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Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics

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Since in-feed antibiotics (IFAs) are being taken out of broiler diets around the world, beginning in Sweden in the year 1986, the search for alternatives to replace IFAs has gained increasing interest in animal nutrition. Gut microflora appears to be the target for IFAs and alternatives to exert health benefits and some growth-promoting effects. In this review the effect of six kinds of alternatives to IFAs on gut microflora is discussed and their working mechanisms and growth-promoting effects are reviewed. This review focuses on mannanoligosaccharides (MOS) as alternative to antibiotics using research results obtained by various authors in recent years.

Keywords: alternatives to in-feed antibiotics; fibre-degrading enzymes; prebiotics; probiotics; mannanoligosaccharides; symbiotics; phytobiotics; gut microflora

Introduction

Antibiotics have been added to poultry and pig diets to maintain health and production efficiency in the last few decades (Rosen, 1995). However, because of the development of resistance by pathogenic bacteria, which can impact on public health, antibiotics are being taken out of poultry and pig diets around the world, beginning in Sweden in the year 1986 (Dibner and Richards, 2005). The search for alternatives to replace IFAs has gained increasing interest in animal nutrition in recent years. Bedford (2000) pointed out that the growth-promoting effects of antibiotics in animal diets are clearly related to the gut microflora because they exert no benefits on the performance of germ-free (GF) animals.

Gut microflora has significant effects on host nutrition, health, and growth performance (Barrow, 1992) by interacting with nutrient utilization and the development of gut system of the host. This interaction is very complex and, depending on the composition and

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activity of the gut microflora, it can have either positive or negative effects on the health and growth of birds. For example, when pathogens attach to the mucosa, gut integrity and function are severely affected (Droleskey *et al.*, 1994) and immune system threatened (Neish, 2002). Chicks grown in a pathogen-free environment grow 15% faster than those grown under conventional conditions where they are exposed to bacteria and viruses (Klasing, 1987). Furthermore, it is generally agreed that gut microflora is a nutritional "burden" in fast-growing broiler chickens (Dibner and Richards, 2005; Lan *et al.*, 2005) since an active microflora component may have an increased energy requirement for maintenance and a reduced efficiency of nutrient utilization.

The focus of alternative strategies has been to prevent proliferation of pathogenic bacteria and modulation of indigenous bacteria so that the health, immune status and performance are improved (Ravindran, 2006). In this review, we will evaluate dietary modulation of gut microflora through the use of fibre-degrading enzymes, probiotics, prebiotics, MOS, phytobiotics, as well as their mechanisms of action and effectiveness in promoting growth in broiler chickens.

Fibre-degrading enzymes

Enzymes are naturally occurring and are produced by all living organisms for catalyzing chemical reactions. Enzymes were discovered in the later part of the 19th century and have been used in industry and food processes since the early 1900s (Clarkson *et al.*, 2001). The majority of enzyme products are fermentation products of basophilic micro-organisms (Clarkson *et al.*, 2001). Ferket (1993) defined enzymes as special proteins that catalyse or accelerate the rate of specific chemical reactions in which the enzyme activity may be dependent on the substrate in a random manner or it may be through very specific sites on substrates such as fat, protein, or carbohydrates. In monogastric animal diets, exogenous enzymes are used to improve digestibility of a wide range of feed components such as fibre, phytate, protein, etc. Fibre-degrading enzymes are used to break down specially non-starch polysaccharides (NSP), which are large polymers, to smaller polymers to alleviate their anti-nutritive activities (Choct and Annison, 1992).

The effects of enzymes on gut microflora were classified by Bedford (2000) into two phases: an ileal phase and a caecal phase. In the ileum, enzymes simply reduce the number of bacteria by increasing the rate of digestion and limiting the amounts of substrates available to the microflora. In the caecal phase, enzymes produce soluble, poorly absorbed sugars which feed beneficial bacteria. The volatile fatty acids (VFAs) produced by such bacteria may be of benefit not only in controlling populations of *Salmonella*, and perhaps, *Campylobacter* species, but also in providing an energy source for the bird (Snel *et al.*, 2002).

However, the effects of enzymes on the gut microflora may be far more than those two phases. The composition of gut microflora in the proximal small intestine as well as those associated with the gut wall was shown to be changed by the addition of xylanase (Vahjen *et al.*, 1998; Danicke *et al.*, 1999; Hubener *et al.*, 2002). The authors correlated those effects of xylanase on the gut microflora with its effects on the viscosity of diet, which is well known as one of the major modes of action of enzymes. Inclusion of cereals rich in NSP increases the viscosity of the digesta, reduces apparent nutrient digestibility, alters bacterial profiles and gut physiology. By adding enzymes into a diet, the viscosity of the content is reduced and nutrient uptake and animal performance are improved (Bedford, 2001). Elimination of cell wall encapsulation is another major mode of action of enzymes (Bedford, 2001). It relies on the fact that the feed manufacturing process of grinding and pelleting does not break

open all the cell walls of the endosperm. The addition of enzymes can remove such "encapsulated" material in the gut and hence improve nutrient utilization by the birds.

Therefore, the responses of birds to enzymes depend mainly on dietary cereal quality and quantity. However, quantity and quality of fat, microbial status, bird age and antimicrobial agents can also modify the effects of enzymes (Bedford, 2001). Furthermore, because of cereal quality (*e.g.* the complexity of carbohydrate) and the thermolabile characteristic of enzymes, improvements are not always observed (Acamovic, 2001).

Supplementation of enzymes generally can lead to 2-5% improvement in feed/gain ratio and 2-3% improvement in growth rate (Broz and Beardsworth, 2002). Reduced incidence of sticky excreta and improved litter conditions are also the benefits of using enzymes, which makes the problem of NSP and associated increased ingesta viscosity more manageable (Morrow, 2001). The development of enzymes is towards a specially-designed stage to use NSP as energy sources and to deactivate anti-nutrients in feed (Choct, 2006).

Prebiotics

Gibson and Roberfroid (1995) defined a prebiotic as a non-digestible food ingredient which beneficially affects the host by selectively stimulating the growth of and/or activating the metabolism of one or a limited number of health-promoting bacteria in the intestinal tract, thus improving the host's microbial balance. The growth of endogenous microbial population groups such as bifidobacteria and lactobacilli is specifically stimulated and these bacteria species are perceived as beneficial to animal health. Prebiotics have the advantage, compared with probiotics, that bacteria are stimulated which are normally present in the GIT of that individual animal and therefore already adapted to that environment (Snel *et al.*, 2002). The dominant prebiotics are fructo-oligosaccharide products (FOS, oligofructose, inulin) (Patterson and Burkholder, 2003); gluco-oligosaccharides, stachyose, malto-oligosaccharides, and oligochitosan have also been investigated in broiler chickens (Zhang *et al.*, 2003; Gao and Shan, 2004; Jiang *et al.*, 2006; Huang *et al.*, 2007).

Compared to the application of prebiotics in human and pet food, the use of prebiotics in broiler chicken diets does not have a long history. Reports on the effects of prebiotics on the activity of the microflora of broilers are limited and the effects are variable, depending on the type of prebiotic. Fructo-oligosaccharides were shown to support the growth of beneficial bacteria, such as lactobacilli (Xu *et al.*, 2003; Yusrizal and Chen, 2003; Zhan *et al.*, 2003), but failed to stimulate the growth of bifidobacteria (Vidanarachchi *et al.*, 2006). When supplemented into a dextrose-isolated soy protein diet, short-chain fructo-oligosaccharides decreased caecal populations of *C. perfringens* (Biggs *et al.*, 2007). Reduced susceptibility to *Salmonella* colonization was noticed in birds on fructo-oligosaccharide treatments compared to controls (Bailey *et al.*, 1991; Fukata *et al.*, 1999). The addition of isomalto-oligosaccharides and stachyose did not affect crop and/or caecal bacterial populations such as lactobacilli, *E. coli* and total aerobes (Zhang *et al.*, 2003; Jiang *et al.*, 2006). It was shown that prebiotics can bring about "bifidogenic" effects and a shift in microbial metabolism from "proteolytic" to the more favourable "saccharolytic" in mice (Gibson and Roberfroid, 1995). A combination of various substances with different rates of fermentation will be effective in mimicking some of the antibiotic effects in pigs (Williams *et al.*, 2001). However, no similar research has been reported in broiler chickens.

Among the prebiotics examined in chicken, fructo-oligosaccharides were shown to

improve growth performance of birds but positive effects are not always observed (*Table 1*).

Table 1 Effects of prebiotics on growth performance of broilers .

Parameter	Prebiotics	Response (%)	Reference
Weight gain (g/bird)	FOS	+5	Li *et al.,* 2008
		-2	Biggs *et al.*, 2007
		+6	Zhan *et al.*, 2003
		+8	Xu *et al.*, 2003
	transgalacto-oligosaccharide	0	Biggs *et al.*, 2007
	stachoyse	-3	Jiang *et al.*, 2006
	chitosan	+2	Huang *et al.*, 2005
	isomalto-oligosaccharide	+5	Zhang *et al.*, 2003
Body weight (g/bird)	FOS	+3	Yusrizal and Chen, 2003
		-1	Waldroup *et al.*, 1993
FCR (g/g)	FOS	+2	Li *et al.*, 2008
		+3	Biggs *et al.*, 2007
		0	Yusrizal and Chen, 2003
		+6	Zhan *et al.*, 2003
		+6	Xu *et al.*, 2003
		-1	Waldroup *et al.*, 1993
	transgalacto-oligosaccharide	0	Biggs *et al.*, 2007
	stachoyse	-1	Jiang *et al.*, 2006
	chitosan	+3	Huang *et al.*, 2005
	isomalto-oligosaccharide	+4	Zhang *et al.*, 2003

FCR: Feed conversion ratio.

Also, dietary supplementation of fructo-oligosaccharide (0.3% dose) or oligochitosan (0.1% dose) showed growth-promoting effects similar to antibiotic treatments based on flavomycin (Huang *et al.*, 2005) or aureomycin (Li *et al.*, 2008). The optimal dose for prebiotics to exert growth-promoting effects is not easy to define; however feeding a higher level (0.8%) of inulin and short-chain fructo-oligosaccharide depressed growth performance, digestibility of amino acids as well as metabolisable energy of birds (Biggs *et al.*, 2007). Some positive changes in digestive enzymes, gut morphology, and immune system were noticed in birds given prebiotic-supplemented feed (Xu *et al.*, 2003; Zhang *et al.*, 2003; Huang *et al.*, 2007).

However, there are many considerations in supplementing prebiotics in animal feed. These include the type of diet (*i.e.*, the content of non-digestible oligosaccharides); the type and inclusion level of the supplements; the animal characteristics (species, age, stage of production); and the hygiene status of the farm (Verdonk *et al.*, 2005). The primary ones are the type and inclusion level of the supplement as high dosage of prebiotics can have negative effects on the gut system and retard the growth rate of birds as observed by Biggs *et al.* (2007). It is reported that rapid fermentation of prebiotics, leading to high concentrations of organic acids, impaired the barrier function, which reduced the ability of rats to resist salmonella infection (Ten Bruggencate *et al.*, 2003). It may also be worthwhile to examine the interaction between prebiotics(s) and bird sex. In the report by Yusrizal and Chen (2003), body weight and feed conversion ratio (FCR) of female birds were improved by 10% and 9%, respectively, on oligofructose treatment but no such effects were observed in males.

Probiotics

In animal nutrition, probiotics are defined as viable micro-organisms used as feed additives, which lead to beneficial effects for the host by improving its microbial balance (Fuller, 1989) or the properties of the indigenous microflora (Havenaar and Huis In 't Veld, 1992). A variety of microbial species have been used as probiotics, including species of *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Escherichia*, *Lactobacillus*, *Lactococcus*, *Streptococcus*, a variety of yeast species, and undefined mixed cultures. *Lactobacillus* and *Bifidobacterium* species have been used most extensively in humans, whereas species of *Bacillus*, *Enterococcus*, and *Saccharomyces* yeast have been the most common organisms used in livestock (Simon *et al.*, 2001). There has been an increase in research on feeding *Lactobacillus* to broiler chickens (Jin *et al.*, 2000; Kalavathy *et al.*, 2003).

The possible modes of action of probiotics were extensively reviewed (Jin *et al.*, 1997; Simon *et al.*, 2001; Ghadban, 2002; Edens, 2003). Two basic mechanisms by which probiotics act to maintain a beneficial microbial population include "competitive exclusion" and immune modulation. Competitive exclusion involves competition for substrates, production of antimicrobial metabolites that inhibit pathogens, and competition for attachment sites. Based on this mechanism, probiotics have been tested for their efficacy at controlling *Salmonella* colonization in broilers and the results are positive and consistent (*Table 2*).

Table 2 Effectiveness of probiotics in the prevention of *Salmonella* colonization in broilers in research station and/or commercial field tests.

Researcher	Number of Chickens	Reduction (%) in the colonization
Higgins *et al.* (2007)	840	60
Stern *et al.* (2001)	210	44
Pascual *et al.* (1999)	344	50
Stern *et al.* (1998)	100,000	9
Blankenship *et al.* (1993)	Six commercial blocks	31
Bolder *et al.* (1992)	420	20
Hinton *et al.* (1991)	720	39
Goren *et al.* (1988)	8 million	31

Further, a product of competitive exclusion specially designed at gut mucosal level showed much stronger effectiveness than a general competitive exclusion product; the number of affected birds was reduced by 50% and 10%, respectively (Stern *et al.*, 2001). Probiotic supplementation, especially with *lactobacillus* species, has also shown beneficial effects on resistance to the other infectious agents such as *Clostridium* population (Decroos *et al.*, 2004) and *Campylobacter* (Stern *et al.*, 2001). Regarding the gut microbiota of normal birds, the results of probiotics supplementation are variable because of the difference in origin, strain as well as species of probiotics. Reduced caecal coliform populations were noticed in chickens given a diet supplemented with lactobacilli strains, isolated from chicken intestine, but the populations of other kinds of bacteria were not affected (Watkins and Kratzer, 1984; Jin *et al.*, 1998a, 1998b). In contrast, Murry *et al.* (2006) reported that birds supplemented with botanical probiotic containing lactobacilli had higher lactobacilli but lower *C. perfringens* compared to the control birds. When multi-strain and/or multi-species probiotics were applied, no significant change(s) in bacterial populations was noticed (Priyankarage *et al.*, 2003; Mountzouris *et al.*, 2007).

By directly interacting with gut mucosal immune system, probiotics can modulate either innate or acquired immunity, or both (Dugas *et al.*, 1999). Further, specific immune modulatory effects of probiotics are dependent on the strain or species of bacteria included in the probiotics (Edens, 2003; Huang *et al.*, 2004). Lactobacilli, the most studied strain of probiotic in both animal and human, have been implicated to increase the activity of certain innate immune functions, specifically the activity of macrophages and natural killer cells (McCracken and Gaskins, 1999). In accordance with this notion, Koenen *et al.* (2004) reported that feeding *L. paracasei* to broilers enhanced the phagocytic activity of the gut cells (caecum, ileum). However, *L. plantarum*, rather than *L. paracasei*, exerted stronger stimulating effect on antigen-specific titre (Koenen *et al.*, 2004). In general, feeding probiotics could improve antibody titres against Newcastle disease; infectious bursal disease virus and/or sheep red blood cell (SRBC, Panda *et al.*, 2000; Zulkifli *et al.*, 2000; Huang *et al.* 2004) but no responses were also observed by Panda *et al.* (1999). The results from Zulkifli *et al.* (2000) further indicated that these effects of probiotics could be affected by the age and strain of broilers. In addition, *Lactobacillus*-based probiotic may strengthen gut defence function via activation and enhancement of local cell-mediated immunity to against certain enteric pathogen (Dalloul *et al.*, 2003). However the exact mechanisms for probiotics to enhance immune function remain largely unknown.

Probiotics did not consistently improve growth performance and/or mortality rate of birds (*Table 3*).

Table 3 Growth performance and/or mortality rate of birds to probiotic supplementation.

Item	Control	Probiotics	Improvement (%)
Liu *et al.* *(2007)*			
BWG (g/bird)	1892	1920	+1
FCR (g/g)	1.75	1.74	0
Mountzouris *et al.* *(2007)*			
BWG (g/bird)	2216	2237	+1
FCR (g/g)	1.81	1.78	+2
Murry *et al.* *(2006)*			
BWG (g/bird)	2784	2720	- 2
FCR (g/g)	1.62	1.63	0
Mortality (%)	7.02	4.76	+32
Timmerman *et al.* *(2006)*			
ADG (g/bird)	49.99	49.65	0
FCR (g/g)	1.93	1.87	+3
Mortality (%)	8.84	7.27	+18
Kalavathy *et al.* *(2003)*			
BWG (g/bird)	2151	2251	+5
FCR (g/g)	1.96	1.78	+9
Zulkifli *et al.* *(2000)*			
BWG (g/bird)	1379	1545	+12
FCR (g/g)	2.08	2.17	-4
Mortality (%)	1.7	2.2	-29
Jin *et al.* *(1998a)*			
BWG (g/bird)	1290	1388	+8
FCR (g/g)	2.27	2.10	+7
Mortality (%)	6.7	5.3	+21
Mohan *et al.* *(1996)*			
BWG (g/bird)	1046	1128	+8
FCR (g/g)	2.01	1.84	+8

BWG: body weight gain. FCR: feed conversion ratio. ADG: average daily gain.

The inconsistency may become more complex because of rearing environment. For example, under heat stress condition, lactobacilli probiotic supplementation improved body weight gain (BWG) of female birds by 12% but increased FCR and mortality rate by 4% and 29%, respectively (Zulkifli *et al.*, 2000). However, growth-promoting effects of certain probiotics were reported to be comparable to antibiotic treatments (virginiamycin, Cavazzoni *et al.*, 1998; oxytetracycline, Zulkifli *et al.*, 2000; avilamycin, Mountzouris *et al.*, 2007); a meta-analysis of 35 studies involved in probiotics across Brazil between 1995 and 2005 seems to indicate that probiotics are a technically viable alternative to IFA in broiler feeding (Faria Filho *et al.*, 2006). However, the growth-promoting effects of probiotics are dependent on the specific probiotics, the application level of probiotics, the age of birds as well as the delivery method (*i.e.* via water and/or feed). Moreover, there are many factors from nutrition, environment (sanitary condition), to management that could compromise the effectiveness of probiotics (Edens, 2003). This can probably explain the inconsistent results in the growth performance and gut bacterial responses described above.

Mannanoligosaccharides

Mannanoligosaccharides, derived from yeast cell wall, are more complex than the name suggests; they are components of the outer layer of yeast cell walls and their components include proteins, glucans and phosphate radicals as well as mannose (Klis *et al.*, 2002). The basic composition of the wall consists of mannan (30%), glucan (30%) and protein (12.5%). While the ratio of one component to another remains relatively constant from strain to strain, the degree of mannan phosphorylation and the interaction among the mannan, glucan and protein components vary (Lyons, 1994). Mannanoligosaccharides contain protein which has relatively high proportions of serine, threonine, aspartic and glutamic acids, and a paucity of methionine (Song and Li, 2001).

Hooge (2004a) reviewed pen trials conducted with a commercially available dietary MOS (Bio-MOS, Alltech Inc.) from 1993 to 2003 and the meta-analysis showed that Bio-MOS improved the growth performance of birds compared to the negative control (*Table 4*).

Table 4 Effects of MOS on growth performance of broiler chickens (adapted from Hooge, 2004a).

Parameter	**Negative Control**	**MOS**	**Relative change (%)**	**Note**
Body weight (kg/bird)	2.231	2.267	+1.61	29 pen trials
FCR (g/g)	1.808	1.772	-1.99	29 pen trials
Mortality (%)	4.494	3.534	-21.4	21 pen trials
Parameter	**Antibiotic Control**	**MOS**	**Relative change (%)**	**Note**
Body weight (kg/bird)	2.246	2.238	-0.36	21 pen trials
FCR (g/g)	1.822	1.820	-0.11	21 pen trials
Mortality (%)	5.404	4.426	-18.1	16 pen trials

FCR: feed conversion ratio.

Compared to a wide range of antibiotics (including avilamycin, bacitracin, bambermycin or virginiamycin at prophylactic concentrations), a significant decrease in mortality was observed for Bio-MOS treatment (*Table 4*). The optimal dose of

Bio-MOS for broiler production appears to be around 2 g/kg, depending on the production stage of birds (Rosen, 2007; Yang *et al.*, 2007b). Three major modes of action by which broiler performance is improved by MOS are proposed: 1) control of pathogenic or potential pathogenic bacteria which possess type-1 fimbriae (mannose-sensitive lectin), 2) immune modulation, and 3) modulation of intestinal morphology and expression of mucin and brush border enzymes (Ferket, 2004).

Mannanoligosaccharides not only prevent those pathogenic bacteria possessing type-1 fimbriae, such as *E. coli*, from attaching to gut wall but also displace them from the gut wall. This reduces sub-clinical or lethal infection. Pathogens possessing type-1 fimbriae are much more virulent than non-fimbriated bacteria. Duguid *et al.* (1976) demonstrated that fimbriation in *S. typhimurium* significantly increases both the number of infections (a 26% increase) and deaths (a 40% increase) in mice inoculated orally compared to non-fimbriated organisms from the same parent strain.

The immune modulatory effects of MOS are based on the following two aspects: 1) its mannan and glucan components have antigenicity characteristics, and 2) MOS prevents colonization of specific pathogens but allow them to be presented to immune cells as attenuated antigens (Ferket, 2004). The immuno-stimulatory effects of MOS were demonstrated by Privulescu (1999) in GF vs. conventional piglets (*Table 5*). A unique character of MOS in immune modulation is that it enhances the protective antibody response to enhance disease resistance while at the same time suppress the acute phase (fever) response (Ferket *et al.*, 2002). Mannanoligosaccharides have also been shown to enhance macrophage response in different animal species (Spring and Privulesu, 1998).

Table 5 Effects of MOS (Bio-MOS) on immune responses of conventional and germ-free piglets. (Adapted from Privulescu, 1999)

Item	Without MOS	With MOS	Change
Cellular immune responses			
Phagocytotic index	41.4	78.2	89% increase
Lymphoblast transformation	1.60	3.09	93% increase
Proportion T lymphocytes	37.6	24.7	34% decrease
Cytokines dose in lymphocyte cultures (relative doses)			
IL-2	1	2	100% increase
IFN-γ	1	3	200% increase
Humoral immune responses			
Serum IgG (mg/100mL)	200	916	358% increase
Serum IgM (mg/100mL)	Trace	106	>500% increase
Serum IgA (OD by ELISA)	163	364	123% increase
Lysozyme (μg/mL)	1.38	3.22	133% increase

Positive effects of MOS on growth performance and gut physiology are observed; however, the information available is limited or/and variable. A number of studies demonstrated that MOS are effective in reducing *Salmonella* infection of birds (Fernandez *et al.*, 2000; Spring *et al.*, 2000; Fernandez *et al.*, 2002; Kumar *et al.*, 2002). A few studies investigated the effect of MOS on intestinal and faecal microbial populations of broilers (Spring *et al.*, 2000; Song and Li, 2001; Jamroz *et al.*, 2004; Kocher *et al.*, 2004) but the results are inconsistent. Yang *et al.* (2008a, 2008b, 2008c) showed that MOS inhibited the development of lactobacilli and coliforms; in particular the colonization of mucosa-associated coliforms was inhibited by MOS as early as 7 days of age. In addition, MOS reduced the abundance of *C. perfringens* in the caeca of birds at 21 days of age (Yang *et al.*, 2008c), which is in agreement with the report from Jamroz *et al.* (2004) and Kocher *et al.* (2004). The effect of MOS on the

development of mucosa-associated bacteria appears to closely relate to the age of birds and rearing environment but less independent of the type of diet; whereas the effect of MOS on luminal bacteria mainly interacts with the type of diet and the age of birds.

Mannanoligosaccharides, specifically Bio-MOS, were shown to alter mucosal architecture and longer villi were noticed in birds fed MOS-supplemented diets (Iji *et al.*, 2001; Loddi *et al.*, 2002; Yang *et al.*, 2007b). Further, Bio-MOS consistently reduced the crypt depth of the mucosa of the small intestine where its growth-promoting effects were observed (*Figure 1*). Also, this effect was dependent on the age of birds.

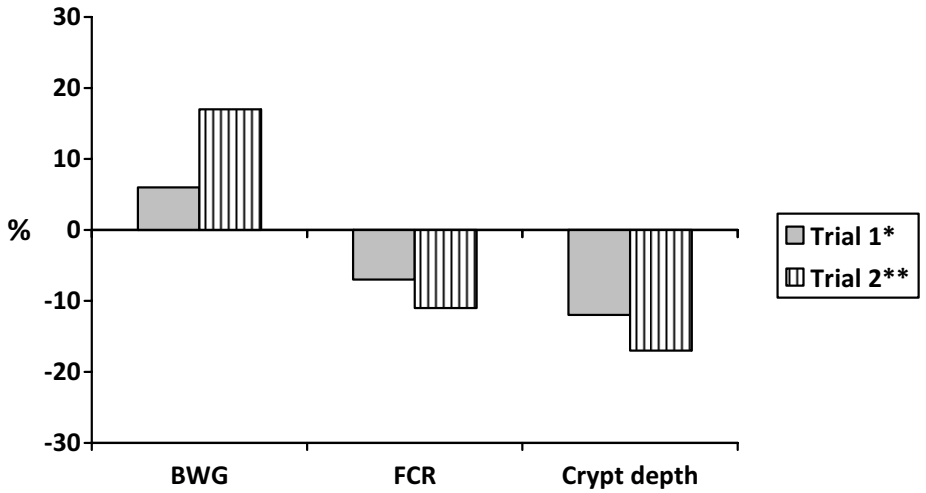

Figure 1 Relative change (%) of jejunal crypt depth and growth performance of birds supplemented with Bio-MOS compared to a negative control.
BWG: Body weight gain (g/bird), FCR: Feed conversion ratio (g/g). BWG and FCR were measured from day 8 to day 21. Crypt depth (μm) was measured with 7-day-old birds.
***The experiment was conducted with day-old broilers under a challenge model of pathogenic *E. coli*. The basal diet was mainly composed of wheat, sorghum and barley (Yang *et al.*, 2008b).**
****The experiment was conducted with day-old broilers fed on a wheat basal diet (Yang *et al.*, 2008c).**

There is scant information on the effect of MOS on nutrient digestion, availability and retention of birds. Studies by Kumprecht and Zobac (1997) showed that total tract digestibility of fibre was increased by MOS but the digestibility of fat, nitrogen-free extract and crude protein was not affected. Similarly, Yang *et al.* (2008a) did not observe any effect of MOS on total tract digestibility of starch, protein, fat and NSP in birds given a sorghum-wheat based diet but MOS significantly improved apparent metabolisable energy and numerically improved the net energy value of the diet, which agrees with the reports from Samarasinghe *et al.* (2003) that energy utilization was improved by MOS. However, the results on protein utilization are variable; while Shafey *et al.* (2001a) reported that MOS supplementation had no effect on nitrogen utilization of birds, Samarasinghe *et al.* (2003) noticed that MOS increased protein utilization. Yang *et al.* (2008c) also observed that MOS largely increased ileal starch digestibility in birds on a wheat-based diet. This effect of MOS seemed to be related to its modulatory effects on gut microflora but the exact working mechanism(s) is unclear.

The development of brush-border membrane bound enzyme activities and mucin mRNA expression was influenced by MOS (Smirnov *et al.*, 2005; Yang *et al.*, 2007a, 2008c). However, the information is too limited to draw any conclusion(s). The effects of

MOS on the immune organ or response were observed by Kumar *et al.* (2002), Kocher *et al.* (2004) but not Shafey *et al.* (2001b), an area that requires more research.

However, based on the available information, it can be concluded that the responses of birds to MOS supplementation are influenced by the type of diet, rearing conditions as well as the age of birds. Therefore, these factors need to be carefully considered in order to obtain maximal growth-promoting effects of MOS in broiler production.

Symbiotics

A symbiotic is, in its simplest definition, a combination of probiotics and prebiotics (Collins and Gibson, 1999; Schrezenmeir and De Vrese, 2001). This combination could improve the survival of the probiotic organism, because its specific substrate is available for fermentation. This could result in advantages to the host through the availability of the live micro-organism and the prebiotic. Bengmark (2001) regards symbiotics as products of fermentation. Since in mixtures of pre- and probiotics, the prebiotics will be fermented when the appropriate choice of products is used, this definition may also be plausible. Examples of symbiotics are FOS and bifidobacteria, and lactitol and lactobacilli (Collins and Gibson, 1999). Bailey *et al.* (1991) used a combination of FOS and competitive exclusion flora to reduce *Salmonella* colonization in chickens. The combination was more effective in reducing *Salmonella* colonization than FOS or competitive probiotic alone. While applying the combination of FOS and bacillus to a corn-soybean basal diet, Li *et al.* (2008) observed that average daily gain (ADG) and FCR were improved by 6% and 2 %, respectively; diarrhoea and mortality rate were reduced by 58% and 67%, respectively, which were very comparable to aureomycin treatment (the relative changes are 4% for ADG, 2% for FCR, 69% for diarrhoea rate and 33% for mortality rate). To our knowledge, this is the only experiment published regarding the growth-promoting effects of symbiotic in broiler chickens thus far. Therefore more research is warranted on this kind of products in order to achieve the application significance in the industry.

Phytobiotics

Plant products have been used for centuries by humans as food and to treat ailments. Natural medicinal products originating from herbs and spices have also been used as feed additives for farm animals in ancient cultures for the same length of time. To differentiate from the plant products used for veterinary purposes (prophylaxis and therapy of diagnosed health problems), phytobiotics were redefined by Windisch and Kroismayr (2006) as plant-derived products added to the feed in order to improve performance of agricultural livestock. Around the world, phytobiotics have been investigated as natural sources of biologically important chemicals since efforts are being made to ban all types of IFAs in many countries. Compared with synthetic antibiotics or inorganic chemicals, these plant-derived products have proven to be natural, less toxic, residue free, and are thought to be ideal feed additives in food animal production (Wang *et al.*, 1998).

With respect to biological origin, formulation, chemical description and purity, phytobiotics comprise a very wide range of substances and four subgroups may be classified: 1) herbs (product from flowering, non-woody and non-persistent plants), 2) botanicals (entire or processed parts of a plant, *e.g.*, root, leaves, bark), 3) essential oils (hydro distilled extracts of volatile plant compounds), and 4) oleoresins (extracts based

on non-aqueous solvents) (Windisch and Kroismayr, 2006). The active compounds of phytobiotics are secondary plant constituents.

Antimicrobial activity and immune enhancement probably are the two major mechanisms by which phytobiotics exert positive effects on the growth performance and health of animals. Compounds (phytochemicals) in phytobiotics are well known to have antimicrobial ability (Cowan, 1999). Polysaccharide components are considered to be the most important immunoactive components (Xue and Meng, 1996). In diseased chickens (either infected with avian *Mycoplasma gallisepticum* or *Eimeria tenella*), Guo and his colleagues (Guo *et al.*, 2004a, 2004b, 2004c) demonstrated that plants and their extracts could improve the growth performance, reduce the populations of coliforms and/or *C. perfringens*, and enhance both cellular and humoral immune responses of chickens. Some herbal extracts have also been shown to possess a coccidiostatic activity (Allen *et al.*, 1997; Youn and Noh, 2001; Christakia *et al.*, 2004).

A common feature of phytobiotics is that they are a very complex mixture of bioactive components. For example, hawthorn fruit, a common growth-enhancing and digestion modifier, has been shown to contain more than 70 kinds of organic chemicals along with some unidentified factors and active bio-active compounds (Wang *et al.*, 1998). Therefore they may exert multiple functions in the animal body. Increased feed intake and digestive secretions are also observed in animals offered phytobiotic-supplemented feed (Windisch and Kroismayr, 2006). Growth enhancement through the use of phytobiotics is probably the result of the synergistic effects among complex active molecules existing in phytobiotics (Gauthier, 2002). However, the exact growth-promoting mechanisms of phytobiotics in broiler chickens are poorly understood.

Among phytobiotics, essential oils (EO) have been applied into chicken feed in Europe and USA (Hooge, 2004b). However, bird growth responses to EO supplementation are still controversial. No EO effects on growth performance were reported by Botsoglou *et al.* (2002); Zhang *et al.* (2005), Jang *et al.* (2007); whereas improved growth performance were observed at different ages of birds fed certain EO-supplemented diet(s) by Jamroz *et al.* (2003), Hernandez *et al.* (2004), and Cross *et al.* (2007). On the other hand, some EO(s) induced growth improvements similar to or even better than an antibiotic treatment (*Table 6*).

Table 6 Effects of essential oils on growth performance in broilers.

Item	Negative control	Antibiotic control	Essential oil treatment
Garcia *et al.* *(2007)*			
ADG (g/bird)	68.9	66.5	68.8
FCR (g/g)	1.92	1.54	1.59
Ertase *et al.* *(2005)*			
ADG (g/bird)	61.3	65.8	71.3
FCR (g/g)	1.61	1.50	1.41
Jamroz *et al.* *(2003)*			
ADG (g/bird)	48.1	48.9	49.2
FCR (g/g)	1.85	1.81	1.79

ADG: average daily gain. FCR: feed conversion ratio.

While comparing the effects of various herbs and oils on broiler performance, Cross *et al.* (2007) concluded that the quality as well as the quantity of active chemicals in plant extract determines bird response. In addition, the efficacy of dietary EO can be affected by intrinsic and extrinsic factors such as nutritional status of animals, infection, diet

composition and environment (Giannenas *et al.*, 2003; Lee *et al.*, 2004b). Essential oils function mainly as antimicrobials and antioxidants; their antimicrobial ability may modulate the gut ecosystem to affect fat digestibility (Lee *et al.*, 2004a), starch or/and protein digestibility of feeds (Jamroz *et al.*, 2003; Hernandez *et al.*, 2004). A commercial preparation of essential oil components reduced faecal *C. perfringens* counts of broilers in a field study (Mitsch *et al.*, 2002). In addition, dietary supplementation of EO reduced the intestinal populations of *E. coli* (Jamroz *et al.*, 2003; Jang *et al.*, 2007) and increased digestive enzymes in either pancreas and/or intestinal mucosa (Lee *et al.*, 2003; Jang *et al.*, 2007); however intestinal mucosal morphology was not affected by EO supplementation (Garcia *et al.*, 2007).

Four factors may affect the effectiveness of phytobiotic additives: 1) plant parts and their physical properties, 2) source, 3) harvest time, and 4) compatibility with the other ingredient (s) in the feed (Wang *et al.*, 1998), which may also explain why 50% difference in BWG and 63% difference in FCR could happen when different kinds of phytobiotics are used in chicken diet (Xing, 2004). Although phytobiotics are a group of natural additives, research into their mechanisms of action, compatibility with diet, toxicity and safety assessment (based on the fact that some phytobiotic might have harmful substance(s)) needs to be done before they can be applied more extensively in poultry feed.

Conclusions

The withdrawal of IFAs from poultry feed requires the industry to look for various alternatives to maintain or improve the health and performance of birds. Although the efficacy of antibiotic substitutes needs to be assessed by setting up standards and comprehensive multi-factorial models (Rosen, 2003), it is encouraging to know that most of the alternatives reviewed in the context can exert growth-promoting effects; also some of the effects are comparable to those of IFAs. However the other side of the coin is these growth-promoting effects are (very) variable; under certain circumstances the alternatives can even negatively affect the performance. Further, the effects of the alternatives on gut microflora and/or digestive physiology are often inconsistent. These facts require us to carefully examine the modes of action of the alternatives.

By increasing the growth of beneficial microbes or by reduction and removal of potential pathogens, the alternatives to IFAs possibly can improve the health and performance of birds. However, their effects on gut microflora interact with digestive physiology and thus growth in many complex ways, which can be further influenced or even determined by many other factors such as the compatibility between the diet and the alternative, hygiene standards and animal husbandry practices. There possibly remain many questions to be answered or barriers to be overcome so that the alternatives can be applied (more) successfully in the industry in future.

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