheat shorts plus 15% raw potato starch; 25% beet pulp), non-fermentable fibre (15% ground oat hulls), or low fibre (corn-soyabean meal; parboiled rice and animal protein; cooked rice and animal protein).

The findings presented above are inconsistent. A possible explanation offered by Kirkwood et al. (2000) is that different strains of Brachyspira hyodysenteriae were used by the different workgroups and that these strains may respond differently to the experimental conditions. Moreover, Brachyspira hyodysenteriae obviously requires other bacteria before it can colonise the intestinal mucosa (Harris et al., 1978). It is hence possible that the indigenous microflora differed per pig operation and thus interacted differently with Brachyspira (Kirkwood et al., 2000). Another possibility is that ensiled feed as used by Prohászka and Lukács (1984) offers more protection than unfermented dietary fibre. Besides, it might be simply the change of dietary parameters that reinforced the indigenous microflora in some trials. In conclusion, it is still unclear if feed strategies can protect pigs from swine dysentery.

**Campylobacter**

**Occurrence**

Campylobacter is found in the reproductive organs, intestinal tract, and oral cavity of humans and animals (Holt et al., 1994). Campylobacter jejuni can infect several animal species (including cattle and sheep) and is a major cause of abortions. It is highly successful in colonizing and multiplying in the gastrointestinal tract. At least some strains are able to invade across the bowel wall and spread to various sites throughout the body (Field et al., 1986). Campylobacter jejuni is mainly found in poultry, whereas Campylobacter coli is found in pigs. Campylobacter jejuni and Campylobacter coli can even in low numbers cause food-borne infections in man (Dijk et al., 1999). Livestock and especially poultry are commonly colonised by Campylobacter and are therefore considered major reservoirs for human infections (Jacobs-Reitsma, 1997; Prescott and Munroe, 1982), but also domestic animals such as cats and dogs can carry the bacterium (Stern, 1992). Colonisation of the intestinal tract of animals is mostly not associated with disease (Stern, 1992).

**Attractants**

Mucin chemotaxis plays a critical role in mucosal localisation of Campylobacter jejuni. 10 mM L-fucose, 10 mM L-aspartate, 100 mM L- (+)-cysteine hydrochloride, 100 mM L-glutamate (monosodium), 10 mM L-serine, but also some organic acids, produced a positive chemotactic response of Campylobacter jejuni (Hugdahl et al., 1988).

**Susceptibilities**

Environmental stresses, such as a low pH, damage cells and hinder recovery of campylobacters to a greater degree than many other bacteria. Blaser et al. (1980) demonstrated that Campylobacter jejuni was not able to survive at a pH below 3.0. In weak acidic conditions, i.e. at a pH between 4.0 and 6.0, Campylobacter survived well and showed only a slight decrease in culturability (Doyle and Roman, 1981). The optimum pH range for growth of Campylobacter jejuni is 5.5 to 6.5 (Albrecht, 2005; Fletcher et al., 1983). Hence, ensiling or acidifying feed and supporting a low stomach pH seem promising measures to suppress campylobacters (Heres, 2003; Mroz, 2005). In addition, campylobacters are very heat-sensitive; they die at temperatures above 48°C (Dijk et al., 1999). That suggests that slight heating can possibly reduce the risk of feed and food poisoning.

Combinations of formic, acetic, and propionic acid (1:2:3 and 1:2:5) showed a synergistic bactericidal activity towards different strains of Campylobacter jejuni (C144, C186, C350, C591, C690, C2146, C2150; ID-Lelystad) and Campylobacter coli (C4596, C4601, C4602; ID-Lelystad), which was stronger than the one of each single acid and the commercial products Hyalus (Envirotec. Co., Hereford, UK) and Selko-DWB (Selko Co., Tilburg.
The Netherlands) (Chaveerach et al., 2002). The lower the pH, the faster the antimicrobial effects became visible among the four pHs tested (4.0, 4.5, 5.0, 5.5).

Campylobacter jejuni is sensitive to the fatty acids C10:0, C12:0, C14:0, C18:1, and C18:2 in concentrations of 500 µmol/l at pH 5 (Sprong et al., 2001). These fatty acids are released during gastric digestion of fat. Sphingolipids present in milk fat or degradation products of them as sphingosine, lysophosphatidylcholine, and galactosylsphingosine killed Campylobacter jejuni in a concentration of 100 µmol/l at pH 7 (Sprong et al., 2001).

Competitive exclusion
Administration of material from the autochthonous intestinal microflora of adult birds to chicks during the early period after hatching is a widely accepted prophylactic method to control Campylobacter, E. coli, Salmonella, and Clostridium perfringens infections in poultry by competitive exclusion. Examples of commercial competitive-exclusion products are Aviguard® (Bayer Microbial Developments Ltd, Worcs, UK), Broilact® (Orion Pharma, Turku, Finland), and PREEMPT™ (Bioscience, Dundee, Illinois, USA). Aviguard® consists of freeze-dried uncharacterised micro-organisms derived from healthy, specific pathogen free chicken intestinal bacteria (Bayer M.D.L., 2005). Broilact® is a freeze-dried bacterial formulation consisting of a selected mixture of strictly and facultatively anaerobic bacteria derived from the caeca of an adult healthy hen (Orion Pharma, 2005). A total of 32 different pure cultures were isolated and characterised from Broilact®, including 22 strictly anaerobic bacteria and 10 facultatively anaerobic bacteria. Broilact® is free from spore-formers (Orion Pharma, 2005). PREEMPT™ is a non-defined competitive exclusion product that contains more than 29 species of bacteria originally isolated from the intestinal tract of healthy mature chickens (Bioscience, 2005; Methner, 2000; Nisbet, 2002).

Broilact® and microaerophilic K-bacteria alone were inactive against Campylobacter (Aho et al., 1992). However, when Broilact®, which contains anaerobic bacteria, was supplied together with K-bacteria, onset of campylobacter infection in treated chicks was delayed by 1.5 weeks and subsequent colonisation was lower than in control birds (Aho et al., 1992). In contrast to Aho et al. (1992), Hakkinen and Schneitz (1999) observed that broilers treated with Broilact® only had a significantly lower Campylobacter jejuni incidence in their caecal contents than control animals when orally challenged. It is not known if the composition of Broilact® was subject to changes.

Reducing crop contamination
The crop of market-age broiler chickens is an important source of carcass contamination after slaughter in the processing plant. Byrd et al. (1998) observed that preslaughter feed withdrawal increased the frequency of Campylobacter in the crop, but not in the caecum of market-age broiler chickens. Incorporation of 0.44% lactic acid in the drinking water reduced Salmonella and Campylobacter contamination in crops (Byrd et al., 2001) by 73% and 27%, respectively. However, concentrations of 0.5% acetic, lactic, and formic acid reduced water consumption (Byrd et al., 2001). Provision of a nutrient broth-like cocktail with 4% sucrose instead of water (or a glucose-cocktail) during feed withdrawal reduced the incidence of Salmonella typhimurium ST-10 (challenge strain) and natural campylobacters in crops (Hinton et al., 2002). According to Hinton et al. (2002), this effect might have been caused by stimulation of growth and acid production of slime-producing lactic acid bacteria.

Helicobacter

Occurrence
Helicobacters that cause gastritis have been isolated from humans and animals. Apart from the stomach, Helicobacter pylori was isolated from dental plaque in the oral cavity of man, being a possible source of re-infection (Desai et al., 1991). Helicobacter pylori has been detected in tissues from experimentally infected piglets with gastric ulcers (Bertram et al., 1991) and cats (Fox et al., 1995). Helicobacter acinonyx has been associated with gastritis in cheetahs (Eaton et al., 1993). Helicobacter mustelae causes hypergastrinaemia in ferrets (Perkins et al., 1996).

Helicobacters have also been isolated from animals with diarrhoea, including Helicobacter canis from dogs (Burnens et al. 1993; Stanley et al., 1993), Helicobacter sp. from kittens (Foley et al., 1998), Helicobacter pullorum from poultry and humans (Burnens et al., 1994; Stanley et al., 1994), and Helicobacter bilis and Helicobacter rodentium from mice (Shomer et al., 1998). Since helicobacters appear to be a subgroup within the campylobacters, the niche within the enteric mucus may be the primordial conditions for the campylobacter-helicobacter group, with inflammatory gastric niches being possibly a secondarily evolved character (Foley et al., 1998).

Phylogenetic analyses indicated that Gastrospirillum hominis, later named Helicobacter heilmannii (human isolate) and Helicobacter bizzozeronii (frequently found in dogs) are highly related, which suggests that transfer of Helicobacter to humans can take place from infected animals (Jalava et al., 2001; Švec et al., 2000). Other Helicobacter species with a zoonotic potential are Helicobacter canadensis (Waldenström et al., 2003) and Helicobacter pullorum (Gibson et al., 1999).

Susceptibilities
A methylene chloride extract of cinnamon (100 g powdered stem bark of Chinese cinnamon/l, dried, re-dissolved in 100 ml ethanol; 15 – 50 mg/l; Tabak et al., 1999), rose oil products (Rosanol, BASF, Germany; >2 mg/l; Boyanova and Neshev, 1999), allixin (12.5 – 50 mg/l; Mahady et al., 2001), present in garlic bulbs only after stress-treatment, and bisulphite or sulphite (0.96 mM; Hawrylik et al., 1994) were found to inhibit the growth of Helicobacter pylori in vitro.

Attractants
Helicobacter pylori shows chemotactic responses to urea, flurofamide (a potent urease inhibitor), and sodium bicarbonate. Since urea and sodium bicarbonate are secreted by gastric epithelia and hydrolysis of urea by
urease on the bacterial surface is essential for colonisation, the chemotactic response of *Helicobacter pylori* may be crucial for its colonisation and persistence in the stomach (Mizote *et al*., 1997).

**Adhesion and colonisation**

*Helicobacter pylori* and *Helicobacter mustelae* bind to phosphatidylethanolamine, a constituent of host gastric mucosal cells, and to ganglio-triaosylceramide and ganglio-tetraosylceramide, glycosphingolipids in cell membranes. A lyophilised bovine colostrum concentrate, prepared by removal of cholesterol, casein, and lipoproteins from colostrum, blocked the attachment of *Helicobacter pylori* in vitro to these constituents of gastric mucosal cells (Bitzan *et al*., 1998).

Bovine milk, even in a hundred- to twohundredfold dilution, obviously contains active substances that inhibit in vitro adhesion of *Helicobacter pylori* to sulfatide, a mucosal lipid. Hata *et al.* (1999) concluded that bovine milk may have a protective effect on the gastric mucosa in *Helicobacter*-associated gastritis.

In a mouse model, milk fat globule membrane fractions, prepared from bovine buttermilk by removal of casein, whey proteins, and carbohydrates with subsequent drying, showed potencies to inhibit in vitro adhesion of *Helicobacter pylori* strains to immobilised human mucus, erythrocytes, and cultured gastric epithelial cells (Burger *et al*., 2000, 2002). Cranberry juice may also inhibit adhesion of bacteria to the stomach in vivo.

*Helicobacter pylori* has to exert urease enzyme activity in order to colonise the gastric mucus of gnotobiotic piglets (Eaton *et al*., 1994). An ethanol extract of cinnamon (100 g powdered stem bark of Chinese cinnamon/l; filtered, concentrated ten times) proved to inhibit the urease activity of *Helicobacter pylori* in vitro (12 – 25 mg/l; Tabak *et al*., 1999).

**Haemagglutination**

*Helicobacter pylori* and *Helicobacter mustelae* can agglutinate red blood cells of several species, whereas *Helicobacter felis* did not show this ability (Taylor *et al*., 1992). Mucin preparations, separated from human gastric juices and isolated from different colon regions, showed high inhibitory activity for *Helicobacter pylori* haemagglutination. High molecular mass mucin-like components from bovine buttermilk, prepared by removal of casein, whey proteins and carbohydrates with subsequent drying, showed inhibitory potencies comparable to gastric mucins. These mucin-like preparations could possibly serve as anti-infectious components against *Helicobacter pylori* (Hirmo *et al*., 1998). Wang *et al.* (2001) found that 31 – 65 µg/ml milk fat globule membrane fraction from bovine buttermilk (prepared in the same way as the mucin-like components, optionally defatted) caused 50% inhibition of haemagglutination. Iron-saturated lactoferrin also inhibited haemagglutination of *Helicobacter pylori* in vitro with 0.2 mg/ml causing 50% inhibition, whereas iron-free lactoferrin did not inhibit haemagglutination at a concentration of 33 mg/ml (Wang *et al*., 2001).

**Escherichia coli**

**Occurrence**

Bacteria of the genus *Escherichia* occur as normal flora in the lower intestine of warm-blooded animals. Some *E. coli* strains are pathogenic to animals, such as pigs (Suárez *et al*., 1995; Vázquez *et al*., 1996), cattle (China *et al*., 1996; Sandhu *et al*., 1996; Vázquez *et al*., 1996; Wieler *et al*., 1996), sheep (Kudva *et al*., 1996), dogs (Beaudry *et al*., 1996), or rabbits (Pillien *et al*., 1996).

Human-pathogenic *E. coli* are often present in the intestinal tract of cattle. Via contamination of meat during slaughtering, pathogens can be transferred to human beings, if the meat is not thoroughly heated. Other risky products are those, which might have been in contact with faeces, as raw milk, raw milk cheese, and vegetables (Dijkstra *et al*., 1999).

**Non-metabolisable compounds**

Nearly all isolates of the human pathogenic *E. coli* O157: H7 ferment D-sorbitol slowly, or not at all (Dijkstra *et al*., 1999; De Vaux *et al*., 2002). An exception and example of a sorbitol-utilising enterohaemorrhagic *E. coli* is *E. coli* O157:H7 (Brundell *et al*., 2001). Sorbitol is a sweet-tasting alcohol sugar that is naturally present in apples and pears, for example (Rieger, 2004). When *E. coli* O157: H7 was grown in non-sterile rumen broth containing 3 g sorbitol/l, it was displaced within 72 h. The same was the case when the sugars L-arabinose, rhamnose, and trehalose were supplied in non-sterile rumen medium inoculated with *E. coli* O157:H7. L-Arabinose is a natural sugar found in several plants, among them *Prosopis juliflora* (Natura Internacional, 2005). In cell wall residues of feed ingredients, Carré and Brillouet (1986) found L-arabinose contents of 19.7, 19.1, 18.3, 17.4, 14.6, 13.6, and 12.8% for peas, maize, wheat bran, wheat, barley, soyabean meal, and rapeseed meal, respectively. Van der Meulen *et al.* (2000) registered an increase in arabinose after treating a wheat bran based diet with cellulase. In contrast to arabinose, however, common feed ingredients contain at best small amounts of rhamnose (Carré and Brillouet, 1986). Commercially available rhamnose is produced by chemical hydrolysis of arabic and karaya gums, or from rutin or citrus fruit which contain by weight 10–30% rhamnose (Natura Internacional, 2005). Apart from the compounds already mentioned, L-glucose and D-tagatose are not catabolised at all by *Escherichia coli* O157:H7 (Bautista *et al*., 2000). D-Tagatose is present in small amounts in milk; it is commercially produced from galactose in whey. If distinct sugars shall be applied in the large intestine of animals, it is essential to supply them in a form, which guarantees that they pass the upper intestinal tract without being transformed or absorbed (e.g. as ileal non-digestible polysaccharides).

When *E. coli* O157:H7 was grown in a sterile broth with sorbitol, sorbitol-positive mutants appeared (De Vaux *et al*., 2002). A possible explanation lies in the fact that all species produce viable mutants in minor percentages.
The large cell numbers in which bacteria can appear bring about a corresponding proportion of mutants. A sterile medium is highly selective for mutants, because a physiologically adapted mutant is the only organism present that can grow. However, if other bacteria are present that can use the sugar substrates, they most likely competitively outgrow E. coli O157:H7.

Susceptibilities
E. coli O157:H7 was sensitive to the fatty acids C10:0 and C12:0 in a concentration of 0.5 mmol/l of at pH 5 (Sprong et al., 2001). These fatty acids are released during gastric digestion of fat. Sphingolipids present in milk fat or degradation products of them as sphingosine, lysophosphatidylcholine, and galactosylsphingosine showed bactericidal activities in a concentration of 0.1 mmol/l at pH 7 (Sprong et al., 2001). Moreover, E. coli O157:H7 proved sensitive to 4 µl of allyl isothiocyanate (component of food grade oil of mustard) at 37°C when tested in an agar disk assay (Park et al., 2000).

Harmful products
Porcine, bovine, and human enterotoxigenic E. coli (ETEC) produce STIa (also called STIp), a heat stable enterotoxin. STI production is subject to catabolite repression, being optimally produced in the absence of glucose (Bettelheim and Thomas, 2005), i.e. that in the presence of glucose, production of STIa is impeded.

Spray-dried whey of colostrum from non-immunised cows (Lactobin®) had high antibody titers against Shigella-like toxins (SLTs) and blocked the cytotoxic effect of SLT-I and SLT-II of E. coli O157 in concentrations of 125 mg Lactobin®/l and 62.5 mg Lactobin®/l, respectively (Lissner et al., 1996).

Iron starvation and the presence of bile salts and trypsin in the concentrations normally present in the intestine stimulate release of heat-labile enterotoxin (LT) from E. coli (Bettelheim and Thomas, 2005). Supplying sufficient iron in feed can possibly prevent iron starvation of E. coli.

Attractants
In E. coli, chemotaxis is mediated by certain bacterial membrane-proteins, the so-called methyl-accepting chemotaxis proteins MCP I, MCP II, and MCP III. MCP I processes information about the attractants α-aminoisobutyrate, L-alanine, glycine, and L-serine and the repellents acetate, benzoate, H+, indole, and L-leucine (Silverman and Simon, 1977; Springer et al., 1977). MCP II transduces information about the attractants L-aspartate and maltose and the repellents Co2+ and Ni2+ (Silverman and Simon, 1977; Springer et al., 1977). MCP III processes signals from the attractants D-galactose and D-ribose (Kondo et al., 1979). For the attractants L-alanine, α-aminoisobutyrate, L-aspartate, glycine, maltose, D-ribose, and L-serine, in vitro effective concentrations were 170 mM, 50 mM, 10 mM, 170 mM, 30 mM, 30 mM, and ≥ 3 µM, and for the repellents acetate, benzoate, indole, and L-leucine, Co2+, and Ni2+, 20 mM, 30 mM, 0.3 mM, 30 mM, 0.5 mM and 0.5 mM, respectively (Hedblom and Adler, 1980; Springer et al., 1977).

Adhesion and colonisation
The sugar mannose can bind to type 1 fimbriae, which are found in the majority of clinical isolates of E. coli, and thus prevent adherence to epithelial cells (Iida et al., 2001). Mannose-sensitive adhesion of E. coli was also inhibited by the fructose present in different juices (guava, pineapple, mango, grapefruit, blueberry juice, and cranberry cocktail; Ofek et al., 1996; Zafiri et al., 1989). However, the effect of fructose was weaker than the one of mannose. Apart from E. coli with type 1 fimbriae, there are also E. coli, such as enteropathogenic E. coli (EPEC), that show a mannose-resistant attachment to epithelial cells (Cravioto et al., 1979; Iida et al., 2001).

Wild-type EPEC strains attached to epithelial cells when grown on glucose as carbon source. When grown on galactose, they did not adhere to epithelial cells (Vannmaele and Armstrong, 1997). In practice, it does not seem possible to make feeds without glucose, because most polysaccharides contain plenty of it.

Mucin-like components of milk prevented adhesion of S-fimbriated E. coli (Hacker et al., 1985) to epithelial cells of the oral cavity (Schroten et al., 1992). Holmgren et al. (1981) and Ashkenazi and Mirelman (1987) showed that the non-immunoglobulin-fraction of human milk significantly inhibited E. coli cell adhesion mediated by CFA/I, CFA/II, or K88 fimbriae, but not type 1 pili. The non-immunoglobulin-fraction had a stronger effect than the immunoglobulin-fraction.

Haemagglutination
D-Mannose and α-methylmannoside inhibit haemagglutination of the enteropathogenic E. coli strain 0125: K 70 at concentrations of 3 to 12 mg/l (Rivier and Darekar, 1975). However, haemagglutination of other enteropathogenic E. coli proved to be resistant to mannose (Evans et al., 1979; Svanborg et al., 1983). Holmgren et al. (1981) showed that the non-immunoglobulin-fraction of human milk significantly inhibited E. coli/haemagglutination mediated by CFA/I, CFA/II, or K88 fimbriae, but not type 1 pili.

Haemolysis
Lissner et al. (1996) showed that spray-dried whey of colostrum from non-immunised cows (Lactobin®) inhibited the haemolytic activity of E. coli O157 by 50% in a concentration of 1 g Lactobin®/l. For practical application, this concentration seems rather high.

Haemoglobin, haematin, haem, or iron salts have a virulence-enhancing effect on E. coli (Bullen et al., 1968; Waalwijk et al., 1985). However, at iron concentrations above 100 µM Fe2+, an inverse relationship between medium iron and haemolytic activity was found. Waalwijk et al. (1985) registered that under conditions of plentiful iron, haemolysin production of E. coli was repressed. Lebek and Gruenig (1985) confirmed these results by showing that the haemolysin secretion of different E. coli strains was clearly reduced after addition of 100 µM FeCl3. They, however, suggested that haemolysin secretion is differentially regulated among E. coli strains. Hence, a sufficient iron supply in feed can possibly reduce haemolysin synthesis.
Competitive exclusion
Moulds isolated from cattle feeds showed only weak or no antagonistic reactions toward *E. coli* (Tiwari, 1981). The commercial competitive-exclusion product Broiact® (see chapter “*Campylobacter*” for details) reduced the caecal incidence of both the poultry pathogen *E. coli O20*:*K*:*H8* and the human pathogenic *E. coli O157*:*H7* in a challenge trial with newly-hatched broiler chicks (Hakkinen and Schneitz, 1996). Aviguard® (see “*Campylobacter*” for details) was shown to significantly reduce the incidence and number and acid resistance of *E. coli* passage through the human stomach than non-resistant *E. coli* O78:K80 in the small intestine, large intestine, and caeca in newly-hatched chickens (Hofacre et al., 2002). Genoves et al. (2001) reported a reduction of mortality in neonate piglets challenged with an ETEC strain by prior administration of an undefined competitive exclusion culture from the cecal contents of a healthy pig that contained, inter alia, enterococci, streptococci, clostridia, and *Bacteroides* species.

Colonisation/infection-preventive feed
Nutritional experiments with non-challenged young chickens fed with normal chicken feed or budgerigar feed suggested that a diet consisting exclusively of seeds has an inhibitory effect on intestinal colonisation of *E. coli* (Glünder, 2002).

In fattening cattle, grain feeding seems to promote growth and acid resistance of *E. coli* due to a decrease of the colonic pH down to 5.4 (Diez-Gonzalez et al., 1998). Acid-resistant *E. coli* are more likely to survive passage through the human stomach than non-resistant *E. coli*. When cattle were fed hay for only five days, the number and acid resistance of *E. coli* decreased (Diez-Gonzalez et al., 1998; Russel et al., 2000). According to Diez-Gonzalez et al. (1998), with hay only little starch reaches the colon, which has a lowering effect on the *E. coli* population.

Salmonella
Occurrence
*Salmonella* occurs in humans, warm and cold blooded animals, foods, and the environment. It can cause typhoid fever, enteric fevers, gastroenteritis, and septicaemia in humans and many animal species (Holt et al., 1994). *Salmonella* can be transmitted to humans by eating foods contaminated with animal faeces and cause food poisoning.

Non-metabolisable compounds
Both L-glucose and D-tagatose are not catabolised by *Salmonella enterica* serovar Typhimurium (Bautista et al., 2000). D-Tagatose is present in small amounts in milk; it is commercially produced from galactose in whey. If distinct sugars shall be applied in the large intestine of animals, it is essential to supply them in a form, which guarantees that they pass the upper intestinal tract without being transformed or absorbed (e.g. as ileal non-digestible polysaccharides).

Susceptibilities
*Salmonella enterica* serovar Enteritidis is sensitive to the fatty acids C10:0 and C12:0 in a concentration of 500 µmol/l at pH 5. These fatty acids are released during gastric digestion of fat, if present in fat. Sphingolipids present in milk fat or degradation products of them as sphingosine, lysophosphatidylcholine, and galactosylsphingosine showed bactericidal activities in a concentration of 100 µmol/l at pH 7 (Sprong et al., 2001).

Cocoa powder in a concentration of 5% proved bactericidal to salmonellae in commercial nutrient broth and lactose broth (Zapatka et al., 1977). However, the bactericidal effects were minimised by either 5% casein or non-fat dry milk. In rat trials, consumption of 5% cocoa for three generations had no adverse effect on reproduction (Hostetler et al., 1990).

*Salmonella enterica* serovar Paratyphi B, *Salmonella enterica* serovar Typhimurium, and *Salmonella enterica* serovar Venezuela were sensitive to the oil constituent pulegone when supplied on paper disks, which were placed on the surface of solid media inoculated with these bacteria (component in *Mentha pulegium* oil for example; Flamini et al., 1999). The minimum inhibitory amounts were 0.5 µl for *Salmonella enterica* serovar Venezuela and 1 µl for *Salmonella enterica* serovar Paratyphi B and *Salmonella enterica* serovar Typhimurium.

Adhesion and colonisation
The small intestine of the chicken has mannosreceptors for *Salmonella enterica* serovar Typhimurium with type 1 fimbriae (Baba et al., 1993; Oyofo et al., 1989b). In vitro, *Salmonella enterica* serovar Typhimurium exerts a D-mannose-sensitive cytotoxic effect on the mucosal epithelium of chicks (Droleskey et al., 1994). Addition of 2.5% D-mannose to the incubation medium inhibited the loss of mucosal epithelial integrity affected by *Salmonella enterica* serovar Typhimurium (Droleskey et al., 1994).

Dietary calcium phosphate (CaHPO₄ x 2H₂O; 60–180 mmol/kg diet) evidently has a trophic effect on the intestinal microflora and strongly protects against salmonella infection by improving colonisation resistance in rats (Bovee-Oudenhoven et al., 1996, 1997c, 1999). Calcium supplementation also reduced translocation of *Salmonella* to the systemic circulation (Bovee-Oudenhoven et al., 1997a,b,c, 1999). The reduced colonisation resistance found with low calcium diets in rats might be explained by higher cytotoxicity (to *Lactobacillus* for example) caused by bile acids, which calcium precipitates, or a higher soluble iron concentration, which calcium decreases (Bovee-Oudenhoven et al., 1996, 1997c, 1999). The protective effect of calcium might also be based on the induction of gastric acid secretion (Floor et al., 1991).

Line et al. (1997) studied the effect of dried yeast, supplied as 1 g of *Saccharomyces boulardii* (Levucell™SB20, Lallemand Inc., Rexdale, Canada) per kg feed, on experimental caecal colonisation of broilers with *Salmonella enterica* serovar Typhimurium and *Campylobacter jejuni*. Frequency of *Salmonella* colonisation was significantly reduced due to yeast treatment, *Campylobacter* colonisation was not significantly affected.
Competitive exclusion
The commercial competitive-exclusion product Aviguard® (see chapter “Campylobacter” for details) was shown to give a significant protection and reduce shedding of salmonellae by chicks (Ferreira et al., 2003; Nakamura et al., 2002). Broilact® (see chapter “Campylobacter” for details) gave good protection against both caecal colonisation and subsequent invasion of specific organs (heart, liver and spleen) by Salmonella enterica serovar Enteritidis PT4, Salmonella enterica serovar Infantis or Salmonella enterica serovar Typhimurium (Boiler et al., 1992; Cameron and Carter, 1992; Methner et al., 1997; Nuotio et al., 1992; Schneitz and Hakkinen, 1996). PREEMPT™ (see chapter “Campylobacter” for details) was shown to significantly reduce caecal colonisation by Salmonella enterica serovar Enteritidis (PT4) and Salmonella enterica serovar Typhimurium (PT4 and PT13) (Nisbet, 2002), and mortality and horizontal transmission of Salmonella enterica serovar Gallinarum in broiler chickens (Nisbet et al., 1998). According to Nisbet (2002), a similar product was developed that decreases shedding and reduces mortality of salmonellae in neonate and weaned pigs.

In some cases, the effects of single bacteria or small consortia were studied. Mossel (1971), for example, named E. coli as an antagonist of Salmonella. According to Mossel (1971), E. coli naturally inhibits Salmonella by competition. Van der Wielen et al. (2002) suggested that it was the production of acetic acid and propionic acid by Lactobacillus crispatus and Clostridium lactatifermentans in co-culture that inhibited the growth of Salmonella enterica serovar Enteritidis.

Reducing crop contamination of broilers
The crop of market-age broiler chickens is an important source of carcass contamination after slaughter in the processing plant. Crops empty of ingesta within 6 h after broilers are denied access to feed (Hinton et al., 2000; Wabeck, 1972). Crop emptying is followed by a decrease of fermentation products, an increase of the pH, and growth of food-borne pathogens (Hinton et al., 2002). Ramirez et al. (1997) observed that preslaughter feed withdrawal increased the incidence of Salmonella in crops, but not in caeca. Incorporation of 0.44% lactic acid in the drinking water reduced Salmonella and Campylobacter contamination in crops by 79% and 27%, respectively (Byrd et al., 2001). However, concentrations of 0.5% acetic, lactic, and formic acid reduced water consumption (Byrd et al., 2001). Provision of a nutrient broth-like cocktail with 4% sucrose instead of water during feed withdrawal reduced the incidence of Salmonella enterica serovar Typhimurium ST-10 (challenge strain) and natural campylobacters in crops (Hinton et al., 2002). The competitive-exclusion culture CF3 (PREEMPT™, see chapter “Campylobacter” for details) was shown to reduce Salmonella enterica serovar Typhimurium-positive crops in chicks by up to 56% after 5 weeks, after having been administered once on the day of hatch (Hume et al., 1996).

Colonisation-preventive feed
In the caecal contents of chicks challenged by contaminated feed, Allen et al. (1977) observed that the dietary inclusion of 15 and 25 g/kg mannose was associated with a reduction in the numbers of Salmonella enterica serovar Enteritidis (PT4). The same benefit was not recorded for Salmonella enterica serovar Infantis because this strain lacks mannose-sensitive fimbriae. The addition of 5 – 25 g palm kernel meal/kg, but not 20 g desiccated coconut/kg, to the feed also reduced the degree of salmonella colonisation in the intestinal tract of broiler chicks. Palm kernel meal contains inter alia oligosaccharides that contain mannose (Allen et al., 1977). Mannose-supplemented drinking water (2.5% mannose) had the same effect: colonisation of Salmonella enterica serovar Typhimurium was significantly reduced (Oyofo et al., 1989a). Carré and Brilouet (1986) showed that cell wall material from soyabean meal and sunflower meal contained the highest amounts of mannose (5.4 and 3.4%, respectively) among the different feed ingredients tested. Soyabean meal cell wall material moreover contained 24.9% galactose (Carré and Brilouet, 1986), which can be used as carbon source by health-promoting lactic acid bacteria, but also by certain enterobacteria and almost all Aeromonas strains. Hence, the latter products might also reduce Salmonella adhesion. According to Heres (2003), fermented feed increased the infectious dose of Salmonella for broiler chickens and can reduce the transmission of Salmonella in chickens flocks, but it will not prevent the occurrence of major outbreaks.

The risk for Salmonella shedding of pigs is reduced according to Belœil et al. (2004), Kranker et al. (2001), and Van der Wolf et al. (1999), when wet feed instead of dry feed is provided. This effect might be explained by the low pH caused by natural fermentation of liquid feed (Belœil et al., 2004).

Shigella
Occurrence
Shigellae are water- and foodborne intestinal pathogens of humans and other primates, causing dysentery.

Susceptibilities
About 1.8 mM of undissociated acetic acid completely inhibited Shigella flexneri multiplication (Baskett and Hentges, 1973). Adjustment of the medium pH from 6 to 7 reversed inhibition due to a shift from the undissociated to the dissociated form. The reason for the difference in effectiveness is that undissociated acetic acid penetrates cells better. Tetteh and Beuchat (2001), performing acid tolerance studies, found that the order of lethality of organic acids for Shigella flexneri at a given pH was lactic acid < acetic acid < propionic acid. Rough estimates of putatively effective concentrations of undissociated acids from the findings of Tetteh and Beuchat (2001) resulted in about 14 µM propionic and acetic acid (pk1 = 3.14), and 72 µM lactic acid (pk1 = 3.86) in unbuffered medium.

According to Park et al. (1979), a total of 102 bacteria were inhibited by 5% cocoa powder, which was the only concentration tested. Shigella, Staphylococcus, Micrococcus, and Bacillus were the most sensitive...
bacteria. The inhibitory effect increased with temperature. Non-fat dry milk, tryptone and casein reduced the toxicity of cocoa for bacteria. In rat trials, consumption of 5% cocoa for three generations of rats had no adverse effect on reproduction (Hostettler et al., 1990). The plant phenolics caffeic acid, chlorogenic acid, and ferulic acid had a dose-dependent bactericidal effect on Shigella sonnei (group D) (Tsou et al., 2000). Concentrations of each 400 µM proved to inhibit growth by > 50%.

Adhesion
Low concentrations of whey, secretory immunoglobulin A, lactoferrin and free secretory component from human milk inhibited both adhesion and invasion of HeLa cells in vitro by Shigella dysenteriae, S. flexneri and S. sonnei isolated from children with diarrhoea (Willer et al., 2004). Tigyi et al. (1992) showed that bovine lactoferrin bound slightly better than human lactoferrin to 45 S. flexneri clinical isolates.

Competitive exclusion
Hentges (1967) found that Shigella flexneri type 2a was inhibited by Klebsiella (Aerobacter aerogenes) in a mixed culture. Fomric and acetic acids produced by Klebsiella and a strong reduction of the culture medium were responsible for the inhibition. Kuroiwa et al. (1990) observed that Clostridium butyricum MIYAIRI 588 inhibited the growth of Shigella flexneri (1b and 2a) in a mixed culture. Apella et al. (1992) studied the inhibitory effect of different lactobacilli on growth of Shigella sonnei; all bacteria were isolated from human faeces. Lactobacillus casei and Lactobacillus acidophilus together showed a higher inhibiting effect than the single bacteria.

Plesiomonas shigelloides, an organism only rarely associated with diarrhoea in man and commonly found in water, is antigenically similar to Shigella sonnei. In contrast to non-immunised rabbits, rabbits immunised by feeding two doses of 10<sup>10</sup> Plesiomonas shigelloides O:17 (SVC O1) cells at an interval of six days were completely protected against shigellosis in an oral challenge trial with Shigella sonnei (Sayeed et al., 1992).

Plesiomonas shigelloides
Occurrence
Plesiomonas is commonly isolated from drinking water, aquatic environments, and aquatic animals (Foster et al., 2000; Schubert, 1984; Van Damme and Vandepitte, 1980). Plesiomonas shigelloides has been associated with gastroenteritis and bacteraemia in humans and diarrhoea in cats, dogs, goats, swine, and monkeys (Arai et al. 1980; Bardon, 1999; Foster et al., 2000; Jagger et al., 2000). Although originally considered a tropical or subtropical bacterium, isolations of Plesiomonas shigelloides outside those regions have increased during recent years (González-Rey et al., 2004).

Susceptibilities
Mannose was shown to be toxic to Plesiomonas shigelloides. Plesiomonas shigelloides transports mannose into the cell and phosphorylates it to mannose-6-phosphate. In contrast to glucose-6-phosphate, mannose-6-phosphate accumulates and is not further metabolised, what probably causes the toxic effect (Rager et al. 2000). Carré and Brillouet (1986) analysed, inter alia, the mannose contents in the cell wall residues of different plant materials, which might be a suitable mannose source in the intestine; see chapter “Salmonella” for details.

Haemolysis
Most Plesiomonas shigelloides strains secrete a β-haemolysin under reduced oxygen tension (Baratéla et al., 2001; Janda and Abbot, 1993). Maximal haemolysin synthesis occurs under iron limitation (Baratéla et al., 2001; Daskaleros et al., 1992; Santos et al., 1999). In contrast to 200 µM FeCl<sub>3</sub>, 2 mM CaCl<sub>2</sub> was found to enhance the haemolytic activity (Baratéla et al., 2001). Calcium is also required for the binding of the E. coli haemolysin Hly A to erythrocytes (Ludwig et al., 1988). Baratéla et al. (2001) suggested that the mechanism might be the same in Plesiomonas.

Yersinia enterocolitica
Occurrence
Yersinia occurs in a broad spectrum of habitats including humans, animals, especially rodents and birds, soil, water, dairy products, and other food (Holtt et al., 1994). Enteropathogenic yersiniae, like Yersinia enterocolitica and Yersinia pseudotuberculosis, are faecal-oral pathogens of animals and humans. Yersinia enterocolitica and Yersinia pseudotuberculosis survive in moist natural environments or in food by means of highly adaptable metabolic pathways (Straley and Perry, 1995). In Europe, Yersinia enterocolitica is the main causative agent of yersiniosis, which is the collective term for invasive gastrointestinal diseases caused by yersiniae, in pigs, chinchillas, cattle, small ruminants, cats, and dogs (Neubauer et al., 2001). Apart from Yersinia enterocolitica, Yersinia pseudotuberculosis is also associated with diarrhoea in pigs (Neeff, 1993).

Non-metabolisable compounds
Yersinia enterocolitica does not ferment lactose or L-rhamnose (Dijk et al., 1999; Shehee and Sobsey, 2004). Also, L-glucose and D-tagatose are not catabolised at all by Yersinia enterocolitica (Bautista et al. 2000). In contrast to nonpathogenic Yersinia enterocolitica, most pathogenic strains lack the ability to ferment D-arabitol (Shehee and Sobsey, 2004). These compounds might stimulate outgrowth of Yersinia enterocolitica by competing bacteria.

Susceptibilities
Acetic acid (0.156% v/v) proved to be the most effective antimicrobial agent against Yersinia enterocolitica among bicarbonate, vinegar, acetic and citric acid (Karapınar and Gönül, 1992).

Harmful products
Pathogenic Yersinia isolates contain chromosomal and plasmid-encoded virulence factors. To be able to establish in a host, the presence of the virulence plasmid pYV is obligatory (Cornelis et al., 1998). The virulence
plasmid encodes the low-Ca\(^{2+}\) response stimolus LCRS. The LCRS is important in inhibiting phagocytosis by host phagocytes and it tempers inflammatory response. In the presence of Ca\(^{2+}\), synthesis of anti-host proteins is downregulated at gene level in *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* (Straley et al., 1993; Straley and Perry, 1995). Most human isolates of *Yersinia enterocolitica* produce the heat-stable, chromosome-encoded enterotoxin YST. According to Amirmozafari and Robertson (1993), Ca\(^{2+}\) and Mg\(^{2+}\) had no effect on the synthesis of YST by *Yersinia enterocolitica*; however, concentrations of Fe\(^{3+}\) above 10 µM inhibited the synthesis of the enterotoxin. Mixtures of adenosine and guanine, each at a concentration of 2.0 mM, also inhibited the synthesis of YST (Amirmozafari and Robertson, 1993).

**Adhesion**

*Yersinia enterocolitica* and *Yersinia pseudotuberculosis* possess chromosomal as well as plasmid-encoded mechanisms to promote adhesion to epithelial cell surfaces (Pærregaard et al., 1990). Jensen and Pærregaard (1991) observed a significant reduction of adhesion of *Yersinia enterocolitica* O:3 (YeO301P+pYV plasmid) to rabbit ileal brush border membrane vesicles after incubation with human milk at a final dilution of 1:10. This effect was not attributed to immunoglobulins, but to milk fat, proteins, and factors resistant to proteolytic and heat-treatment.

**Haemin as iron source**

*Yersinia pseudotuberculosis, Yersinia enterocolitica, and Yersinia pestis* can all utilise picomolar quantities of haemin as sole source of iron (Perry and Brubaker, 1979; Perry, 1993). The haemin-transport system of *Yersinia enterocolitica* is repressible by iron (Perry, 1993; Straley and Perry, 1995).

**Competitive exclusion**

Asplund et al. (1996) observed that the growth of *Yersinia enterocolitica* serotype O:3 was substantially inhibited by the ileal or caecal microflora from pigs used as an inoculum in an in vitro model of porcine caecum. After 3 days of inoculation at 39°C, *Yersinia enterocolitica* was not detected any more. Among Enterobacteriaceae, especially *Hafnia alvei* and environmental *Yersinia* organisms inhibited growth of *Yersinia enterocolitica* serotype O:3 at 6 and 25°C (Fukushima and Gomyoda, 1986). Schiemann and Olson (1984) found that *Yersinia enterocolitica* serotype O:8 was outgrown by other Gram-negative organisms at 32°C. At lower temperatures, growth rates of *Yersinia enterocolitica* and the *Pseudomonas, Providencia, Enterobacter, Klebsiella, Citrobacter, Achromobacter*, and *Acinetobacter* strains tended to be more comparable. Özbaş and Aytaç (1996) observed that different lactobacilli had an antimicrobial effect on *Yersinia enterocolitica*.

**Aeromonas spp.**

**Occurrence**

*Aeromonas* occurs in fresh water, in sewage, in meats, poultry, and fresh produce (Annapurna and Sanyal, 1977; Hao et al., 1998). Gray and Stickler (1989) observed aeromonads (*Aeromonas hydrophila, Aeromonas sobria, Aeromonas caviae*) in faeces from healthy pigs and cows in a pattern comparable to the one of the natural waters that the animals drank. Annapurna and Sanyal (1977) isolated *Aeromonas hydrophila* from man, from animals and waters and found that the majority of the strains were enterotoxigenic irrespective of the source of isolation. The human diseases caused by *Aeromonas* ssp. range from diarrhoea to life-threatening illnesses (Janda and Abbott, 1998). In animals, *Aeromonas hydrophila* has been associated with enteric lesions in cattle (Al-Mashat and Taylor, 1983), bovine abortion (Wohlgemuth et al., 1972), and hemorrhagic septicaemia in farmed rabbits (Paniagua et al., 1998). *Aeromonas salmonicida* ssp. salmonicida is the causative agent of the fish disease furunculosis. The gastrointestinal tract is a possible site of colonisation and port of entry of this pathogen (Nikoskelainen et al., 2001).

**Susceptibilities**

A combination of ascorbic acid (0.1%) and citric acid (0.03%) most effectively inhibited the growth of *Aeromonas caviae* and *Aeromonas sobria* in fish homogenates among different mixtures of sodium chloride, citric acid, ascorbic acid, potassium sorbate, and thyme extract (Abu-Ghazaleh, 2000). Commercially available eugenol ( clove extract) and pimento, applied to the surface of refrigerated cooked beef, significantly inhibited the growth of *Aeromonas hydrophila* (Hao et al., 1998).

**Competitive exclusion**

*Lactobacillus rhamnosus* ATCC 53103, *L. casei* Shirot, *L. bulgaricus*, *L. rhamnosus* LC 705, *L. johnsonii* La1, *Bifidobacterium lactis* Bb12, and *Enterococcus faecium* Tehobak inhibited growth of *Aeromonas salmonicida* in coculture. The inhibition was mediated by competition for nutrients rather than secretion of inhibitory substances by the probiotic bacteria (Nikoskelainen et al., 2001). Özbaş and Aytaç (1996) observed that growth of *Aeromonas hydrophila* was retarded or inhibited during fermentation of skim milk by lactobacilli. Especially *Lactobacillus bulgaricus* IFO 13953 and *Lactobacillus cremoris* 708 caused sharp decreases of the number of *Aeromonas salmonicida*. Kuroiwa et al. (1990) found that *Clostridium butyricum* MIYAIRI 588 inhibited the growth of *Aeromonas hydrophila*.

**Colonisation/infection-preventive feed**

Infection with *Aeromonas hydrophila* was found in 1.9% of granivorous and herbivorous bird species, in 7.1% of omnivorous and in 12.4% of carnivorous and insectivorous birds, with a total of 3500 wild and pet birds tested. According to Glünder (2002), these data indicate a negative correlation between a vegetarian diet and the frequency of occurrence of *Aeromonas hydrophila*.

**Vibrio cholerae**

**Occurrence**

Visser et al. (1991, 1997) described *Vibrio cholerae* of neither serotype O1 nor O139 (serotypes found with
humans) as causative agent of severe watery diarrhoea in lambs and from a goat with enterotoxicosis in the Netherlands. Rhodes et al. (1985) isolated non-O1 Vibrio cholerae from a horse, a lamb, and two American buffalos with enteric diseases in western Colorado. In Romania, haemorrhagic diarrhoea in piglets due to Vibrio cholerae serovars O6 and O37 was reported (Rapantean et al., 1992). In Argentina, non-O1 Vibrio cholerae was found to be associated with two outbreaks of sudden death and one of chronic diarrhoea in cattle (Fain Binda et al., 1993). Another non-O1 Vibrio cholerae was isolated from diseased fish in Japan (Kiiyukia et al., 1992). Giving an overview of the Vibrio cholerae groups isolated from diseased animals in the Netherlands Visser et al. (1999) ask for more awareness of these pathogens as a possible participant in enteric infections of farm animals.

**Attractant/adhesion/haemagglutination**

L-Fucose inhibited the adhesion of Vibrio cholerae (Ogawa serotype) to membranes from rabbit intestinal epithelial cells at a concentration of about 100 mg/l by blocking chemotactic receptors on the vibrio surface (Freter and Jones, 1976; Jones and Freter, 1976; Freter et al., 1981). Jones and Freter (1976) also observed that L-fucose inhibited Vibrio cholerae haemagglutination completely at a concentration of 160 mg/l. Bound fucose was an even more effective inhibitor; methyl-L-fucoside had an inhibiting concentration of 78 mg/l (containing 72 mg fucose/l), for p-nitrophenyl-L-fucose it was 39 mg/l (22 mg fucose/l), and for bovine serum albumine-bound L-fucose it was 2.2 mg/l (1.2 mg fucose/l). Other monosaccharides as D-fucose, D-glucose, N-acetyl-D-glucosamine, D-galactose, N-acetyl-D-galactosamine, and N-acetyleneuramic acid were not inhibitory in haemagglutination assays (Jones and Freter, 1976). However, haemagglutinating activity of fimbriate Vibrio cholerae O1 was completely inhibited by 1% of D-mannose, D-glucose, D-fructose, or N-acetyl-D-glucosamine, but not by L-fucose (Ehara et al., 1991). According to Sanchez and Jonson (1990), binding of classical and El Tor vibrios to L-fucose beads was found to correlate with fucose-sensitive agglutination of human O erythrocytes, while binding to beads with D-mannose was consistent with mannose-sensitive agglutination of chicken erythrocytes (bead size 45 – 165 µm, agarose was consistent with mannose-sensitive agglutination of human erythrocytes to its membrane receptor G, ganglioside. The authors suggest the presence of structural analogues to cell membrane receptors, functioning as competitive inhibitors, in milk.

Toda et al. (1991) reported that black tea extract had bactericidal activity against Vibrio cholerae O1 and inhibited the haemolysin activity of Vibrio cholerae O1 and O1 El Tor. Tea extract was prepared by suspending 20 g of tea leaves in 80 ml buffered solution for 3 h at room temperature. After that, the leaves were removed by centrifugation and the pH of the supernatant was adjusted to 7.0. The resulting tea concentration of the extract was about 10 times higher than in beverage. A final concentration of 3.3 ml tea extract/l totally inhibited the haemolytic activity of Vibrio cholerae. In an animal model, i.e. in rabbit intestinal loops of 7 cm length, injection of 0.5 ml of tea extract was shown to prevent cholera infection when challenged (Toda et al., 1991).

**Competitive exclusion**

Kuroiwa et al. (1990) observed that Clostridium butyricum MIYAIRI 588 inhibited the growth of Vibrio cholerae O1 and Vibrio cholerae non-O1.

**Clostridium perfringens**

**Occurrence**

Clostridia are an important cause of illness and death in humans and animals (Borriello, 1995). Some of the most common clostidial infections are those of the gut. In chickens, Clostridium perfringens types A and C are causative agents of clinical and subclinical necrotic enteritis (NE) (Al-Sheikhly and Truscott, 1977; Engström et al., 2003; Ficken and Wages, 1997). In the absence of antibiotics, NE is the major problem for poultry producers. Clostridium perfringens has also been associated with diarrhoea, enterotoxaemia, or NE in sheep and goats (C. perfringens types B, C and D; Miserez et al., 1998; Niilo, 1980), cattle (C. perfringens types C, D and E; Atkinson, 1998; Hermann, 2002; Niilo, 1980), a foal (C. perfringens type C; Niilo, 1980; Pearson et al., 1986), pigs (C. perfringens type C; Collins et al., 1989; Jestin et al., 1985; Klaasen et al., 1999; Sidoli and Guarda, 1982); dogs (Weese et al., 2001), and cats (El-Sanousi et al., 1992; Werdeling et al., 1991). Other important pathogens are Clostridium difficile that can cause diarrhoea in neonatal pigs (Nagy and Bilkei, 2003; Yaeber et al., 2002), Clostridium septicum causing braxy (high fever, coma, and inflammation of the fourth stomach) in sheep, Clostridium colinum causing ulcerative enteritis in birds, and Clostridium spiroforme causing enterotoxaemia in rabbits (Borriello, 1995; Borriello and Carman, 1983).

However, not all clostridia are pathogens. Clostridium herbivorans, isolated from the intestinal tract of pigs, for example, transforms cellulose to butyrate, formate, hydrogen, and ethanol (Varel et al., 1995). While cellulose cannot be degraded by higher organisms, short-chain fatty acids can both be absorbed as nutrients by the animal (Arzenio and Southworth, 1975; Stevens, 1978) and have a gut health-stimulating effect (Kanauchi et al., 1999; Venkatraman et al., 1999).

**Susceptibility**

In contrast to E. coli O157:H7, Salmonella enteritidis, Campylobacter jejuni, and Listeria monocytogenes, Clostridium perfringens (isolated from surface water; NIZO BS42) was completely killed at pH 5 in buffer (Sprong et al., 2001).
Non-metabolisable compounds
According to Chou (1971), *Clostridium perfringens* A S107 (NCTC 8237) does not ferment salicin, xylose, arabinose, mannitol, and dulcitol. In a non-sterile environment, these compounds might stimulate outgrowth of *Clostridium perfringens* by competing bacteria.

Harmful products
In addition to four major toxins used to biotype *Clostridium perfringens* (α, β, ε, ι), a further eight soluble proteins (γ, δ, η, θ, κ, λ, μ, ν) were identified with biological activities that may justify their consideration as toxins (Shone and Hambleton, 1989). Factors that promote the toxin production in *Clostridium perfringens* are present in enzymatic digests of meat and casein, like proteose peptone and tryptone (Chou, 1971). In *vitro*, *Clostridium perfringens* type C strain CN 5384 produced less ι-toxin in media with 1% dextrin, fructose, or raffinose than in media containing glucose, starch, or sucrose (Sakurai and Duncan, 1979). In addition, distinctly more toxin was formed when the pH was maintained at 7.5 compared with an uncontrolled, decreasing pH (Sakurai and Duncan, 1979).

Competitive exclusion
According to Hofacre et al. (1998), the commercial competitive-exclusion product Aviguard® (see chapter “Campylobacter” for details) significantly reduced gross lesions induced in an NE infection model for chicken. In addition, chicks treated with Aviguard® ate more feed and had better feed efficiencies than groups treated differently (Hofacre et al., 1998). Broilact® (see chapter “Campylobacter” for details) decreased mortality due to NE and necrotic hepatitis when broiler chicks were given a diet with animal protein instead of vegetable protein (Elwinger et al., 1992). Moreover, use of Broilact® increased slaughter yield of birds in this trial (Elwinger et al., 1992).

Generally, growth of proteolytic bacteria is impeded by growth of lactic acid bacteria (Dijk and Hambleton, 1989). When carbohydrates are fermented, large quantities of acids are produced and the resulting reduction in pH value inhibits proteolytic organisms. The presence of carbohydrates and proteins may affect the digestion of protein (Mossel, 1971). According to Mossel (1971), *Clostridium perfringens* is inhibited by *Clostridium sporogenes* and Lactobacillaceae. Gibson and Wang (1994) reported that a *Bifidobacterium infantis* strain exerted an inhibitory effect on *Clostridium perfringens* (NCTC 8237) in *vitro*, which was not necessarily related to acid production. La Ragione et al. (2004) showed that *Lactobacillus johnsonii* FI9785 suppressed all aspects of colonisation and persistence of *Clostridium perfringens* strain FD00385, isolated from a clinical case of avian NE, in chicks.

Necrotic enteritis-preventive feed
NE in chickens, caused by *Clostridium perfringens*, is promoted by high dietary levels of wheat (Branton et al., 1987, 1997; Riddell and Kong, 1992), barley (Kaldhusdal and Hofshagen, 2002; Kaldhusdal and Skerve, 1996; Riddell and Kong, 1992), rye (Riddell and Kong, 1992), oat groats (Riddell and Kong, 1992), fish-meal (Truscott and Al-Sheikhly, 1977; Drew et al., 2004), and damage to the intestinal mucosa due to high-fibre litter (Al-Sheikhly and Truscott, 1977). Corn-based diets, on the other hand, reduced the incidence of NE (Branton et al., 1987, 1997; Riddell and Kong, 1992). Drew et al. (2004) stated that it is the increased viscosity of digesta, caused by cereal grains like wheat and barley, that predisposes broiler chickens to NE. However, proliferation of *Clostridium perfringens* type A was not only in vivo, but also in vitro significantly higher in the supernatants from digested wheat and barley diets than in the one from a digested corn diet (Annett et al., 2002), suggesting a physiological rather than a mechanical reason. Among non-digested diets, no significant differences in clostridial proliferation were observed (Annett et al., 2002). Because oat groats have a higher level of fat than corn, the possibility that fat in corn may have a protective effect was discounted (Riddell and Kong, 1992). Addition of the enzyme pentosanase, which increases the digestibility of complex carbohydrates, to a wheat-based diet did not affect the mortality level due to NE (Riddell and Kong, 1992). Thus, no evidence was obtained that complex carbohydrates in wheat may explain the increased susceptibility to NE. Diets with pectin or guar gum added were not fully consumed or severely reduced growth (Branton et al., 1997; Riddell and Kong, 1992; Wagner and Thomas, 1977). Addition of glucose to a corn-based diet caused a slight, but not significant, increase in mortality due to NE (Riddell and Kong, 1992). Apart from carbohydrate sources, protein sources seem to be decisive as a predisposing factor for NE. Drew et al. (2004) and Truscott and Al-Sheikhly (1977) reported higher incidences of *Clostridium perfringens* and symptoms of NE in chickens fed fishmeal-based diets than in birds fed soy protein-based diets. Elevated glycine and methionine contents in the fishmeal diet might have lead to the higher Clostridium numbers (Drew et al., 2004). Effects of distinct feed ingredients or compounds on passage time of digesta, colonisation of *Clostridium perfringens*, or formation of clostridial toxins have yet to be elucidated.

Conclusions
Resources for counteractive agents, which can possibly be applied to weaken pathogenic bacteria, include sugars, polysaccharides, dietary fibre, amino acids, organic acids, fatty acids, lipids, minerals, milk components, plant extracts, essential oils, and antagonistic bacteria. In practice, there are some restrictions to the application of certain compounds. For metal ions such as iron and copper, EU feed regulations prescribe the maximum admissible content in animal diets. Compounds that are not yet authorised as feed additives must qualify in a registration procedure.

Most protective strategies in this review were described for a distinct bacterium or a bacterial strain. To be able to make good use of the Achilles’ heels of enteropathogenic bacteria (Fig. 1), it is essential to identify the etiologic agent of a current disease or an infection risk, or the human-pathogenic stowaway. A quick diagnosis is indispensable when animal herds are affected, because gastrointestinal problems can cause substantial losses. The pathogen is commonly diagnosed by the disease
symptoms, the production of specific antibodies, by biotyping, serotyping or nucleic acid-based techniques. In general, these approaches give veterinarians a good idea about the kind of infection they are dealing with. In pig production, enterotoxigenic E. coli are primarily held responsible for diarrhoea in young pigs, whereas Brachyspira hyodysenteriae is a suspect in growing and finishing pigs. In addition, Salmonella, clostridia and coccidiosis are familiar causes of diarrhoea in pigs. In poultry, some enterotoxin-producing E. coli, Clostridium perfringens, Clostridium colinum, and Campylobacter cause enteric diseases with economic consequences. Moreover, poultry meat is a possible carrier for campylobacteriosis and salmonellosis in humans. In cattle herds, E. coli is regarded as the most important bacterial enteropathogen in neonate calves. Apart from E. coli, Clostridium perfringens types A, B and C are known to cause enteric diseases in calves. Salmonellosis forms a problem in cattle, because clinically normal animals can chronically carry and shed persisting Salmonella species. Non-bacterial infectious agents such as viruses and parasites might also be involved in the development of diarrhoea. Despite all efforts, in some cases pathogens are not identified because they are not included in routine tests or not sufficiently recognized up to now. Although knowing the enemy is a prerequisite for its abatement, the fragmentary character of information about the Achilles’ heels of specific animal-pathogenic bacteria forms a problem that has yet to be solved. Agents showing an inhibiting effect on pathogens include plant extracts, essential oils and organic acids (Abu-Ghazaleh, 2000; Basket and Hentges, 1973; Boyanova and Neshev, 1999; Burger et al., 2000, 2002; Byrd et al., 2001; Chaveerach et al., 2002; Flamini et al., 1999; Hao et al., 1998; Karapinar and Gönül, 1992; Mahady et al., 2001; Mroz, 2005; Park et al., 2000; Sprong et al., 2001; Tabak et al., 1999; Tetteh and Beuchat, 2001; Zapatka et al., 1977). Unfortunately, little is known about the specificity of these substances, because they were for the most part tested with only a limited number of bacteria. However, even a low specificity might qualify a substance as a potential carrier for campylobacteriosis and salmonellosis in humans. In cattle herds, E. coli is regarded as the most important bacterial enteropathogen in neonate calves. Apart from E. coli, Clostridium perfringens types A, B and C are known to cause enteric diseases in calves. Salmonellosis forms a problem in cattle, because clinically normal animals can chronically carry and shed persisting Salmonella species. Non-bacterial infectious agents such as viruses and parasites might also be involved in the development of diarrhoea. Despite all efforts, in some cases pathogens are not identified because they are not included in routine tests or not sufficiently recognized up to now. Although knowing the enemy is a prerequisite for its abatement, the fragmentary character of information about the Achilles’ heels of specific animal-pathogenic bacteria forms a problem that has yet to be solved. 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However, the problem with both toxic and non-metabolizable carbohydrates (Bautista et al., 2000; Chou, 1971; De Vaux et al., 2002; Sheheee and Sobsey, 2004), which stimulate outgrowth of the pathogen by competing bacteria, is that a solution must yet be found that guarantees their integral passage through the upper intestinal tract. Minerals such as zinc, copper, iron, or calcium showed protective effects at higher concentrations (Baratéa et al., 2001; Bovee-Oudenhoven et al., 1996, 1997a,b,c, 1999; Dupont et al., 1994; Straley et al., 1993; Straley and Perry, 1995; Zhang et al., 2001). Because of the well-known growth-promoting properties of copper and zinc, commercially available diets for monogastrics usually include maximum admissible concentrations of the latter minerals. Calcium phosphate for protection against Salmonella has only been tested in rats up to now and its mode of action is still subject to speculation (Bovee-Oudenhoven et al., 1996, 1997c, 1999). The latter seems also the case with various feed strategies tested in trial-and-error-like manners, with the complexity of an in vivo experiment probably hampering clear conclusions. Nevertheless, those strategies aimed at reducing the crop contamination of broilers before slaughter (Byrd et al., 2001; Hinton et al., 2002) seem promising enough to raise hope for the successful development of further effective dietary strategies.

In conclusion, thorough testing will be needed both in vitro and in vivo under standardized experimental conditions to evaluate promising strategies aimed at warding off specific pathogenic bacteria and/or their virulence factors. Multifactorial approaches seem particularly promising, because by tackling different Achilles’ heels of one pathogen, the treatment becomes more target-specific and the combined virulicidal actions might have a synergistic effect.

In addition to attacking individual bacteria, general strategies are conceivable that can possibly be applied to repel different pathogenic bacteria collectively in the intestinal tract. In the following, an overview is given of some general strategies.

**Milk constituents**

Apart from being a nutrient source, milk protects newborns from infections until their own immune system is sufficiently developed to cope with pathogens. Spray-dried whey from bovine colostrum, for example, which is rich in immunoglobulins (IgG), was shown to block the cytotoxic effect of E. coli O157 (Lissner et al., 1996). However, it is not only the immunoglobins in milk that seem to be responsible for strong antibacterial effects (Holmgren et al., 1981; Ashkenazi and Mirelman, 1987). Fat fractions, like sphingolipids, can be bactericidal to Escherichia coli, Salmonella enteritidis, and Campylobacter (Sprong et al., 2001). Milk fat, in the form of membrane fractions (Hirmo et al., 1998; Wang et al., 2001) and mucin-like fat globule membrane components (Schroten et al., 1992) can, moreover, play a role in inhibition of adhesion of pathogens to host cells. The milk protein lactoferrin has also adhesion-inhibiting effects (Tigyi et al., 1992; Wang et al., 2001; Willer et al., 2004).

**Antagonistic bacteria for competitive exclusion**

Although the antagonistic bacterial strains that are recommended as growth-inhibiting to pathogens differ, it is a general principle that a pathogen cannot establish when its ecological niche is already occupied (Fig. 2), i.e. when binding sites are already covered, inhibitory compounds are present that are synthesised by other bacteria, or nutrients are depleted by others. Especially for chickens, different commercial competitive-exclusion products of undefined compositions are available that were enriched from the intestinal microflora of healthy animals. The competitive exclusion method was first described by Nurmi and Rantala (1973). At present, mostly complex cultures with an undefined composition or preparations consisting of a multitude of enteric bacteria are used for competitive exclusion. According to Methner (2000), knowledge of the mechanisms of action of competitive exclusion cultures as well as the effective species of the various bacterial genera is still inadequate.
**Mucin-related attractants**

A protective mucus layer covers the gastrointestinal epithelium. Its main part consists of mucin, i.e. protein with a substantial amount of carbohydrate that is secreted by the intestine and produced as part of the epithelial cell membrane (Fogg et al., 1996; Deplancke and Gaskins, 2001). Intestinal microvillus membranes and mucin contain the sugar fucose (Babbar et al., 1989; Milner and Sellwood, 1994; Kennedy and Yancy, 1996). L-Fucose is a powerful attractant for *Brachyspira*, *Campylobacter*, and some *Vibrio cholerae* (Jones and Freter, 1976; Hugdahl et al., 1988; Kennedy and Yancy, 1996; Witters and Duhamel, 1999). Bound fucose proved even more effective than pure L-fucose in inhibiting cell adhesion of these *Vibrio cholerae* (Jones and Freter, 1976). Also, the vibrios were found to specifically adhere to agarose beads that carried covalently linked L-fucose on their surfaces (Sanchez and Jonson, 1990). However, other *Vibrio cholerae*, some *E. coli*, *Salmonella typhimurium*, and *Salmonella enteritidis* (PT4) do not attach to L-fucose, but to D-mannose (Rivier and Darekar, 1975; Allen et al. 1977; Cravioto et al., 1979; Naughton et al., 2001; Oyofo et al., 1989b; Sanchez and Jonson, 1990; Baba et al., 1993). Thus, it might be possible to prevent adherence of different pathogens to the gut epithelium by misguiding and trapping them (Fig. 3). To this end, suitable attractants or binding sites could be orally supplied in a digestion-resistant form to animals (Kiers et al., 2002; Naughton et al., 2001; Ofek et al., 2003; Spring et al., 2000).

**Iron**

The impact of iron can be different: Iron can promote growth, toxicity, and haemolytic activity of pathogens (Bullen et al., 1968; McCardell et al., 1986; Waalwijk et al., 1985), or reduce haemolysin synthesis and pathogenicity (Daskalaros et al., 1992; Santos et al., 1999; Baratéla et al., 2001). The effect seems to depend strongly on its concentration. While low concentrations have a virulence-stimulating effect (Bullen et al., 1968), high concentrations evidently inverse this relationship (Waalwijk et al., 1985). Waalwijk et al. (1985) and Lebek and Gruenig (1985) observed under conditions of plentiful iron (i.e. >100 μM Fe³⁺) a repression of haemolysin production in *E. coli*.

A marked decrease in haemolytic activity caused by iron was also detected in *Serratia marcescens* (Poole et al., 1988) and *Plesiomonas shigelloides* (Daskalaros et al., 1992; Santos et al., 1999; Baratéla et al., 2001). The question, whether a pathogen that grows slower but is highly virulent, or the same bacterium that multiplies faster and is less virulent causes more damage, has to be weighed. Moreover, Van Asbeck and Verhoef (1983) compiled evidence suggesting that iron deficiency as well as hyperferraemia might impair the immunologic response of the host.

Paying attention to the Achilles’ heels of enteropathogenic bacteria (Fig. 1) in livestock might open up new vistas for the management of gut health via animal nutrition. Valuable lessons can be learned from health-stimulating milk components in terms of their mode of functioning. Also, current knowledge about attractants and receptors of enteropathogenic bacteria offers appealing prospects for the development of new antibacterial strategies. To be able to react quickly to disease symptoms and because of the current lack of details or confirmation concerning methods to repress specific pathogens, it seems reasonable to preliminary focus on more general antipathogenic strategies.

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