

Phytobiotics: alternatives to antibiotic growth promoters in monogastric animal feeds

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Summary

Phytobiotics represent a wide range of bioactive compounds that can be extracted from various plant sources. In recent years, some interesting and novel applications of phytobiotics in the production and well-being of monogastric animals have emerged, and these aspects are reviewed in the present paper. Recent studies at the University of New England indicate that water-soluble carbohydrate extracts from some Australian and New Zealand native plants can be used as potential phytobiotic compounds to modify the gut microflora in broiler chickens. Compared with other in-feed antibiotic alternatives, the evaluation of phytobiotics is still in its infancy and their potential use needs to be investigated with broader emphasis.

Keywords: phytobiotics, plant extracts, antibiotic, gut microflora, monogastric animals

Introduction

A variety of synthetic feed additives including drugs and antibiotics are used in poultry feeds to maximize the efficiency of production, product quality and to control diseases (Bedford 2000; Whitehead 2002). Although the modes of action of antibiotic growth promoters are not fully understood, the main effects are thought to be mediated via the gut associated bacteria (Gaskins *et al.* 2002). The poultry and pig industries are currently moving towards a reduction in use of synthetic antibiotics due to public concerns regarding development of antibiotic-resistant bacteria in humans (Barton 1998). In the European Union, the use of antibiotic growth promoters as feed additives is now restricted. This restriction, however, has resulted in an increased incidence of enteric disorders such as necrotic enteritis in poultry. Countries such as Australia and the USA are following the European example by either regulating the use of antibiotics in feeds or setting up programs to reduce their overall use. In this context, there has been increased interest in the use of biological products, including enzymes, probiotics, prebiotics,

synbiotics, organic acids and plant extracts (phytobiotics), as alternatives to antibiotic feed additives in diets for monogastric animals (Bedford 2000; Wenk 2003). The aim of this review is to highlight the research findings from various groups working in the area of phytobiotics and to present some findings from our on-going studies.

Phytobiotics and possible modes of action

Phytobiotics are plant-derived natural bioactive compounds that can be incorporated into diets in order to enhance the performance and well-being of animals. Phytobiotics represent a wide range of plant-derived compounds. Wenk (2003) reported that herbs, spices and their extracts can stimulate appetite and endogenous secretions such as enzymes or have antimicrobial, coccidiostatic or anthelmintic activities in monogastric animals. Oligosaccharides and polysaccharides such as inulin (fructan), fructo-oligosaccharides (FOS) and arabinogalactans, which are extracted from plants, and sulfated fucans, which are extracted from seaweeds, are potential substitutes for currently used antibiotic growth promoting compounds (Tringali 1997; Iji and Tivey 1998).

Direct antimicrobial effects

Some bioactive substances from plants, like most antimicrobial agents, exert their effects by modulating the cellular membrane of microbes (Kamel 2000). *In vitro* studies of Kamel (2001) indicate that the minimum inhibitory concentration (MIC₅₀) and minimum bactericidal concentration (MBC₅₀) are linked to the level of active substance and purity of the plant extract. Furthermore, a strong increase in hydrophobicity of the microbial species in the presence of some plant extracts may influence the surface characteristics of microbial cells and thereby affect the virulence properties of the microbes (Kamel 2001).

This may be an important antimicrobial mechanism of some plant extracts. This concept may have implications for the gut, in which adhesion of microbes to intestinal mucosal cells is of vital importance for some pathogenic microflora and is strongly influenced by the hydrophobic surface properties of microbial cells (Pusztai *et al.* 1990; Kamel 2001). Various essential oil mixtures, which contain natural polyphenolic compounds or flavonoids as major active ingredients, have been identified as potential antimicrobial and antioxidant agents (Cruickshank 2001; Friedman *et al.* 2004). Thus, supplementation of broiler diets with essential oil mixtures can create a healthier gut microflora, aiding optimum digestion and improving bird performance (Cruickshank 2001).

Prebiotic effects

The effects of phytobiotics are often indirectly mediated by metabolites generated by gut microflora that use the bioactive compounds for their own metabolism (Kamel 2000). It has been observed that prebiotic oligosaccharides and some plant extracts can influence the growth of commensal gut microflora by facilitating a continuous supply of specific substrates for the protective intestinal flora or by minimizing the risk of development of populations in which opportunistic pathogens can thrive (Mul and Perry 1994; Lan *et al.* 2005). The protection of the gut environment is now known to play an important role in reducing disease in animals.

Several bioactive compounds from mushrooms and plants have been identified as compounds that differentially stimulate favourable bacteria such as lactobacilli and bifidobacteria without promoting the growth of pathogenic species (Jamroz *et al.* 2003a; Guo *et al.* 2004; Lan *et al.* 2004). Stimulation of these beneficial bacteria could contribute to a balanced gut microflora and may provide an optimal precondition for effective protection against pathogenic microorganisms and an intact immune system (Piva and Rossi 1998; Wenk 2003). This, in turn, may be associated with the reduction of gut-associated diseases

in chickens such as coccidiosis and necrotic enteritis. Cross *et al.* (2004) found that caecal counts of *Clostridium perfringens* were reduced when the broiler diets were supplemented with thyme and yarrow extracts. Similarly, Jamroz *et al.* (2003a) reported that dietary supplementation of plant extracts containing capsaicin (1.98 g/100g), carvacol (4.95 g/100g) and cinnamic aldehyde (2.97 g/100g) reduced *C. perfringens* and *E. coli* counts in colonic contents to the same degree as birds treated with the antibiotic, avilamycin. Guo *et al.* (2004) observed that dietary supplementation with mushroom- and herb polysaccharide extracts stimulated the growth of beneficial caecal bacteria such as bifidobacteria and lactobacilli in broiler chickens and reduced the number of potentially harmful bacteria such as *Bacteroides* spp. and *E. coli*. Recently, Lan *et al.* (2004) showed that dietary inclusion of oligosaccharides and water-soluble polysaccharides extracted from soyabean meal promoted the growth of lactic acid bacteria in the caeca. They suggested that this approach might be used as a method of competitive exclusion in the caeca of *E. tenella*-challenged broilers.

Prebiotic- and bioactive compounds are believed to exert their beneficial effects by production of short chain volatile fatty acids (SCVFAs) that inhibit the growth of a range of putrefactive proteolytic bacteria. It is known that the undissociated SCVFAs penetrate bacterial cell walls and disrupt the normal physiology of certain types of bacteria (Alakomi *et al.* 2000; Fons *et al.* 2000). A recent study indicated that acetate, isovalerate and succinate decrease the sporulation of *C. perfringens*, and isobutyrate decreases vegetative cell numbers as well as sporulation of this species (Wrigley 2004). In addition, Roe *et al.* (1998) reported that the accumulation of dissociated anions (A^-) within cells can cause internal osmotic problems and toxicity in bacteria. Supplementation of the diets of chickens with butyric acid impregnated microbeads resulted in a decrease in colonization of salmonella in the caeca (van Immerseel *et al.* 2003). Furthermore, van der Wielen *et al.* (2002) reported that undissociated forms

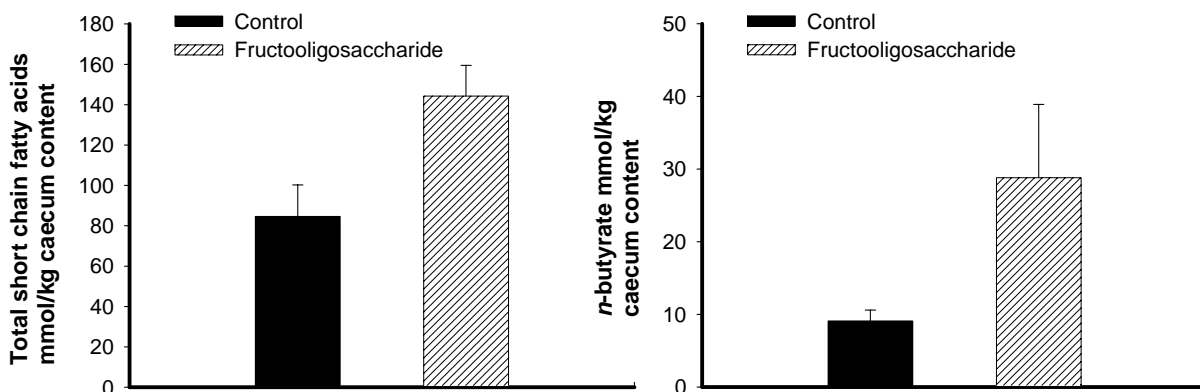


Figure 1 The effect of fructooligosaccharides on volatile organic fatty acid production in caecal content of pigs (adapted from Tsukahara *et al.* 2003).

of acetate and propionate produced in the mixed culture of *Lactobacillus crispatus* and *Clostridium lactifermentans* isolated from chicken caecal contents inhibited the *in vitro* growth of *Salmonella enterica* serovar Enteritidis. Certain anaerobic bacteria in the colon and caeca convert various prebiotic compounds to SCVFAs that have a trophic effect on the caecal and colonic epithelium (Sakata 1987; Flickinger *et al.* 2003). Butyrate is a preferred energy source for colonic mucosal cells (Hara 2002; Tsukahara *et al.* 2003).

According to Manzanilla *et al.* (2004), the volatile fatty acid profile in the caeca and colon was modified by dietary supplementation of a plant extract mixture (carvacrol, cinnamaldehyde and oleoresin) in early-weaned pigs. Furthermore, these authors observed decreased ileal total microbial mass and increased lactobacilli:enterobacteria ratio in the jejunal digesta of pigs fed a diet supplemented with plant extracts compared to that of unsupplemented controls. Recently, Cardozo *et al.* (2004) documented that cinnamon,

garlic, oregano and anise extracts modified the SCVFA production between two- and six days of fermentation in a dual-flow continuous-culture fermentation system. *In vitro* fermentation and animal studies indicate that inulin and oligofructose decrease colonic and caecal pH, and increase the size of the colonic and caecal pool of SCVFAs (Flickinger *et al.* 2003; Juskiewicz *et al.* 2004). Recently, Tsukahara *et al.* (2003) reported that adding FOS to diets of weaning piglets causes a rapid increase in the concentration of total SCFAs and *n*-butyrate in caecal contents (Figure 1). Certain plant extracts can act as acid-sparing agents against the highly basic components in animal diets that can cause poor feed digestibility and potential overgrowth of pathogenic bacteria such as *E. coli* (Kamel 2001). Moreover, a reduction in pH of the gut contents may stimulate the production of gut-associated protective agents such as mucus (Cummings and Macfarlane 1991) and cause beneficial rather than harmful bacteria to dominate. Table 1 summarizes the

Table 1 Effects of plant-derived bioactive compounds on gut-associated microflora.

Prebiotic or phytobiotic compound	Animal	Evidence of prebiotic or phytobiotic effects	Reference
Thyme and yarrow extracts	Broiler chickens	Decrease in <i>C. perfringens</i> in caecal content	Cross <i>et al.</i> (2004)
Capsaicin, carvacol and cinnamic aldehyde mixture	Broiler chickens	Decrease in <i>C. perfringens</i> and <i>E. coli</i> in rectal content	Jamroz <i>et al.</i> (2003a)
Mushroom extracts	Broiler chickens	Increase in bifidobacteria and lactobacilli and decrease in <i>E. coli</i> in caecal content	Guo <i>et al.</i> (2004)
Carvacrol, cinnamaldehyde and capsicum oleoresin mixture	Weaner pigs	Increased lactobacilli:enterobacteria ratio in jejunal digesta	Manzanilla <i>et al.</i> (2004)
Oligosaccharides and water-soluble polysaccharides extracted from soyabean meal	Broiler chickens	Increase in lactic acid bacteria and decrease in <i>E. tenella</i> in caecal content	Lan <i>et al.</i> (2004)
Carbohydrate-based cocktails	Broiler chickens	Decrease in <i>Salmonella typhimurium</i> and campylobacter in crop content	Hinton <i>et al.</i> (2002)
Chicory fructans	Broiler chickens	Increase in lactobacilli in gizzard and small intestine; decrease in <i>E. coli</i> and campylobacter in large intestine	Yusrizal and Chen (2003b)
Jerusalem artichoke fructans	Broiler chickens	Decrease in <i>C. perfringens</i> in caeca	Kleessen <i>et al.</i> (2003)
Fructooligosaccharides	Broiler chickens	Increase in bifidobacteria and lactobacilli and decrease in <i>E. coli</i> in ileal and caecal content	Xu <i>et al.</i> (2003)
Larch arabinogalactans	Dogs	Increase in bifidobacteria and lactobacilli and decrease in <i>C. perfringens</i> in fecal matter	Grieshop <i>et al.</i> (2002)
Larch arabinogalactans	Humans	Increase in lactobacilli in stool	Robinson <i>et al.</i> (2001)
Acacia arabinogalactans	Humans	Increase in faecal lactic acid bacteria, lactobacilli and bifidobacteria	Cherbut <i>et al.</i> (2003)

effects of various plant-derived bioactive compounds on gut-associated microflora.

Competitive blocking of bacterial adhesion

Lectin-carbohydrate receptor interactions are the main mechanism in adhesion of pathogens to the brush border of the gut mucosal epithelium. Many prebiotic and phytogetic bioactive substances can have a direct effect on certain pathogenic bacteria either by specific adhesion of pathogens through the 'lectin-receptor' mechanism (agglutination) by blocking the adhesion of pathogens onto the mucosal layer of the intestine (Pusztai *et al.* 1990). One such group of bioactive compound that has been studied extensively is the phosphorylated mannanoligosaccharides (MOS) from yeast cell walls (Spring *et al.* 2000; Fernandez *et al.* 2002). Dietary MOS have been shown to decrease the prevalence of strains of salmonella expressing type-1 fimbriae in young chicks under laboratory conditions (Spring *et al.* 2000). Recently, Piva and Rossi (1998) postulated that compounds such as oligomannans and lectins could bind to the enterocyte receptors that are present on the cell walls of pathogenic bacteria, thus preventing them from colonizing the gut. Moreover, Bengmark (1998) suggested that certain prebiotic compounds such as pectin, guar gum and oat gum, which have a protective function in the mucosal layer of the intestine, act by preventing the colonization of pathogenic bacteria.

Immunostimulatory effects

It is also known that certain plant polysaccharides act as immunostimulatory (adjuvant effect) substances (Paulsen 2002; Chen *et al.* 2003; Guo *et al.* 2003). The gut-associated lymphoid tissue (GALT) plays a key role in immunomodulation in farm animals. Recent data from several animal models clearly indicate that prebiotic compounds can exert beneficial effects on gut health by enhancing GALT responses directly (systemic and local immunological effects) or indirectly by the mediation of short chain fatty acids

such as butyrate and lactic acid producing bacteria (Kaminogawa 2002; Hosono *et al.* 2003). A recent review by Guo *et al.* (2003) suggests that polysaccharides obtained from two mushroom species; *Lentinus endodes* and *Tremella fuciformis*, and a herb, *Astragalus membranaceus radix*, may be used as immune enhancers or modulators in chickens to activate both innate- and adaptive-, or specific immunity including the cellular or humoral immunity. Hoshi *et al.* (1999) observed that saponins extracted from *Quillaja* (*Quillaja saponaria*) are a useful mucosal adjuvant in chickens following oral immunization with a non-replicating antigen. Moreover, Chen *et al.* (2003) found that the low-molecular weight Chinese herbal polysaccharide, achyranthan, was more effective than the high molecular weight polysaccharide, astragalin, in enhancing the immunity of broiler chickens. Findings of Hosono *et al.* (2003) suggest that dietary supplementation of fructooligosaccharides changes the intestinal environment of microflora, and leads to up-regulation of immunoglobulin A (IgA) secretion in murine Peyer's patch cells in the intestinal mucosa. These results were confirmed by Roller *et al.* (2004), who showed that supplementation with prebiotic inulin enriched with oligofructose enhances the production of interleukin-10 in Peyer's patches as well as the production of secretory immunoglobulin A (sIgA) in the caeca of rats (Figure 2). In a similar study, Kelly-Quagliana *et al.* (2003) indicated that dietary inclusion of oligofructose and inulin up-regulates macrophage-dependent immune responses in a dose-dependent manner in rats. Verstegen and Williams (2002) pointed out that in order to exert these immunomodulating properties, non-digestible oligosaccharides and polysaccharides must to act before fermentation and/or escape fermentation in the digestive tracts of monogastric animals.

Stimulation of digestive enzymes

Another possible mode of action of phytogetic bioactive compounds on growth performance of farm animals could be their effects on the activities of

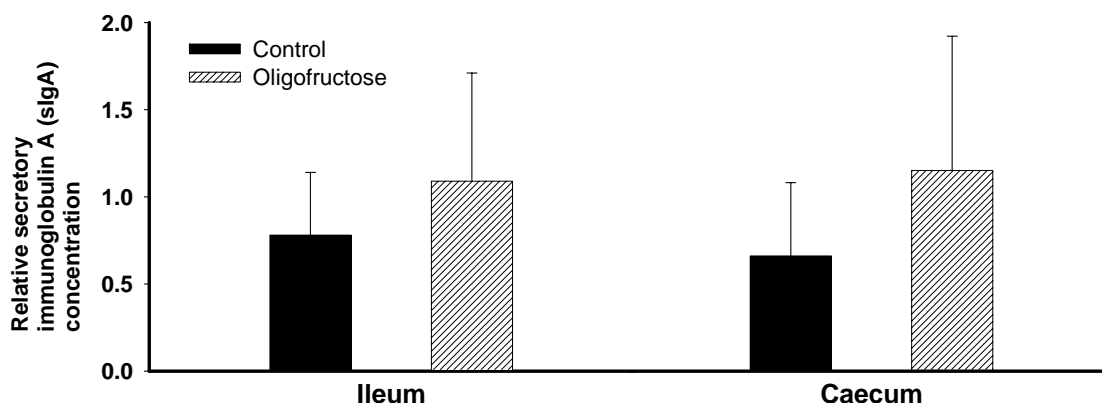


Figure 2 Immuno-enhancing effect of dietary oligofructose in rats (adapted from Roller *et al.* 2004).

digestive enzymes. Xu *et al.* (2003) reported that dietary supplementation of fructooligosaccharides improved daily body weight gain of male broiler chickens by increasing the activities of amylase and protease. Furthermore, a study with broiler chickens indicated that feeding a diet containing a commercial blend of essential oils (CRINA[®]) in combination with lactic acid induced a significant increase in activities of digestive enzymes of the pancreas and intestinal mucosa of birds, leading to a significant increase in growth (Jang *et al.* 2004). Although Lee *et al.* (2003) observed significantly higher amylase activity in the intestinal digesta of broiler chickens fed the same commercial essential oil mixture, they did not observe any improvement in growth.

Phytobiotics as alternatives to in-feed antibiotic growth promoters

Plant and herbal extracts

The use of plants and plant bioactive compounds dates back thousands of years to the ancient Egyptians, Chinese, Indians and Greeks (Gill 1999; Kamel 2000). Chinese herbal medicines have been used for many centuries for treating various human and animal diseases (Li 2000). In recent years, there has been an increased awareness of the potential that natural plant compounds have in the prevention and treatment of poultry diseases (Chen *et al.* 2003; Guo *et al.* 2003; Cross *et al.* 2004; Guo *et al.* 2004).

Hernández *et al.* (2004) observed that supplementation of 200 ppm essential oil extract from oregano, cinnamon and pepper and 5,000 ppm Labiate extract from sage, thyme and rosemary improved the apparent faecal digestibility of dry matter and crude protein in broiler finisher diets. However, Lee *et al.* (2003) did not observe any positive effects on growth performance or macronutrient digestibility in female broiler chickens when diets were supplemented with thymol, cinnamaldehyde or CRINA[®]. In contrast, in a similar study, Williams and Losa (2002) found that feeding CRINA[®] to broilers significantly reduced the concentration of pathogenic microorganisms in the ileum, caecum and colon, which was accompanied by an increased weight gain of birds.

A soluble solution of twelve plant extracts has been shown to improve broiler performance and reduce ascites and necrotic enteritis (Cruickshank 2001). An osmoprotectant extracted from sugar beet, betaine, can offset the negative effects of heat stress in poultry and improve feed efficiency by at least 6.0% (Remus 2002). A recent study by Youn and Noah (2001) has tested extracts from 15 Asian herbs for their anticoccidial activity against *E. tenella* and found that of the species tested, an extract from *Sophora flavescens* Aiton was the most effective in reducing lesion scores and oocyst

production without affecting the growth performance of birds. In a similar study, Du and Hu (2004) found that a Chinese herbal complex can effectively control avian coccidiosis caused by *E. tenella*. According to Gill (1999), oregano essential oil also has anticoccidial activity against *E. tenella*, *E. acervulina*, *E. necatrix*, *E. mivati* and *E. bruneti*.

Ilseley *et al.* (2003) found that the use of a combination of plant extract additives (capsicum, carvacrol and cinnamylaldehyde) in the diets of lactating sows improved piglet performance prior to weaning. In a more recent study (Ilseley *et al.* 2005), the same group observed that supplementation of weanling pig diets with Quillaja (*Quillaja saponaria*) saponins enhanced the immune function of pigs managed under normal commercial rearing situations in the absence of any controlled pathogen challenge. However, in this study, they did not observe any beneficial effect of supplementing weanling pig diets with curcumin (*Curcuma longa*) extract.

Plant oligosaccharides and polysaccharides

Many plant extracts contain carbohydrates, mostly heteroglycans composed of hexoses and pentoses and methylated uronic acids (Delzenne and Roberfroid 1994). Recent studies have shown that various oligosaccharides and polysaccharides act as phytobiotics or prebiotics in poultry feed and exert numerous growth-promoting effects (Xu *et al.* 2003; Lan *et al.* 2004). Prebiotic oligosaccharides are obtained by one of three processes: direct extraction of natural oligosaccharides from plants, controlled hydrolysis of natural plant polysaccharides and enzymatic synthesis, or by using hydrolases and/or glycosyl transferases (Grizard and Barthomeuf 1999).

Inulin and fructooligosaccharides

Fructans are polymers of polyfructosylated sucrose (Figure 3). The majority of their polymeric structures are based on three trisaccharides (1-kestotriose, 6_G-kestotriose and 6-kestotriose) and two branched tetrasaccharides (1,6-kestotetraose and 6,1-kestotetraose). Inulins and fructans, extracted primarily from chicory root (*Cichorium intybus*) and Jerusalem artichoke (*Helianthus tuberosus*) tubers, are homologues of the GF_n type (where G and F represent glucose and fructose, respectively) consisting of β-(2→1) fructosyl residues with an average degree of polymerization (DP) of 10. Partial enzymatic hydrolysis produces FOS, a mixture of small fructofuranose chains of the GF_n and F_m types with an average DP of 5 (Salminen *et al.* 1998). These compounds are not digested enzymatically in the proximal intestinal of monogastric animals, making them available for fermentation to SCVFAs and lactic acid by the intestinal bacteria of the distal parts of the intestine such as the colon and caeca (Flickinger *et al.* 2003). Indeed, low

molecular weight fructooligosaccharides (GF₂₋₄) exhibit a variety of health benefits by stimulating the growth of favourable bacteria in the gut (Kleessen et al. 2001; Biedrzycka and Bielecka 2004).

An *in vitro* study by Gibson and Wang (1994) showed that inulin and FOS are preferentially fermented by bifidobacteria, while populations of *E. coli* and *C. perfringens* are maintained at relatively low levels. In addition, the inclusion of FOS in diets fed to broilers inoculated with *Salmonella typhimurium* causes a reduction in intestinal colonization by salmonella and an improvement in daily gain and feed efficiency (Choi et al. 1994; Oyarzabal and Conner 1996). In a similar study, Fukata et al. (1999) observed that adding a low-level (0.1%) of FOS to the diets of layers receiving a competitive exclusion treatment resulted in reduced susceptibility to *Salmonella enteritidis* colonization in the caeca. Furthermore, Bailey et al. (1991) observed that inclusion of 0.7% (w/w) FOS in chicken diets led to a four-fold reduction in the counts of salmonella in the caeca. Supplementing broiler diets with chicory FOS resulted in improved body weight gain, feed conversion, carcass weight, and a high density of ileal villi (Yusrizal and Chen 2003a). Moreover, it increased the *Lactobacillus* counts in the gizzard and the small intestine, while reducing faecal *E. coli* and campylobacter counts in the large intestine (Yusrizal and Chen 2003b). In contrast, Juskiewicz

et al. (2004) did not observe any improvement in growth parameters due to supplementation of turkey diets with inulin.

Arabinogalactans

Arabinogalactans are found in many plants because they are essential for the strength and elasticity of plant cells (Paulsen 2002). Based on the structural differences, arabinogalactans are divided into two groups, namely, arabinogalactan type I and arabinogalactan type II. The major structural difference between these two types of arabinogalactans is that the galactose monomer units are (1→4) linked in type I, and (1→3) and (1→6) linked in type II (Paulsen 2002). High concentrations of arabinogalactans, with the latter of the two types having been found to exert most of the biological activity, have found in the Western Larch (*Larix occidentalis*), Mongolian Larch trees (*Larix dahurica*) and resins exuded from stems and branches of Acacia trees (Fitzpatrick et al. 2004; Meance 2004). Moreover, arabinogalactans and fucogalactoxylglucans are the major bioactive compounds of *Echinacea purpurea*, a plant that has a long tradition of use against common cold and influenza in humans (Wagner et al. 1988; Paulsen 2001). The arabinogalactan from Acacia trees has an average molecular weight of 300–800 kDa and

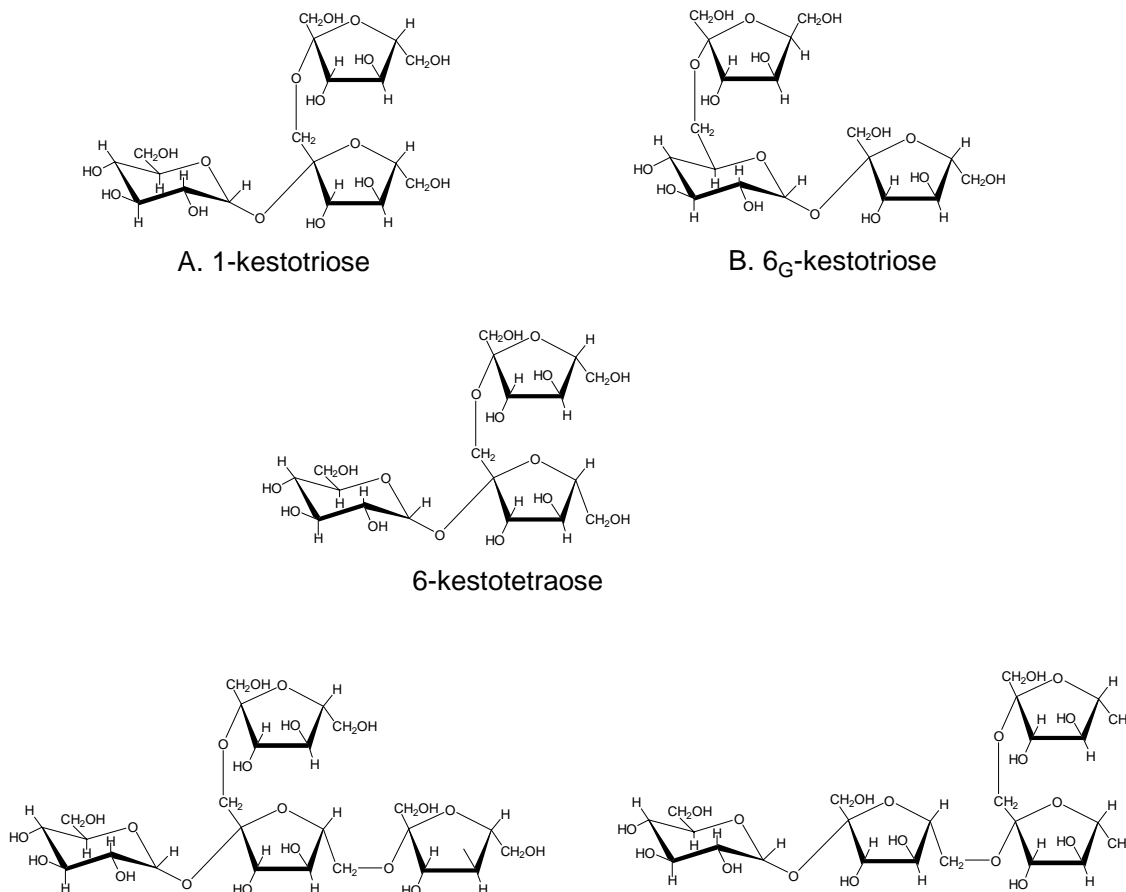


Figure 3 Structure of fructooligosaccharides (adapted from Sims 2003).

constitutes 95% of the dry weight of polysaccharides, which consist of highly-branched galactan polymers, with galactose and/or arabinose side chains and 1–5% of proteins depending on the species (Meance 2004) (Figure 4).

Recent studies indicate that arabinogalactans can be used as bioactive compounds for humans and companion animals. Larch arabinogalactans are actively fermented by the intestinal microflora, thereby increasing the number of beneficial anaerobic bacteria such as bifidobacteria and lactobacilli, while decreasing *Clostridium perfringens* in the faeces of dogs (Grieshop *et al.* 2002). Moreover, Robinson *et al.* (2001) reported that supplementation of an adult human healthy diet with 15 g/day or 30 g/day of Larch arabinogalactans resulted in increased total faecal anaerobic- and *Lactobacillus* populations. Similar observations had been previously reported by Michel *et al.* (1998), who showed a 6.75-fold increase in lactic acid bacteria counts and a 1.5–1.8 log reduction in *Clostridium* sp. counts within 24 h of inoculating chemostatic continuous-cultures of human faecal bacteria with two acacia gums (the arabinogalactans, Fibregum standard and Fibregum AS[®]). Furthermore, an *in vivo* study by Cherbut *et al.* (2003) demonstrated that supplementation with Acacia gum (Fibregum[®]) increases total lactic acid bacteria, and lactobacilli and bifidobacteria counts in stools of healthy adult humans without affecting total anaerobic and aerobic counts. Taguchi *et al.* (2004) postulated that the β -D-(1 \rightarrow 3)-galactan backbone of the arabinogalactans may play an important role in expression of intestinal immune modulating activity against Peyer's patch cells. Moreover, Wagner *et al.* (1988) observed that arabinogalactans from *Echinacea purpurea* specifically

stimulate macrophages to secrete tumour necrosis factor (TNF). According to Currier *et al.* (2003), a neutral arabinogalactan from Larch can act as an immunoenhancer not only on immune (lymphoid) cells, but also on natural killer (NK) lymphoid cells and a variety of other hemopoietic cells in the bone marrow and spleens of healthy, young-adult mice. To date, studies conducted with arabinogalactans have been mainly related to humans and companion animals.

Seaweed sulfated fucans (fucoidans)

Among the vast sources of plant materials that are available for extracting bioactive compounds, seaweeds (macroalgae) represent a rich and largely untapped source (Tringali 1997). Today, many countries, including Australia and New Zealand, are examining seaweeds more closely because they have a number of important uses. Southern Australia is one of the world regions richest in seaweeds; more than 1,000 species of macroalgae exist in the seas around Australia (Edgar 1997). Seaweeds are an abundant source of natural polysaccharides, many of which have commercial uses, particularly in the food, cosmetic and medical industries (Renn 1997). Sulphated polysaccharides are abundant in brown and red seaweeds (Paulsen 2002). The terms, fucan or fucoidan, are generally applied to sulphated polymers with a high fucose content or in instances in which there is a fucosyl backbone (Falshaw *et al.* 1999). The chemical structure of fucoidan from the brown seaweed, *Fucus vesiculosus*, is predominantly a (1 \rightarrow 2)-linked polymer of 4-sulphated fucopyranosyl residues with some (1 \rightarrow 3)- and (1 \rightarrow 4)-linkages (Figure 5).

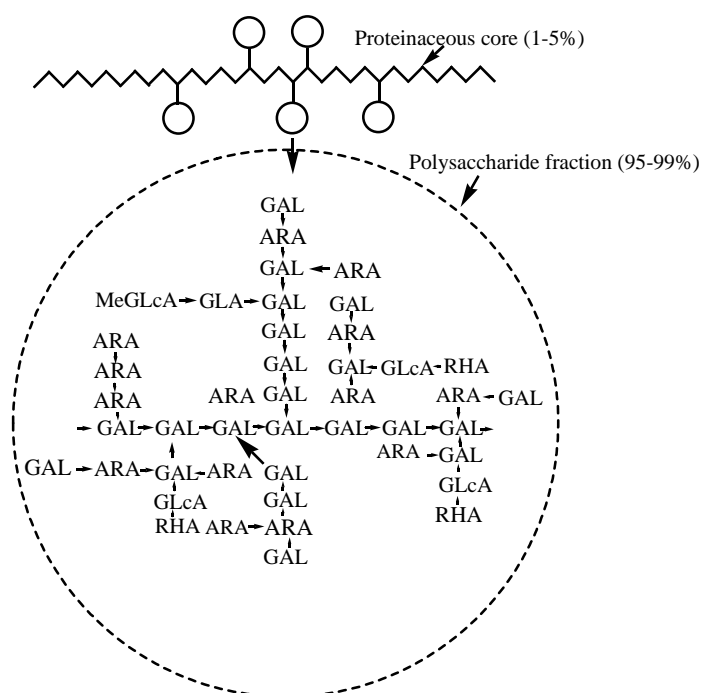


Figure 4 Schematic representation of the structure of acacia gum (adapted from Meance 2004).

The wide range of biological properties exerted by sulfated seaweed polysaccharides is due to their structural conformation, which is similar to that of endogenous sulfated glycosaminoglycans and other sulfated glucans found in mammalian cell membranes (Boisson-Vidal *et al.* 1995; Berteau and Mulloy 2003). Recently, antimicrobial activities of seaweed extracts have been reported (Vlachos *et al.* 1999; Kumar and Rengasamy 2000; Etahiri *et al.* 2001). The immunomodulatory effects of extracts of certain seaweeds are well documented and have been reported to include regulation of cytokine production and activation of macrophages in laboratory species such as rats and mice, but their influence on growth performance of farm animal species is less well documented. Qureshi *et al.* (1996) observed that humoral and cell-mediated immune functions were enhanced in White Leghorn chickens fed 10 000 ppm blue-green algae, *Spirulina platensis*. Moreover, Turner *et al.* (2002) found that the supplementation of weanling pig diets with a seaweed extract of *Ascophyllum nodosum* had minimal effects on growth performance and immune function; however, dietary supplementation with seaweed extracts did not alleviate the negative impact of an oral challenge of *Salmonella typhimurium*.

Studies on Australian and New Zealand native plants

In a recent study at the University of New England, we investigated the effects of water-soluble carbohydrate extracts from third-order branches of the Cabbage tree (*Cordyline australis*; *CorE*), exudates from the Golden wattle tree (*Acacia pycnantha*; *AcaE*) and a seaweed (*Undaria pinnatifida*; *UndE*) on the growth performance and gut microbial populations of broiler chickens. The three extracts were supplemented at

inclusion rates of 5 g/kg or 10 g/kg in starter and grower diets and compared with an antibiotic treatment group (45 mg/kg zinc-bacitracin; Zn-B) and a group of non-supplemented birds (NoS), which resulted in 8 treatment groups.

The analyses showed that *CorE* and *UndE* contained about one third (25–28%) of the water-soluble carbohydrates of *AcaE* (79%). Of the three extracts, *CorE* had the highest crude protein content (14.3%), about 4.5-fold that of *AcaE* and 7-fold that of *UndE*. Sugar composition analysis showed that *AcaE* contained mainly galactose and arabinose with a molar ratio of 7.8:2.2, and *UndE* contained fucose and galactose with a molar ratio of 5.5:4.4. Cabbage tree extract (*CorE*) contained mainly mannose/fructose and glucose/fructose with a molar ratio of 3.2:4.9. Moreover, *CorE* contained a high proportion of fructose and fructose-containing oligosaccharides. These sugars are highly acid labile and were destroyed by the hydrolysing conditions applied in these analyses. *C. australis* extract contains ~34% w/w fructans. The molecular weight of the seaweed fraction (5.11×10^5 Da) was similar to a value (6.8×10^5 Da) previously reported for high-molecular-weight fucans extracted from brown seaweeds (Patankar *et al.* 1993; Nishino *et al.* 1994). The molecular weight of cabbage tree extract was less than DP 10.

In general, no differences in body weight gain or feed intake were observed in broiler chickens fed diets supplemented with these plant extracts, except during the 28 d to 42 d period, when the *UndE* supplemented-group had higher ($P < 0.05$) feed intake than the ZnB, *CorE* or *AcaE* groups (Table 2). Throughout the experimental period, broilers fed diets containing the antibiotics grew faster ($P < 0.05$) and had lower ($P < 0.05$) feed:gain than those fed the un-supplemented or plant-extract diets. Over the period, d 7–21, FCR of birds fed the *AcaE*-supplemented diet was lower ($P < 0.05$) than that of the *CorE*-supplemented group. However,

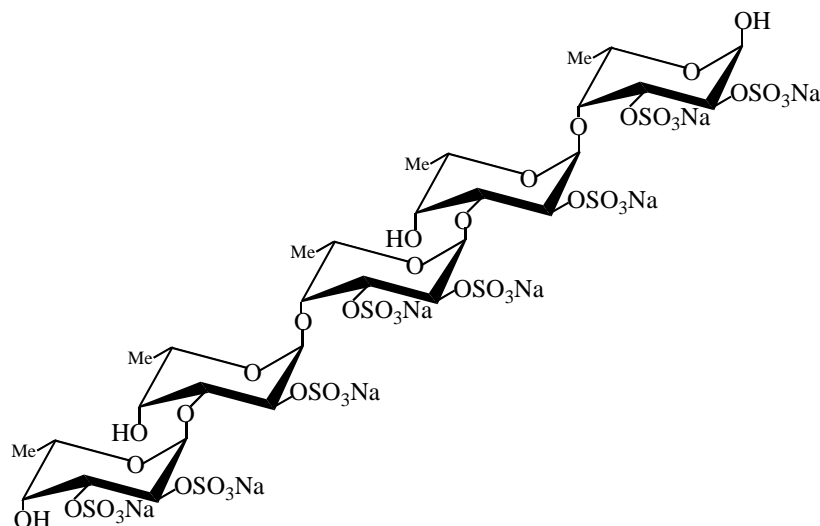


Figure 5 Structure of sulphated oligofucans extracted from brown algae, *Ascophyllum nodosum*, shown as sodium salt (adapted from Chevolut *et al.* 2001).

Table 2 Body weight (BW) gain, feed intake and feed conversion ratio (FCR) of broiler chickens supplemented with the plant extract.

Treatment ¹	d 7 to 14			d 7 to 21			d 28 to 42		
	BW gain (g/bird)	Feed intake (g/bird)	FCR (g/g)	BW gain (g/bird)	Feed intake (g/bird)	FCR	BW gain (g/bird)	Feed intake (g/bird)	FCR (g/g)
Negative control	325 ^b	523	1.61 ^{ab}	685 ^b	1172	1.71 ^{ab}	1758 ^b	3455 ^c	1.97 ^a
Positive control	341 ^a	524	1.54 ^c	716 ^a	1163	1.62 ^c	1792 ^a	3418 ^a	1.91 ^b
<i>CorE</i> *	321 ^b	529	1.65 ^a	682 ^b	1176	1.72 ^a	1760 ^b	3434 ^{ab}	1.95 ^a
<i>UndE</i> **	324 ^b	520	1.60 ^{ab}	686 ^b	1161	1.69 ^{ab}	1764 ^b	3440 ^{bc}	1.95 ^a
<i>AcaE</i> ***	327 ^b	521	1.59 ^b	690 ^b	1160	1.68 ^b	1753 ^b	3423 ^a	1.95 ^a
SEM ²	4.89	8.29	0.03	7.53	18.32	0.035	7.93	18.23	0.03

^{a,b,c}Means with different superscripts within columns are significantly different ($P < 0.05$)

¹Results are given as means (n = 6) of the negative control (non-supplemented), positive control (45 mg/kg Zinc-bacitracin) or plant extract supplemented groups (5 g/kg)

**CorE* = *Cordyline australis* (Cabbage tree shoot extract)

***UndE* = *Undaria pinnatifida* (Wakame seaweed extract)

****AcaE* = *Acacia pycnantha* (Golden wattle extract)

²SEM = pooled standard error of the mean

Table 3 Effect of dietary plant extracts (5 g/kg) on ileal and caecal bacterial counts in broiler chickens on day 14¹.

Bacteria	Negative control	Positive control	<i>CorE</i> *	<i>UndE</i> **	<i>AcaE</i> ***	SEM ²	P-value
(log ₁₀ cfu/g digesta)							
Total anaerobic bacteria							
Ileum	8.59 ^a	7.22 ^b	8.91 ^a	8.56 ^a	8.86 ^a	0.37	0.001
Caeca	9.41 ^{ab}	8.01 ^c	9.75 ^a	9.50 ^{ab}	9.07 ^b	0.54	0.001
Coliform bacteria							
Ileum	7.25 ^a	7.24 ^a	6.72 ^b	6.57 ^b	6.69 ^b	0.27	0.007
Caeca	8.29 ^a	7.85 ^b	8.27 ^a	8.24 ^a	8.27 ^a	0.22	0.03
Lactose-negative enterobacteria							
Ileum	5.94	5.83	5.94	5.96	5.84	0.32	NS
Caeca	6.55	6.21	6.54	6.37	6.29	0.39	NS
Lactobacilli							
Ileum	7.39 ^{bc}	7.00 ^c	8.03 ^a	8.08 ^a	7.64 ^a	0.46	0.001
Caeca	7.83 ^c	7.60 ^c	8.99 ^a	8.58 ^b	8.43 ^b	0.37	0.003
<i>Clostridium perfringens</i>							
Ileum	5.84 ^a	2.91 ^c	5.54 ^a	5.60 ^a	4.92 ^b	0.35	0.001
Caeca	6.70 ^a	4.22 ^c	5.98 ^b	6.66 ^a	5.97 ^b	0.38	0.001

^{a,b,c}Means with different superscripts within columns are significantly different ($P < 0.05$)

¹Results are given as means (n = 6) of the negative control (non-supplemented), positive control (45 mg/kg Zinc-bacitracin) or plant extract supplemented groups

**CorE* = *Cordyline australis* (Cabbage tree shoot extract)

***UndE* = *Undaria pinnatifida* (Wakame seaweed extract)

****AcaE* = *Acacia pycnantha* (Golden wattle extract)

²SEM = pooled standard error of the mean

body weight gain (BWG) was unaffected by *AcaE* supplementation. Interestingly, mortality tended to be lower for the *CorE*- and *AcaE* supplemented groups than that of the NoS group (3.3% and 2.5% vs. 5.5%, respectively; data not shown).

Bacterial counts in ileal and caecal contents are shown in Table 3. The population of lactobacilli in the ileum and caeca was higher ($P < 0.05$) in chickens fed diets supplemented with plant extracts than in those fed the NoS-diet, and total anaerobes in ileal and caecal digesta were not modified. The lactobacilli counts in the ileum and caeca were increased by 4.4- and 14.5-fold, respectively, in birds fed the *CorE* diet compared to the NoS group. Lower ($P < 0.05$) total anaerobic bacterial counts were observed in the ileal and caecal contents of antibiotic- (Zn-B) fed birds. However, supplementation with the antibiotic did not reduce lactobacilli counts in the ileal and caecal contents compared to the NoS group. Significantly lower ($P < 0.05$) ileal coliform counts were observed in birds fed plant-extract supplemented diets than in the NoS- and ZnB fed groups. Coliform count of the caeca was not affected by plant extract supplementation; however, the antibiotic-supplemented group had significantly lower ($P < 0.05$) coliform counts than the NoS group. The counts of *Clostridium perfringens* in the ileal content of chickens fed the NoS-diet were higher ($P < 0.05$) than those of the *AcaE* fed group. The numbers of lactose-negative enterobacteria in the ileum and caeca were unaffected by dietary inclusion of the plant extracts. However, the numbers of bifidobacteria enumerated in the ileal and caecal contents were comparatively lower in this study than other published data on broiler chickens (Xu et al. 2003; Guo et al. 2004). Identification of bifidobacteria isolates by fructose-6-phosphate phosphoketolase (F6PPK) activity also revealed that the population of bifidobacteria in the ileal and caecal contents in the present study was numerically low. The selectivity of the agar media used in some other published studies was not verified or accounted for and therefore the results need to be interpreted with caution due to potential methodological biases as demonstrated by Mikkelsen et al. (2003).

Many studies have demonstrated improved weight gain in broiler chickens after dietary inclusion of Zn-bacitracin (Elwinger et al. 1998; Engberg et al. 2000), as was the case in the present study. Zinc-bacitracin is active mainly against gram-positive bacteria, forming a complex with C_{55} -isoprenyl pyrophosphate, carrier for the *N*-acetylmuramyl peptapeptide intermediates for the synthesis of the peptidoglycan in the bacterial cell wall (Butaye et al. 2003).

The reduction in mortality rates in broiler chickens fed *CorE* and *AcaE* may be associated with an improvement of health status by promotion of the growth of beneficial bacteria (lactobacilli) and reduction of growth of pathogenic bacteria.

Lactobacillus spp. act via a number of mechanisms, including competitive exclusion, to reduce the numbers of pathogenic bacteria in monogastric animals. Recently, an *in vivo* study by La Ragione et al. (2004) reported that a single oral dose of *Lactobacillus johnsonii* is sufficient to suppress all aspects of colonization and persistence of *C. perfringens* in chickens. However, from a nutritional point of view, an increase in the number of *Lactobacillus* species may not be desirable since these bacteria contribute to bile acid deconjugation, impairing lipid digestion and absorption. In accordance with our findings, Jamroz et al. (2003b) observed that dietary supplementation of 300 ppm of a plant extract containing capsaicin, carvacrol and cinnamic aldehyde significantly reduced the counts of *C. perfringens* and *E. coli* in rectal contents of broiler chickens.

The present study shows that coliform counts in the ileum were higher in the NoS group than in the plant-extract supplemented groups. This could be due to elevated counts of lactobacilli in the ileal contents of plant-extract groups. In addition, supplementation of broiler diets with plant extracts increased volatile fatty acid production and decreased the pH of the digesta. The current study failed to demonstrate a growth-promoting effect of plant extracts. It is possible that the birds in our study were not stressed and their health status was good.

This study indicates that, regardless of the type of plant extract supplemented, colonization of non-invasive bacteria such as lactobacilli in the ileum and caeca are stimulated, while the growth of *C. perfringens* is suppressed in the ileum by *AcaE*. It appears that water-soluble carbohydrate extracts (*CorE*, *UndE* and *AcaE*) can be used as potential phytobiotic compounds to modify the gut microflora of broiler chickens.

Conclusions

The exact modes of action by which plant bioactive substances exert their positive effects (antimicrobial, prebiotic or immunomodulatory effects) are not well understood, and further research is warranted. Studies using poultry and swine suggest that dietary supplementation with some of the plant-derived bioactive compounds is capable of inhibiting colonization of the gut by pathogenic bacteria; however, inconsistent effects have been observed in terms of growth performance of animals. Perhaps this could be related in part to sanitary conditions specific to the experimental environment and the nutritional status of the animals. There is no doubt that a number of phytobiotics are capable of modifying the gut microflora substantially, which, in turn, can bring about a cascade of changes in the animal's responses to nutrients, disease challenge and the environment. The question that remains unanswered is what type of extracts can be recommended and under what

circumstances they can be used. The application of phytobiotics to animal nutrition is at an early stage of implementation and will require further research input.

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