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DIETARY MODULATION OF THE RESISTANCE TO INTESTINAL INFECTIONS

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SUMMARY

Gastrointestinal infections are still a major health problem. These infections may be modulated by dietary components. Diet determines the composition of (gastro-)intestinal contents, which in turn affects gastro-intestinal survival of food-borne pathogens, the composition of the autochthonous microflora, and functioning of the mucosal barrier. These non-immunological parameters are essential for host defence, especially during the first encounter with a pathogen. Although numerous *in vitro* studies showed beneficial effects of dietary components on microflora- or infection-related issues, sound scientific evidence to confirm their functionality in animal and human health is surprisingly scarce.

We have studied the protective effects of dietary calcium against salmonella infection. It was already known that calcium precipitates irritating bile acids and fatty acids in the intestinal lumen and diminishes epitheliolysis. We speculated that reduced epitheliolysis might strengthen the barrier function of the gut mucosa and improve the resistance to intestinal infections. In addition, intestinal Gram-positive bacteria like lactobacilli might also benefit from the reduced luminal cytotoxicity, as these lactic acid bacteria are very sensitive to bile acids and fatty acids *in vitro*. Several infection studies were performed with rats consuming purified diets differing only in calcium content. After adaptation to these diets, the rats were orally infected with *Salmonella enteritidis.* Indeed, calcium stimulated the intestinal lactobacilli. More importantly, calcium supplementation decreased colonisation and translocation of salmonella, as judged by the reduced faecal shedding of this pathogen in time and the diminished infection-induced urinary NO_x excretion, respectively.

In literature, prebiotics are claimed to improve resistance to intestinal infections, though evidence is merely lacking. Therefore, we studied the effect of non-digestible carbohydrates on the course of a salmonella infection. Lactulose and oligofructose decreased colonisation of *S. enteritidis* when compared with resistant starch, wheat fibre, and cellulose. However, lactulose and oligofructose concomitantly increased translocation of this pathogen, an effect probably due to impairment of the gut mucosal barrier.

So, diet does modulate the resistance to intestinal infections, but more research is needed to prove the high expectations regarding functional foods and to elucidate the mechanisms involved.

INTRODUCTION

Gastrointestinal infections are still a major health problem. The world-wide incidence of acute infectious diarrhoeal disease is estimated to be 3-5 billion cases per year, resulting in 3-5 million deaths each year (*Gianella*, 1993). It is certainly not a problem of developing countries only. Even in industrialised societies the yearly incidence of food-borne intestinal infections is about 10% of the population. Bacterial pathogens (35%) are the leading cause of food-borne disease outbreaks of known aetiology, followed by toxins, viruses, and parasitic organisms other than bacteria and viruses. In the Western world, the bacterial pathogen most frequently isolated from patients suffering from acute gastro-enteritis is *Campylobacter* spp. (50%), followed by salmonella spp. (25%) (*Altekruse* and *Swerdlow*, 1996; *Lacey*, 1993). Moreover, infections caused by these two pathogens have emerged importantly in the last two decades (*Altekruse* and *Swerdlow*, 1996).

While most intestinal infections result in a self-limiting gastro-enteritis, severe complications like gut-derived septicaemia regularly occur in immuno-compromised people, patients suffering from inflammatory bowel diseases, and patients in intensive care units on (par)enteral nutrition regimen (*Brooks* et al., 1993; *Deitch*, 1994). Treatment of many food-borne intestinal infections is often discouraging, since it hardly ameliorates the severity of illness and often prolongs asymptomatic carriage (*Tenover* and *Hughes*, 1996). Another concern is the growing resistance of bacterial pathogens, including Salmonella species to clinically important antibiotics (*Lee* et al., 1994; *Osterholm*, 2000). The high frequency of antimicrobial resistance today is probably a consequence of the widespread use or the inappropriate therapy of infections in both humans and animals (*Osterholm*, 2000; *Fey* et al., 2000). This stresses the importance of prevention and to search for alternative approaches to cope with the problem of emerging infections. One attractive approach is to improve host resistance by modulation of the diet. Scientific interest in dietary modulation of host resistance to intestinal infections is just emerging. Notwithstanding the results of numerous *in vitro* studies, strictly controlled infection studies showing the importance of the diet (e.g. supplemented with pre- or probiotics) to inhibit or ameliorate intestinal infections *in vivo* are scarce. Without the pretension of being complete, the following review highlights and discusses relevant scientific literature on dietary modulation of the primary (non-immunological) resistance to intestinal infections and combines it with results obtained from infection studies performed by our lab.

NON-IMMUNOLOGICAL HOST DEFENCES OF THE GASTRO-INTESTINAL TRACT

Of course, the most effective way of protecting the human body from pathogenic bacteria is to prevent contact with the bacterium in the first place. As that is a utopia in daily practice, we have to rely on our defences in the gastro-intestinal tract to get rid of swallowed disease-causing bacteria. Especially during the first encounter with a pathogen, the non-immunological defences are very important for host resistance to intestinal infections. The low pH of gastric

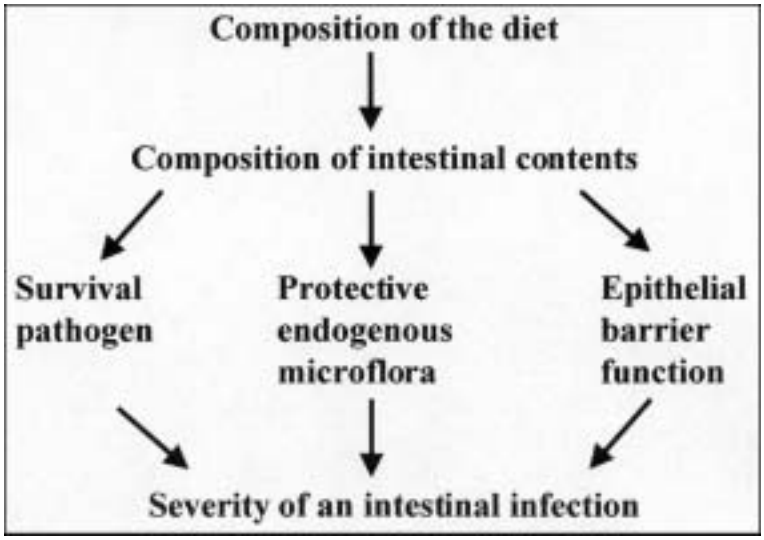

Figure 1: Dietary modulation of non-immunological host defences of the intestinal tract, which determines the severity of an intestinal infection.

juice has strong sterilising properties, because many food pathogens are acid-sensitive (*Gorden* and *Small*, 1993). Hypochlorhydria, in otherwise healthy elderly or in users of H_2 receptor antagonists and proton pump inhibitors, leads to increased intragastric bacterial counts and susceptibility to gastro-intestinal infections (*Duncan* and *Edberg*, 1995; *Gianella* et al., 1973), including salmonella (*Neal* et al., 1994). Buffering of gastric content and physical protection of bacteria by food, in combination with the rate of gastric emptying, are additional factors influencing the amount of pathogens surviving the gastric barrier (*Gianella* et al., 1973; *Sarker* and *Gyr*, 1992). The small intestine is protected to infective bacteria by a thick mucus layer covering the epithelium. Mucus not only acts as a lubricant to protect the delicate epithelial cells from gastric acid, bile acids and physical damage, but is also a trap for microbes to prevent their attachment to the mucosa. The mucus layer obtains its viscosity from mucins. The sugar chains of these glycoproteins mimic epithelial receptors for bacteria and act like a physical sieve trapping microbes and bringing them in close contact with secreted antibodies (sIgA), lactoferrin, lysozyme, and lactoperoxidase (*Duncan* and *Edberg*, 1995). The small intestine has a relatively high motility, which prevents adhesion to the mucosal epithelium and subsequently bacterial overgrowth (*Sarker* and *Gyr*, 1992). Duodenal secretions, such as bile and pancreatic enzymes, have potent bactericidal activity (*Rubinstein* et al., 1985; *Williams* et al., 1975). As a result of the combination of gastric acid, bile salts, and rapid flow of contents, the small intestine is relatively sparsely populated by bacteria. Though not very numerous, the endogenous microflora colonising the ileal mucosa might still be important in host defence, considering that many food-borne pathogens mainly elaborate their noxious effects in this region of the gastro-intestinal tract (*Salyers* and *Whitt*, 1994). In contrast to the small intestine, the most important defence mechanism of the colon is the presence of its luxuriant microflora (*Sarker* and

Gyr, 1992). Probably due to the slow transit of contents, the bacterial density is so great that nearly half of the volume of human colon contents is accounted for by bacteria (*Stephen* and *Cummings*, 1980). Potential invaders have to compete with this extensive established bacterial population for nutrients and adhesion sites on the epithelium. The production of antibacterial substances by the colonic microflora, such as lactic acid, short-chain fatty acids, and bacteriocins, may also inhibit growth of pathogenic bacteria (*Salyers* and *Whitt*, 1994). Animal (*Wells* et al., 1987) and human (*Bartlett*, 1992; *McFarland*, 1998) studies have shown that use of antibiotics, affecting the endogenous microflora, increases the susceptibility to contract intestinal infections. All the above mentioned non-immunological defence mechanisms cooperate with the gut-associated lymphoid tissue (GALT) in eliminating microbial pathogens (*Hazaoui* and *Pringault*, 1998).

DIETARY MODULATION OF THE RESISTANCE TO INFECTION

Diet directly affects the non-immunological host defences of the gastro-intestinal tract (Figure 1). It is obvious that the composition of the diet, especially the amount of non-absorbed nutrients, determines the composition of gastro-intestinal contents. The latter affects the survival of pathogenic bacteria in the gastro-intestinal tract. In addition, intestinal contents may influence the composition and the activity of the protective endogenous microflora and subsequently their antagonistic activity towards pathogens. Besides an effect on bacteria, luminal contents also affect functioning of the intestinal epithelium and its barrier function against invading microbes. Diet also directly affects host defence. For instance, if the habitual diet is deficient in one or more essential nutrients, normal functioning of all cells, including these belonging to the immune system, is impaired (*Kelley* and *Bendich*, 1996). Currently, there is a keen scientific and commercial interest in foods containing specific ingredients that modulate the intestinal microflora and subsequently the resistance to infection. The majority of these ingredients can be divided in two classes, the so-called probiotics and prebiotics. Besides these pro- and prebiotics, other dietary components (like minerals) may influence the course of an intestinal infection as well. Considering that several reviews about probiotics and intestinal health were published recently (e.g. *Erickson* and *Hubbard*, 2000; *Rolfe*, 2000), the discussion below focuses on the effects of non-digestible carbohydrates and minerals (particularly calcium) on host defence against intestinal infections.

NON-DIGESTIBLE CARBOHYDRATES

Most of the predominant species of the intestinal microflora require a fermentable carbohydrate for growth. It is generally assumed that the carbon and energy needed to maintain bacterial mass are derived from host secretions or from dietary carbohydrates (or other components) that escape digestion in the small intestine. Mucins, extensively glycosylated proteins secreted by goblet cells, might be excellent endogenous bacterial growth substrates (*Salyers* and

Leedle, 1983). Dietary fibres (vegetable polysaccharides) and oligosaccharides are more or less fermented in the intestinal tract, depending on their sugar composition, type of glycosidic linkages, and degree of branching. Dietary fibres are essential for maintenance of intestinal mucosal integrity, considering that oral administration of fibre-free elemental liquid diets induces spontaneous translocation of the gut microflora (*Sedman* et al., 1994). The protective effect of fibres on the mucosal barrier function might be independent of their fermentability and mediated by stimulation of the release of trophic gut hormones, preventing mucosal atrophy (*Jenkins* and *Thompson*, 1994). By definition, prebiotics are fermented by the endogenous microflora in contrast to the non- or low-fermentable dietary fibres (e.g. cellulose). Selective stimulation of the growth or the activity of a limited number of resident bacteria (preferably lactobacilli and bifidobacteria) and improvement of the host health are often mentioned criteria a prebiotic should meet. Polysaccharides like inulin, pectin, and resistant starch, and oligosaccharides based on fructose and galactose are potential prebiotics (*Roberfroid*, 1993).

A characteristic all the above mentioned potential prebiotics share is that they are fermented by the intestinal microflora in the lower gut to lactic acid and short-chain (C_2-C_6) fatty acids, resulting in a decreased pH of luminal contents (*Roberfroid*, 1993). Consumption of prebiotics may change the intestinal microflora. For instance, dietary inulin and oligofructose increase human faecal bifidobacteria (*Gibson*, 1999). Whether the *in vivo* growth advantage of bifidobacteria is due to selective utilisation of these non-digestible sugars by these genera or due to their relative resistance to the organic acids formed is not established yet. The latter explanation seems more likely, considering that other genera (for instance *Bacteroides*) can also degrade oligofructose at least *in vitro* (*Roberfroid*, 1993). Indeed, a recent study from our lab showed that oligofructose has a general growth-stimulating effect because significantly higher numbers of lactobacilli as well as *Enterobacteriaceae* were detected in faeces of rats fed these rapidly fermentable oligosaccharides (*Bovee-Oudenhoven* et al., submitted for publication).

It is frequently assumed that stimulation of intestinal lactobacilli or bifidobacteria (e.g. *Gibson*, 1999) is beneficial to the host and will result in an improved resistance to intestinal infections. This is mostly based on results of *in vitro* experiments showing that these carbohydrate-fermenting bacteria produce bactericidal organic acids and possibly other inhibitory compounds (hydrogen peroxide and bacteriocins) which suppress growth of pathogens like salmonella, *Escherichia coli* (*Gorden* and *Small*, 1993), and *Clostridium difficile* (*May* et al., 1994). However, stimulation of intestinal lactic acid bacteria or inducing a change in gut flora composition as such, is not directly a functional effect or a direct health advantage. In our opinion, evidence to establish the protective effect of prebiotics against intestinal infections can only be obtained from strictly controlled infection studies *in vivo*. Surprisingly, studies showing the efficacy of fermentable dietary fibres to reduce the severity of intestinal infections are rare. Lactulose therapy has been investigated in the management of salmonella and Shigella infections in humans. This non-digestible disaccharide increased faecal clearance of these pathogens in a significant proportion of the patients (*Hanssen* et al., 1981; *Liao* et al., 1994). Notwithstanding the beneficial effect of dietary fibre on intestinal

physiology, restraint should be applied in increasing the intake of non-digestible and rapidly fermentable oligo- or polysaccharides. High concentrations of short-chain fatty acids may damage the intestinal epithelium, resulting in an increased permeability and epithelial cell proliferation (*Argenzio* and *Meuten*, 1991; *Rémésy* et al., 1993; *Wasan* and *Goodlad*, 1996). In addition, a recent infection study of our lab actually showed that lactulose and oligofructose stimulated killing of *Salmonella enteritidis* in the intestinal lumen but concomitantly severely reduced the resistance of rats to translocation of this invasive pathogen, which was likely due to significant impairment of the mucosal barrier (*Bovee-Oudenhoven* et al., submitted for publication). The amount of lactulose and oligofructose added to the diets was realistic for human intake of highly fermentable dietary fibres (*Alles* et al., 1999). The mechanism of this unexpected detrimental effect of these oligosaccharides is subject of current research. Another observation of this infection study was that dietary oligofructose, but especially lactulose, greatly increased the number of faecal lactobacilli (> 100 times) when compared with supplemental cellulose. So, in contrast to often made claims, stimulation of intestinal lactobacilli is absolutely no guarantee that the resistance to intestinal infections will be improved.

DIETARY MINERALS

Very little is known about the effects of dietary minerals on the course of an infection. In addition to our studies on the protective effect of dietary calcium against Gram-negative bacterial infections, only data about the role of iron and zinc are reported in literature. Individuals with iron-overload, whether induced by excess dietary iron intake or due to diseases like β-thalassaemia major and sickle cell disease, are more susceptible to infection (*Walter* et al., 1997). Iron is essential for bacterial growth and may increase oxidative damage to host membranes or DNA during inflammation, as it is a catalyst in the production of hydroxyl radicals (*Shenkin*, 1995). The observed drop in plasma iron levels during infectious diseases, the so-called anaemia of infection, is a protective response of the body to combat the infection and to limit oxidative damage (*Beisel*, 1977). On the other hand, there is also evidence that severe iron deficiency is associated with an increased incidence of infections (*Walter* et al., 1997). Iron deficiency compromises host resistance by suppressing the cellular immune response (*Omara* and *Blakley*, 1994).

Zinc is a cofactor of about 120 mammalian enzymes. Zinc deficiency has a pronounced effect on nucleic acid metabolism, thus influencing protein synthesis and cell growth. Furthermore, an inadequate zinc intake is associated with an impaired immune function (*Mocchegiani* et al., 2000). In zinc deficiency, the organism is more susceptible to toxin-producing bacteria or enteroviral pathogens that activate guanylate and adenylate cyclases, which stimulate chloride secretion into the intestinal lumen. The resulting diarrhoea and diminished absorption of nutrients exacerbate an already compromised mineral status (*Wapnir*, 2000). Zinc supplementation can reduce the incidence and prevalence of acute infectious diarrhoea (*Black*, 1998). Besides restoration of immune cell functioning, normalisation of intestinal epithelial cell proliferation and strengthening of the mucosal barrier might be responsible

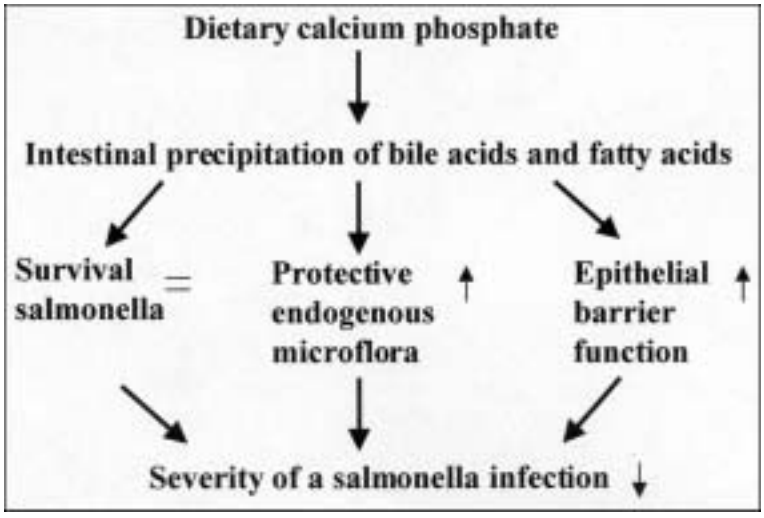

Figure 2: Mechanism by which dietary calcium phosphate may decrease the severity of an intestinal Salmonella infection.

for the observed beneficial effects of zinc supplementation (*Alam* et al., 1994; *Black*, 1998).

Until recently, the effects of calcium on the resistance to intestinal infections were unknown. At the same time, evidence accumulated showing the protective effects of dietary calcium on the development of colorectal cancer (*Baron* et al., 1999; *van der Meer* et al., 1997). A substantial amount of dietary calcium reaches the lower gut because the intestinal absorption of calcium is limited. After passing the stomach calcium forms an insoluble complex with dietary phosphate, which strongly adsorbs and precipitates intestinal surfactants, like bile acids and fatty acids. Bile acids and fatty acids are damaging to the intestinal epithelium and stimulate epithelial cell proliferation, which may increase the risk of colon cancer (*Govers* et al., 1996; *van der Meer* et al., 1997). We speculated that these cytoprotective effects of dietary calcium on the intestinal epithelium are not only relevant tot colon carcinogenesis but also have major implications for the resistance to intestinal infections. Diminishing epithelionlysis, by decreasing the cytotoxicity of gut contents, might strengthen the mucosal barrier. Conversely, increased epithelial cell damage provoked by dietary lectins or chemical irritants leads to disruption of the physical barrier function of the intestinal mucosa and gut-derived septicaemia, as shown by others (*Gardiner* et al., 1993; *Shoda* et al., 1995). By precipitating and thus inactivating intestinal bile acids, calcium may also indirectly affect the intestinal microflora. Bile is a classical supplement used in the preparation of several selective microbiological culture media to suppress contaminating flora. This indicates that bacterial species differ in their sensitivity to bile acids. Additionally, the restraining effect of bile in general on the intestinal microflora can be deduced from *in vivo* studies on obstructive jaundice. A reduced or absent bile flow results in intestinal bacterial overgrowth and an increased risk for endotoxaemia and septic complications. Oral administration of bile acids can normalise intestinal bacterial numbers and alleviate some of the symptoms in such cases (*Erbil* et al., 1999).

DIETARY CALCIUM AND RESISTANCE TO SALMONELLA INFECTION

We performed several animal studies to address whether dietary calcium indeed protects against intestinal infections. The proposed mechanism responsible for this protective effect is shown in Figure 2. *Salmonella enteritidis* was chosen as infective agent, because non-typhoidal salmonellosis is one of the most common, food-borne, bacterial infections in Europe and the United States (*Rampling*, 1993). Equally important, the infective dose and the development and pathology of salmonellosis in humans and rodents is largely similar and well described (*Salyers* and *Whitt*, 1994). Because *S. enteritidis* has invasive properties, dietary modulation of the resistance to colonisation as well as to translocation can be studied. Before performing dietary intervention studies, we first developed a new method to quantify intestinal bacterial translocation because classical organ cultures suffer from some major drawbacks. Bacteriological determination of pathogens in tissue samples (e.g. mesenteric lymph nodes) is invasive, only applicable as a one-point measurement, and rather insensitive considering that more than 99% of the bacteria is rapidly killed by the immune system upon translocation (*Gianotti* et al., 1993). Daily nitric oxide-derived urinary NO_x (sum of nitrite and nitrate) excretion appeared to be a sensitive marker to quantify intestinal bacterial translocation. Total translocation-induced urinary NO_x excretion highly correlated with weight of the mesenteric lymph nodes and its viable bacterial content (*Oudenhoven* et al., 1994). Moreover, determination of urinary NO_x might also be applicable to monitor bacterial sepsis in humans (*Krafte-Jacobs* et al., 1997).

After validation of this new translocation marker, strictly controlled infection experiments were performed to study whether dietary calcium protects against salmonella infection. Specific pathogen-free rats (n=8) were fed purified 'humanised' diets differing only in calcium phosphate content (20 and 180 µmol/g). After adaptation to the diets, they received a single oral infection with *S. enteritidis* (10^8-10^9 CFU) to mimic a food-borne intestinal infection. Calcium supplementation reduced the soluble bile acid and fatty acid concentration in ileal lavages and in faecal water, resulting in concomitantly decreased luminal cytotoxicity, as measured with a bioassay. This coincided with significantly higher numbers of lactobacilli in the intestinal tract: in the intestinal lumen as well as adhering to the intestinal mucosa (*Bovee-Oudenhoven* et al., 1999). Probably due to the absence of an extra outer-membrane, the Gram-positive lactobacilli appeared to be very sensitive to the bactericidal activity of bile acids and fatty acids, as shown in additional *in vitro* experiments. In contrast, the viability of a Gram-negative pathogen, like *S. enteritidis* was totally unaffected by physiologically relevant concentrations of these surfactants (*Bovee-Oudenhoven* et al., 1999). Compared with the low-calcium control group, the calcium-supplemented group shedded 10-1000 times less salmonella in their faeces indicating a significantly decreased intestinal colonisation of this pathogen (Figure 3). Even more important, the translocation of *S. enteritidis* was also significantly inhibited, considering the reduced urinary NO_x excretion in time (Figure 3). As shown in another study, the decreased infection-induced urinary NO_x excretion in

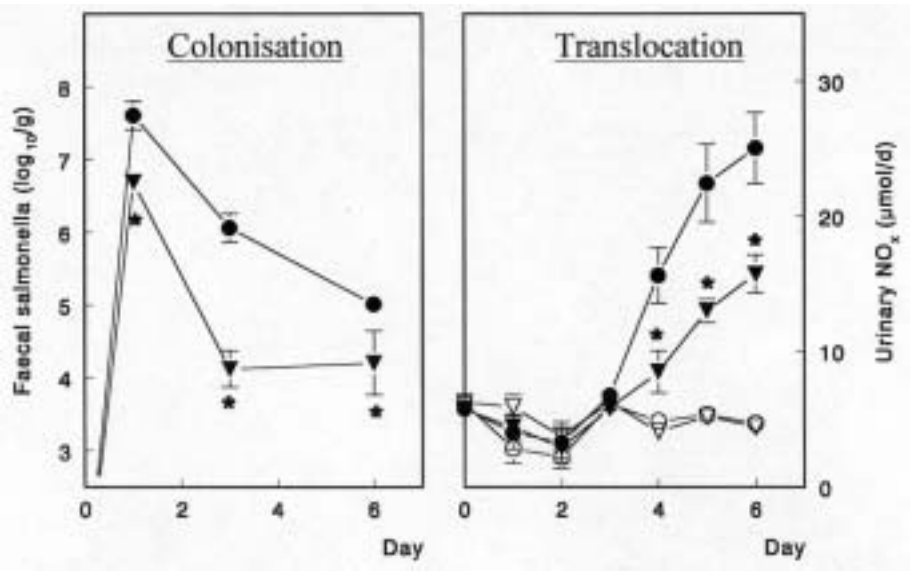

Figure 3: Effect of dietary calcium phosphate (CaP_i) on the colonisation and translocation of *Salmonella enteritidis* after oral infection of the rats (n=8) on day 0. The colonisation of salmonella was determined by measuring the faecal excretion of this pathogen in time and the translocation to extra-intestinal organs was quantified by analysis of urinary NO_x excretion. Symbols: ✿ infected control group, ✿ infected CaP_i-supplemented group, and the corresponding open symbols represent the non-infected groups. No salmonella was detected in faeces of non-infected animals. The asterisk indicates a significant difference from the control group ($p<0.05$).
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the calcium-supplemented groups correlated highly with the significantly less viable numbers of salmonella in ileal Peyer's patches and spleen (*Bovee-Oudenhoven* et al., 1997). The protective effect of calcium against salmonella infection was not only observed during administration of high amounts of calcium as usual in standard rodent diets (*Reeves* et al., 1993), but also during supplementation of 60 µmol calcium per g diet, which is more realistic for the human calcium intake in Western societies (*Becker* and *Kumpulainen*, 1991). Whether these beneficial effects of dietary calcium can also be extrapolated to humans is subject of current research of our lab.

CONCLUSION

Due to emerging problems of antibiotic resistance the need for alternatives to prevent and treat intestinal infections is growing. Though sound scientific evidence is still not very abundant, several *in vivo* experiments now show that the resistance to intestinal infections can be improved by changing the composition of the diet. Combined biochemical and microbiological *in vitro* experiments are very useful to elucidate the mechanisms responsible for protection. However, animal and finally human infection studies are essential to verify whether dietary components (including probiotics) indeed strengthen host resistance and protect against infectious disease.

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