Review

An update on alternatives to antimicrobial growth promoters for broilers

Gerard Huyghebaert[a,*], Richard Ducatelle[b], Filip Van Immerseel[b]

[a] *Ministry of the Flemish Community, Institute Agriculture Fishery Research ILVO Animal Nutrition Sciences, Scheldeweg 68, B-9090 Melle, Belgium.*
[b]*Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium.*

* Corresponding author. Tel.: +32 92522619; fax: +32 92522601.
E-mail address: gerard.huyghebaert@ilvo.vlaanderen.be (G. Huyghebaert)

Abstract

Livestock performance and feed efficiency are closely interrelated with the qualitative and quantitative microbial load of the animal gut, the morphological structure of the intestinal wall and the activity of the immune system. Antimicrobial growth promoters have made a tremendous contribution to the profitability in the intensive husbandry. As a consequence of the increasing concern about the potential for antibiotic resistant strains of bacteria, the European Commission decided to ban all commonly used feed antibiotics. There are a number of non-therapeutic alternatives, including enzymes, (in)organic acids, probiotics, prebiotics, etheric oils and immunostimulants. Their efficacy and mode of action is briefly described.

Keywords: Antimicrobial growth promoters; Alternatives; NSP-enzymes; Inorganic acids; Organic acids; Probiotics; Prebiotics; Etheric oils; Immunostimulants; Broilers

Introduction

Antibiotics have been widely used in animal production for decades. Although some are used therapeutically to improve the health and well-being of animals, a large portion was employed for prophylactic purposes and to improve growth rate and feed conversion efficiency (as antimicrobial growth performance promoters, or AGPs). However, due to the emergence of microbes resistant to antibiotics which are used to treat human and animal infections, the European Commission (EC) decided to phase out, and ultimately ban (January 1st 2006), the marketing and use of antibiotics as growth promoters in feed (EC Regulation No 1831/2003[1]). This political decision was taken by invoking the precautionary principle: '*Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation*' (Principle 15 of the Rio Declaration, 1992[2]).

In other countries, such as the USA, consumer pressure is pushing the poultry industry to rear animals without AGPs (Dibner and Richard, 2005; Castanon, 2007). AGP removal has led to animal performance problems, feed conversion increases, and a rise in the incidence of certain animal diseases, such as (subclinical) necrotic enteritis (Wierup, 2001; Dibner and Richards, 2005). One disease syndrome that is clearly emerging in the EU broiler industry simultaneously with the ban of growth promoting antibiotics is the so-called 'dysbacteriosis'. This is a poorly described condition of the gut and may be synonymous with conditions such as 'wet litter', 'small intestinal bacterial overgrowth', 'malabsorption', 'feed passage syndrome' and others. The common clinical denominator is thinning and ballooning of the small intestine, increased water content of faeces and reduced digestibility of feed with indigested residues visible in the faeces.

The impact of phasing out of animal growth promoters could be minimised provided that adequate attention is given to the implementation of alternative disease-prevention strategies and management factors, such as alternative husbandry practices in food animal production. Indeed, overall disease and performance problems have been rather limited, partly because ionophore anticoccidials are still available, therapeutic antibiotic use (e.g. macrolides and penicillins) has increased, and alternatives for AGPs have been empirically used such that those with the best effects on performance are currently used as feed additives.

Characteristics of good AGP alternatives

Ideally, alternatives to growth promoters should have the same beneficial effect as AGPs. It is however not totally clear how AGPs exert their beneficial action. The most well-known mechanism to be proposed is that AGPs have an antibacterial action that favours performance in different ways: (1) by reducing the incidence and severity of subclinical infections (George et al., 1982; Brennan et al., 2003); (2) by reducing the microbial use of nutrients (Snyder and Wostmann, 1987); (3) by improving absorption of nutrients because of thinning of the intestinal wall, and (4) by reducing the amount of growth-depressing metabolites produced by Gram-positive bacteria (Feighner and Dashkevicz, 1987; Knarreborg et al., 2004). The basis of this mechanistic explanation is that AGPs do not exert growth-promoting effects in germ-free animals (Coates et al., 1963).

Although certain authors reason that AGPs are used in sub-therapeutic or sub-minimum inhibitor concentration (MIC) doses and so any growth-inhibitory action is unclear (Niewold, 2007), clear shifts in the microbiota composition have been demonstrated when AGPs were added to broiler feed (Pedroso et al., 2006; Wise and Siragusa, 2007). Indeed, sub-MIC concentrations do

[1] See: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:268:0029:0043:EN:PDF
[2] See: http://www.unep.org/Documents.Multilingual/Default.asp?DocumentID=78&ArticleID=1163

not mean that growth-inhibition of certain bacterial species in the gut can be excluded but shifts in microbiota composition can, at least in theory, explain the effects of the AGPs. Furthermore, microbiota shifts can affect morphology of the gut wall and induce immune reactions that can have effects on energy expenses of the host (Humphrey and Klasing, 2003; Teirlynck et al., 2009).

Niewold (2007) hypothesized that AGPs may be growth permitting by inhibiting the production and excretion of cytokines by immune cells (macrophages), after AGPs accumulate in these cells. Cytokine release would then lead to an acute phase response leading to loss of appetite and muscle tissue catabolism (Niewold, 2007). Certainly inflammation leads to performance decreases (Humphrey and Klasing, 2003), but equally AGPs may act by shifting the microbiota composition towards one that is less capable of evoking an inflammatory response. AGPs could also simply lower the total microbial load, leading to less inflammation and lower energetic cost for the animal.

Whatever the mechanism of action of AGPs, the main characteristic of a good alternative from a practical point of view is that it must improve performance at least as well as AGPs. Based on the proposed mechanism of action of AGPs, both microbiota modulating and immunomodulatory compounds could have potential. There are many possible ways microbiota modulating compounds could influence the intestinal microbiota population without adding AGPs to the feed. The most obvious method is the use of therapeutic doses of antibiotics under prescription, a practice that will undoubtedly increase and (ironically) probably raise the likelihood of the emergence of resistant human pathogens.

None of the non-antibiotic AGP alternatives suggested below is likely to compensate fully for the loss of AGPs. It must be emphasised that some strategies will only help to compensate partially (but will not replace) AGPs, and will work through indirect mechanisms. The list is by no means exhaustive and there are also other products claiming to be of value in AGP-free diets.

Some alternatives for AGPs and their mode of action

Exogenous enzymes

Non-starch polysaccharides (NSPs) in animal feedstuffs are a complex group of components differing widely in chemical composition, physical properties and physiological activity, many of which have negative effects on growth and performance. NSPs include (hemi)celluloses, pectins and oligosaccharides as well as arabinoxylans and β-glucans (consisting of either a more soluble or a non-soluble fraction).

Different cereal types contain variable NSP levels with concomitant differences in chemical composition. For example, maize contains almost exclusively insoluble NSPs, whereas wheat and barley contain NSPs of which the ratio of soluble to insoluble is about 1/6. This ratio is about 3/4 in rye, making this cereal one with particularly high levels of soluble NSPs (Choct, 2002).

The mechanism by which NSPs exert their anti-nutritive effects is complex, but their viscous nature is considered a primary cause for their anti-nutritive effect in poultry. This is because the increased bulk and viscosity of the intestinal contents decrease the rate of diffusion of substrates and digestive enzymes and hinder their effective interaction at the mucosal surface (Choct et al., 1996). NSPs also induce thickening of the mucous layer on the intestinal mucosa (Hedemann et al.,

153 2009) suggesting that the concentrations of soluble NSPs in wheat are inversely correlated with
154 their metabolisable energy (MEn)-values in broiler chickens (Annison, 1991).

155

156 In addition to the direct effect of viscous NSPs on gut physiology and morphology, there
157 appear to be some indirect effects that could have important implications for the efficient use of
158 nutrients by the chicken (Dänicke et al., 1999). One such indirect effect may be related to
159 stimulation of fermentation of NSPs by the gut microbiota, leading to volatile fatty acid production
160 (VFA) in the small intestine. Under normal circumstances with low NSP-diets, facultative
161 anaerobes predominate in the chicken small intestine and nearly strict anaerobes make up the entire
162 caecal microbiota (Salanitro et al., 1978; Lu et al., 2003; Bjerrum et al., 2006). On a NSP-rich diet,
163 the VFA-concentration increases mainly in the distal ileal lumen due to excess fermentation
164 combined with a proliferation of the fermentative microflora with a rather limited effect on the
165 activity of the hindgut microbiota (Choct et al., 1996; 1999). Small intestinal fermentation indicates
166 competition with the host for digestible nutrients. Enzyme-free diets containing soluble-NSP rich
167 cereals (wheat) have been shown to induce lymphocyte infiltration in the gut wall and induce
168 apoptosis of epithelial cells much more than cereals such as maize that have low levels of soluble-
169 NSPs (Teirlynck et al., 2009).

170

171 Negative effects of diets with high NSP levels can be partly counterbalanced by adding AGPs
172 (Teirlynck et al., 2009). Without these, supplementing the NSP-rich diet with enzymes results in
173 both a reduction in ileal VFA-concentration and an elevation in caecal VFA-concentration (Choct et
174 al., 1996) as more ‘low molecular weight’ fermentable material is entering the caecum. Caecal
175 fermentation suggests the conversion of indigestible compounds into readily absorbable VFAs.

176

Dietary NSP-enzymes work by reducing the viscosity of the digesta in the small intestine, so that digesta passage and nutrient digestion rate increase providing less substrate and less time for the fermentation organisms to proliferate. This may restore the normal and efficient endogenous enzymatic digestion of nutrients in the small intestine. The enzymes are partially counterbalancing the adverse effects of soluble NSP on performance (Bedford and Classen, 1992).

It is not possible to measure the relative contribution following improved nutrient utilisation or the 'selective' reduction in the microbial population (Smits and Annison, 1996). However, there is evidence that the consequence of a NSP-mediated reduced rate of digestion is much more radical in the presence of intestinal microbiota due to the degradation of both digestive enzymes and bile salts and colonisation of the absorptive surface area (Smits and Annison, 1996). In the absence of antimicrobial growth promoters (as in the European Union), there will be a greater response to enzymes, particularly in less well-digested diets (Elwinger and Teglöf, 1991). Furthermore, NSP degrading enzymes will also reduce the proliferation of pathogenic bacteria such as *Clostridium perfringens* (Jackson et al., 2003). These days all broiler feed contains enzymes such as xylanases and beta-glucanases that breakdown NSPs.

Organic acids

Organic acids have been shown to have beneficial effects on performance. Some (e.g. butyric acid) also decrease the incidence of subclinical necrotic enteritis caused by *C. perfringens*, an additional beneficial effect which is highly relevant for the poultry industry (Timbermont et al., 2009). Organic acids are widely distributed in nature as normal constituents of plants or animal tissues. They are also formed through microbial fermentation of carbohydrates predominantly in the caeca of poultry (Van Der Wielen et al., 2000).

A wide range of organic acids with variable physical and chemical properties exists, of which many are used as drinking water supplements or as feed additives (acidifiers). Many are also available as sodium, potassium or calcium salts (and/or partially esterified). The advantage of salts over acids is that they are generally odourless and easier to handle in the feed manufacturing process owing to their solid and less volatile form. They are also less corrosive and may be more soluble in water.

The mechanism of action of organic acids probably reflects their antibacterial nature, such as decreasing the pH of drinking water and reducing the buffering capacity of the feed with subsequent effect on the physiology of the crop and proventriculus (Thompson and Hinton, 1997; Van Immerseel et al., 2006). The ability of organic acids to change from undissociated to the dissociated form (depending on the environmental pH) enhances their antimicrobial effect. When the acid is in the undissociated form it can freely diffuse through the semi-permeable membrane of the microorganisms into the cell cytoplasm (Adams and Hall, 1988; Van Immerseel et al., 2006). Once in the cell, where the pH is maintained near 7, the acid will dissociate and suppress bacterial cell enzymes (e.g. decarboxylases and catalases) and nutrient transport systems. The efficacy of an acid in inhibiting microbes is dependent on its pKa value, which is the pH at which 50% of the acid is dissociated. Organic acids with higher pKa values are more effective antibacterial compounds and their efficacy is generally improved with increasing chain length and degree of unsaturation.

In general, variables that influence antibacterial activity are (1) chemical formula, (2) pKa-value of the acid, (3) chemical form (esterified or not, acid, salt, coated or not), (4) molecular weight, (5) the micro-organism related MIC-value of the acid, (6) the nature of the micro-organism, (7) animal species, and (8) the buffering capacity of the feed (Patten and Waldroup, 1988;

Thompson and Hinton, 1997). It is thus clear that each acid has its own spectrum of microbial activity related to differences in both specific pH-range, membrane structure and in-cell physiology of the microbiota species.

Blends of acids represent an array of pKa values and are used because of the broader spectrum of activity. The physical form of the acids also plays a role in the AGP replacement effect. The coating or micro-encapsulation of fatty acids with a progressive 'slow release' matrix is essential for their anti-microbial activity throughout the distal part of the gastro-intestinal tract. Also additive effects of acids are possible. There are indications that the medium chain fatty acids (with a chain length between 8 and 12 carbon atoms, e.g. caproic acid, has a lower absorption rate because of the higher molecular weight) may improve the efficacy of the short chain fatty acids. In the field, mixtures of organic acids are mainly used, which makes their spectrum broader and combines the good qualities of the different acids.

The possibility that providing additional organic acids in the feed may act as a rapidly absorbed energy source cannot yet be ruled out. Moreover, there is some evidence of increased growth of the gastro-intestinal mucosa in the presence of organic acids, particularly fatty acids such as butyric acid. It has been well documented that butyric acid exerts a wide variety of effects on intestinal function in rodents and humans, and these effects may also be present in livestock. Indeed, butyric acid has been shown to be an important energy source for gut epithelial cells and to stimulate epithelial cell proliferation and differentiation (Dalmasso et al., 2008). Butyric acid also has well documented anti-inflammatory effects (Hodin, 2000) and has been shown to strengthen the gut mucosal barrier by increasing production of antimicrobial peptides in mucous and by stimulating the expression of tight junction proteins (Mariadason et al., 1997; Schauber et al., 2003; Bordin et al., 2004; Peng et al., 2007). Thus for some acids, especially butyric acid, not only

antibacterial but also host effects can play a role in the AGP-replacement effect. Whether other acids, such as medium-chain fatty acids, have similar effects on host cell activities has not yet been investigated.

Probiotics or 'direct-fed microbials'

Probiotics have been defined as *'mono- or mixed cultures of living microorganisms which beneficially affect the host by improving the properties of the indigenous microbiota*' (Fuller, 1992). The available probiotics can be classified into (1) 'colonizing' species, such as *Lactobacillus* and *Enterococcus* spp., and (2) free flowing 'non-colonizing' species, such as *Bacillus* spp. (spores) and *Saccharomyces cerevisiae*. Competitive exclusion (CE) describes the treatment of day-old chicks with an undefined microbiota derived from adult animals resulting in colonisation resistance against pathogenic microorganisms.

There is considerable variation in published studies that evaluate the effect of probiotic strains on performance. It is not the aim of the current review to summarise these data but the search terms PROBIOTICS, PERFORMANCE and POULTRY in Medline leads to numerous publications on this issue. The differences in outcome are most likely due to differences in dose and nature of the administered strains and their persistence (relative intestinal concentration), stability during feed manufacturing (as well as in the gastro-intestinal tract), variation in the physiological state of the bird, and the actual microbiota balance in the gut of the animal. The ideal probiotic should be an image of the indigenous strains resistant to feed processing (a coating might be helpful, e.g. for living yeast cells), as well as acidity, and the effects of bile salts and digestive enzymes. It must also be rapidly proliferating. Bacteria intended for probiotic use should be screened for antibiotic resistance to avoid any potential carriage of undesirable antibiotic resistance into the intestinal environment.

The mechanism of action of probiotics as AGP replacers will depend on the nature of the organism and is not always clear. The different bacterial species in the normal microbiota (colonising on the epithelium of the digestive tract or occurring freely in the gut lumen) of the broiler gut reach a typical equilibrium state after about a week post-hatch, and depends on many factors including location in the gastro-intestinal tract, integrity of the intestinal mucosa and transit time of the chymus (Van Der Wielen et al., 2000; 2002; Teirlynck et al., 2009).

The intestinal microbiota has a specific multifactorial 'barrier' impact, such as (1) induction of anatomical and physiological changes in the intestinal cell wall structure, (2) immunological modifications in the gut, and (3) enhancement of the bird's resistance to enteropathogenic bacteria, such as *C. perfringens* (Nurmi and Rantala, 1973; Hofacre et al., 1998; La Ragione et al., 2004; Kalliomaki et al., 2008; Ng et al., 2009). Depending on the probiotic strain, the mode of action probably involves production of specific metabolites (short organic fatty acids, H_2O_2, intermediary metabolites with antimicrobial activity), interaction with receptor sites, stimulation of the immune system and some others (Madsen, 2001; Sherman et al., 2009).

The most well known group of probiotics are lactic acid bacteria. It has been shown that lactic acid produced in vitro by lactic acid bacteria is used by the strictly anaerobic butyrate producing bacteria of Clostridial clusters IV and XIVa for the production of large concentrations of butyric acid (Duncan et al., 2004). This mechanism is called cross-feeding and is a further reason why lactic acid bacteria administration can beneficially affect performance (see above in the section on butyric acid).

Prebiotics

Prebiotics can be defined as non-digestible feed ingredients with selective effects on the intestinal microbiota. Oligosaccharides are the main components and the range is diverse and may be based on any of the hexose monosaccharides, including glucose, fructose, galactose and mannose (Durst, 1996) with a polymerisation degree of between 2 and 20 monosaccharides. Grain legumes are the most common natural sources of oligosaccharides, being present as raffinose, stachyose and verbascose. 'Synthetic' oligosaccharides are derived from the direct polymerisation of disaccharides or from the fractionation of both vegetable and microbial cells. Oligosaccharides such as arabinogalactose, arabinoxylan and rhamnogalacturonose are derived from polysaccharides of soybean (with about 3-5% galacto-oligosaccharides), wheat and fruit, respectively (Schols et al., 1994; Huisman et al., 2001; Van Craeyveld et al., 2009)..

The mechanism of action of prebiotics as AGP replacers is dependent on the nature of the compound. They are non-digestible feed ingredients that can have a beneficial action because of selective stimulation of the growth or metabolic activity of a limited number of intestinal microbiota species, such as *Bifidobacteria* and *Lactobacillus* spp. (Gibson and Roberfroid, 1995). Thus they may have a similar mechanism of action as probiotics.

Conflicting results obtained with or without oligosaccharides that occur naturally in feed ingredients (for example the raffinose series oligosaccharides) present an unclear scenario regarding the effect of their inclusion in diets for broilers (Coon et al., 1990; Leske et al., 1991; Iji and Tivey, 1998); however, their nutritional impact cannot be separated from other anti-nutritive components in the diet. The lack of any beneficial effect might be related to the non-specificity of the process of hindgut fermentation. When ingested, prebiotics stimulate the growth and/or metabolic activity of

different bacterial species, including species that are both potentially harmful and beneficial (Maczulak et al., 1993).

Results obtained from synthetic materials suggest some benefits using inulin and fructo-oligosaccharides (FOS) that act as substrates for 'desired' microorganisms, for example bifidobacteria (Waldroup et al., 1993; Iji and Tivey, 1998; Verdonk et al., 2005; Ramirez-Farias et al., 2009), whereas manno-oligosaccharides (MOS) have receptor properties for fimbriae of *E. coli* (sensitive to mannose) and *Salmonella* spp., which leads to elimination of these bacteria with the digesta flow instead of binding a mucosal receptor (Ofek et al., 1977; Spring et al., 2000; Parks et al., 2001; Fernandez et al., 2002).

Oligosaccharide beta-glucans of yeast cell wall origin are thought to stimulate performance because of their immunomodulatory effects. Their main action is to enhance phagocytosis and proliferation of monocytes and macrophages (Novak and Vetvicka, 2009). As macrophages play a crucial role in immunomodulation, the interaction of glucans with macrophages can have huge effects in the host. Recent reviews elaborate on the action of glucans on immune stimulation (Schepetkin and Quinn, 2006; Novak and Vetvicka, 2009). Studies with animals have documented significant health benefits from using immune modulating β-1,3/1,6-glucan (from yeast cell walls) as a feed ingredient to protect animals against micro-organisms (Williams et al., 1996).

One approach for future research will be to examine the combination of both probiotics and prebiotics (as 'synbiotics'), which may be defined as a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and persistence of living microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating

348 the metabolism of one or a limited number of health-promoting bacteria (Patterson and Burkholder,
349 2003). This combination would thus combine substrate and bacteria.

350

351 *Herbs and etheric oils*

352 Many plants have beneficial multifunctional properties derived from their specific bio-active
353 components. Biologically active constituents of plants are mostly secondary metabolites, such as
354 terpenoids (mono-and sesquiterpenes, steroids, etc.), phenolics (tannins), glycosides and alkaloids
355 (present as alcohols, aldehydes, ketones, esters, ethers, lactones, etc.). There is a lot of variation in
356 composition due to biological factors (plant species, growing location, and harvest conditions),
357 manufacturing (extraction/distillation, stabilization) and storage conditions (light, temperature,
358 oxygen tension and time). The challenge is to identify and quantify the multitude of actions and
359 claims improving feed utilisation, animal physiology and health status.

360

361 Because of possible 'synergy' between constituents, it remains unclear which components of
362 etheric oil products may stimulate the endogenous digestive enzymes, act as an antioxidant,
363 antimicrobial agent, or immunomodulator. There are experimental data showing the in vitro anti-
364 microbial effects with respective MIC-values and spectrum of activity (see, for example, Penalver
365 et al., 2005; Fu et al., 2007; Barbosa et al., 2009). According to Adams (1999) the antimicrobial
366 activity is rather weak for ginger and pepper, medium for cumin (p-cymene), coriander (lialol),
367 oregano (carvacrol), rosemary (cineol), sage (cineol) and thyme (thymol) and strong for clove
368 (eugenol), mustard (allylisothiocyanate), cinnamon (cinnamaldehyde) and garlic (allicin).

369

370 **Regulations concerning feed additives for animal use**

A zootechnical additive is any additive other than feed material and pre-mixtures used to affect favourably the performance of animals in good health or used to affect favourably the environment. The category 'zootechnical additive' can be further divided into four functional groups: (1) digestibility enhancers; these are substances which, when fed to animals, increase the digestibility of the diet, through action on target feed materials; (2) gut flora stabilisers; these are micro-organisms or other chemically defined substances, which, when fed to animals, have a positive effect on the gut flora; (3) substances which favourably affect the environment; (4) other zootechnical additives.

EC Regulation 1831/2003 will establish a Community procedure for authorising the placing on the market and use of feed additives and to lay down rules for the supervision and labelling of feed additives and pre-mixtures in order to provide the basis for the assurance of a high level of protection of human health, animal health and welfare, environment and users' and consumers' interests in relation to feed additives, whilst ensuring the effective functioning of the internal market. This Regulation will not apply to processing aids and veterinary medicinal products as defined in Directive 2001/82/EC[3], with the exception of coccidiostats and histomonostats used as feed additives. However, prebiotics (inulin, fructo-mannanoligosaccharides, yeast cell walls rich in beta-glucans) or short and medium chain fatty acids are considered as feedstuffs and not as feed additives and so fall under the scope of Regulation 0767/2009[4] concerning the trade of animal feeds. However, the classification of a product as either a feedstuff or a feed additive remains unclear because of differences in the relative impact of technological processing.

Botanical or herbal extracts, flavours and etheric oils (EOs) now fall within the scope of EC Regulation 1831/2003. In general, unprocessed herbs are regarded as feed materials and do not need

[3] See: http://www.echamp.eu/fileadmin/user_upload/Regulation/Directive_2001-82-EC_-_Consolidated_Version_.pdf
[4] See: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:229:0001:0028:EN:PDF

authorisation. Notified plant extracts or components are included in the Community Register of Feed Additives[5]. This register has only informative purposes and does not replace Community legal acts. The Community legal acts concerning the authorisation of each additive entered in the Register constitute the legal basis for the placing on the market and use of additive concerned. This means that before November 2010, a complete scientific dossier for each notified EO or component shall be submitted to EFSA (European Food Safety Authority) which provides guidance on scientific data needed to carry out a safety assessment for botanical agents[6]. After a full evaluation by EFSA, a positive outcome and authorisation by the Standing Committee on the Food Chain and Animal Health (SCFCAH), these EOs can be used legally in the EU in animal nutrition[7]. The actual status of the EFSA evaluation of the scientific dossier can be checked at the Register of Questions[8].

Economical considerations

The general health status determining the performance of broilers is multifactorial. Good management relies on continuous monitoring of the flock for health status and performance. Monitoring of the health status requires regular necropsies with determinations of lesion scores, and identification of pathogens including the make-up of an antibiogram. Monitoring of performance requires information such as feed intake and weight gain, flock uniformity, litter score, climatic and other conditions. This should, at least in theory, provide a view of the cost of the ban in terms of decreased growth rate, higher morbidity and mortality, increased condemnations, and depressed yield.

The return-on-investment for alternatives to AGPs will depend on both the biological impact and the actual market price. It must take into account the fact that the feed cost of these alternatives

[5] See: http://ec.europa.eu/food/food/animalnutrition/feedadditives/registeradditives_en.htm
[6] http://www.efsa.europa.eu/cs/BlobServer/Guidance_of_Panel/sc_op_ej1249_botanicals_en.pdf
[7] http://ec.europa.eu/food/food/animalnutrition/feedadditives/index_en.htm
[8] http://registerofquestions.efsa.europa.eu/roqFrontend/questionsList.jsf

418 is quite variable, ranging from (€/ton[9]): 2-3 for enzymes, 3-12 for organic acids, 4-7 for probiotics,
419 9-17 for immunostimulants, 2-15 for oligosaccharides, 3-25 for herbs and etheric oils (in
420 comparison with 1-2 and 2-4 for respectively feed antibiotics and anticoccidials, depending on dose
421 and type of product). The net economic effect will depend on several factors including the effects
422 on performance levels and the cost of any technologies adopted to compensate for the termination
423 of AGPs, and may be offset by the benefits of increased consumer confidence.
424
425 Unlike with pigs, termination of AGPs in European poultry has not resulted in increases in
426 therapeutic use of antimicrobials largely because of the continuing use of ionophores for the
427 prophylaxis of coccidiosis (Grave et al., 2006). Indeed both chemical and ionophore anticoccidials
428 are almost universally used in the broiler industry. Without these drugs (e.g. during the withdrawal
429 period), birds will become infested and damage to the intestinal epithelial cells will provide further
430 opportunities for 'subclinical' necrotic enteritis and performance problems. Ionophore
431 anticoccidials also have an additional antibacterial potential. In article 11 of the European Council
432 regulation 1831/2003, the European Union states that the use of anticoccidials as feed additives
433 should be phased out by December 2012. However, in 2008, the European Commission submitted a
434 report on the use of these substances as feed additives and existing alternatives to the Council and
435 the European Parliament (COM/2008/0233[10]). In this report, the European Commission clearly
436 recommends maintaining the current legislation and allowing the use of ionophore anticoccidials as
437 feed additives because of the lack of alternatives and to preserve the economic viability of the
438 poultry industry.
439
440 AGP replacers seem to be adequate when other control measures that beneficially affect gut
441 health are also applied. The question remains whether the current AGP replacers have enough

[9] €1 = approx. £0.89 , $1.43, as at 07 January 2010.

potential to replace both AGPs and anticoccidials. There is a need to set and then to meet standards for the replacement of antibiotic compounds in poultry, in terms of product type, identification of suppliers, poultry response criteria, regulatory status and veterinary definition (Rosen, 2003).

Conclusions

Alternatives for AGPs are only of practical significance when they improve animal performance at levels comparable to AGPs. Microbiota modulating and immunomodulatory compounds have potential and are used as feedstuff of feed additives. Enzymes, acids, per- and probiotics and herbs or etheric oils are some examples of product classes which are used as alternatives for AGPs. Within each product class, numerous products are on the market, and while some products clearly have potential, for others the efficacy is not clear. There is thus an urgent need to describe the mechanism of action of these compounds in a scientific way and to set and meet standards for AGP alternatives for broilers.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

References

Adams, C., 1999. Nutricines: Food components in health and nutrition. Nottingham University Press, UK.

Adams, M.R., Hall, C.J., 1988. Growth inhibition of food-borne pathogens by lactic acid and acetic acids and their mixtures. International Journal of Food Science and Technology 23, 287-292.

Annison, G., 1991. Relationship between the levels of soluble nonstarch polysaccharides and the apparent metabolisable energy of wheats assayed in broiler chickens. Journal of Agricultural Food Chemistry 39, 1252-1256.

[10] See: http://www.ipex.eu/ipex/webdav/site/myjahiasite/groups/CentralSupport/public/2008/COM_2008_0233/COM_COM(2008)0233_EN.pdf

Barbosa, L.N., Rall, V.L., Fernandes, A.A., Ushimaru, P.I., da Sliva Probst, I., Fernandes, A.Jr., 2009. Essential oils against foodborne pathogens and spoilage bacteria in minced meat. Foodborne Pathogen Diseases 6: 725-728.

Bedford, M.R., Classen, H.L., 1992. Reduction of intestinal viscosity through manipulation of dietary rye and pentosanase concentration is effected through changes in the carbohydrate composition of the intestinal aqueous phase and results in improved growth rate and food conversion efficiency of broiler chicks. Journal of Nutrition 122, 560-569.

Bjerrum, L., Engberg, R., Leser, T.D., Jensen, B.B., Finster, K., Pedersen, K., 2006. Microbial community composition of the ileum and cecum of broiler chickens as revealed by molecular and culture-based techniques. Poultry Science 85, 1151-1164.

Bordin, M., D'Atri, F., Guillemot, L., Citi, S., 2004. Histone deacetylase inhibitors up-regulate the expression of tight junction proteins. Molecular Cancer Research 2, 692-701.

Brennan, J., Skinner, J., Barnum, D.A., Wilson, J., 2003. The efficacy of bacitracin methylene disalicylate when fed in combination with narasin in the management of necrotic enteritis in broiler chickens. Poultry Science 82, 360-363.

Castanon, J.I., 2007. History of the use of antibiotic growth promoters in European poultry feeds. Poultry Science 86, 2466-2471.

Choct, M., Hughes, R.J., Wang, J., Bedford, M.R., Morgan, A.J., Annison, G., 1996. Increased small intestinal fermentation responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. British Poultry Science 37, 609-621.

Choct, M., Hughes, R.J., Bedford, M.R., 1999. Effects of a xylanase on individual bird variation, starch digestion throughout the intestine, and ileal and caecal volatile fatty acid production in chickens fed wheat. British Poultry Science 40, 419-422.

Choct, M., 2002. Non-starch polysaccharides: effect on nutritive value. In : McNab, J.M., Boorman, K.N. (Eds.), Poultry feedstuffs. CABI Publishing, New York, US.

Coates, M.E., Fuller, R., Harrison, G.F., Lev, M., Suffolk, S.F., 1963. A comparison of the growth of chicks in the Gustafsson germ-free apparatus and in a conventional environment, with and without dietary supplements of penicillin. British Journal of Nutrition 17, 141-150.

Coon, C.N., Leske, K.L., Akavanichan, O., Cheng, T.K. 1990. Effect of oligosaccharide free soybean meal on true metabolisable energy and fiber digestion in adult roosters. Poultry Science 69, 787-793.

Dalmasso G., Nguyen H.T., Yan Y., Charrier-Hisamuddin L., Sitaraman S.V., Merlin D., 2008. Butyrate transcriptionally enhances peptide transporter PepT1 expression and activity. PLoS ONE. 3: e2476.

Dänicke, S., Vahjen, W., Simon, O., Jeroch, H., 1999. Effects of dietary fat and xylanase supplementation to rye-based broiler diets on selected bacteria groups adhering to the intestinal epithelium, on transit time of feed, and on nutrient digestibility. Poultry Science 78, 1292-1299.

Dibner, J.J., Richards, J.D., 2005. Antibiotic growth promoters in agriculture: history and mode of action. Poultry Science 84, 634-643.

Duncan, S.H., Louis, P., Flint, H.J., 2004. Lactate-utilizing bacteria, isolated from human feces that produce butyrate as a major fermentation product. Applied Environmental Microbiology 70, 5810-5817.

Durst, L., 1996. Inclusion of fructo- and galacto-oligosaccharides in broiler diets. Archives fur Geflügelkunde 60, 160-164.

Elwinger, K., Teglöf, B. 1991. Performance of broiler chickens as influenced by a dietary enzyme complex with and without antibiotic supplementation. Archives fur Geflügelkunde 55, 69-73.

Feighner, S.D., Dashkevicz, M.P., 1987. Sub-therapeutic levels of antibiotics in poultry feeds and their effects on weight gain, feed efficiency, and bacterial cholyltaurine hydrolase activity. Applied and Environmental Microbiology 53, 331-336.

Fernandez, F., Hinton, M., Van Gils, B. 2002. Dietary mannan-oligosacccharides and their effect on chicken caecal microflora in relation to *Salmonella* Enteritidis colonisation. Avian Pathology 31, 49-58.

Fu, Y., Zu, Y., Chen, L., Shi, X., Wang, Z., Sun, S., Efferth, T., 2007. Antimicrobial activity of clove and rosemary oils alone and in combination. Phytotherapy Research 21, 989-994.

Fuller, R. 1992. History and development of probiotics. In: Fuller, R. (Ed.), Probiotics - The scientific basis, Chapman & Hall, London.

George, B.A., Quarles, C.L., Fagerberg, D.J., 1982. Virginiamycin effects on controlling necrotic enteritis infections in chickens. Poultry Science 61, 447-450.

Gibson, G.R., Roberfroid, M.B., 1995. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotica. Journal of Clinical Nutrition 125, 1401-1412.

Grave, K., Jensen, V.F., Odensvik, K., Wierup, M., Bangen, M., 2006. Usage of veterinary therapeutic antimicrobials in Denmark, Norway and Sweden following termination of antimicrobial growth promoter use. Preventive Veterinary Medicine 75, 132-132.

Hedemann, M.S., Theil, P.K., Bach-Knudsen, K.E., 2009. The thickness of the intestinal mucous layer in the colon of rats fed various sources of non-digestible carbohydrates is positively correlated with the pool of SCFA but negatively correlated with the proportion of butyric acid in digesta. British Journal of Nutrition 102, 117-125.

Hodin, R., 2000. Maintaining gut homeostasis: the butyrate-NF-kappaB connection. Gastroenterology 118, 798-801.

Hofacre, C.L., Froyman, R., Gautrias, B., George, B., Goodwin, M.A., Brown, J., 1998. Use of Aviguard and other intestinal bioproducts in experimental *Clostridium perfringens*-associated necrotizing enteritis in broiler chickens. Avian Diseases 42, 579-584.

Huisman, M.M., Brül, L.P., Thomas-Oates, J.E., Haverkamp, J., Schols, H.A., Voragen, A.G. 2001. The occurrence of internal (1-5)-linked arabinofuranose and arabinopyranose residues in arabinogalactan side chains from soybean pectic substances. Carbohydrate Research 330, 103-114.

Humphrey, B. D., Klasing, K.C., 2003. Modulation of nutrient metabolism and homeostasis by the immune system. In: Proceedings of the European Symposium on Poultry Nutrition, Lillehammer, Norway.

Iji, P.A., Tivey, D.R., 1998. Natural and synthetic oligosaccharides in broiler chicken diets. World Poultry Science Journal 54, 129-143.

Jackson, M.E., Anderson, D.M., Hsiao, H.Y., Mathis, G.F., Fodge, D.W., 2003. Beneficial effect of β-mannanase feed enzyme on performance of chicks challenged with *Eimeria* sp. and *Clostridium perfringens*. Avian Diseases 47, 759-763.

Kalliomäki, M., Salminen, S., Isolauri, E., 2008. Positive interactions with the microbiota : probiotics. Advances in Experimental Medical Biology 635, 57-66.

Knarreborg, A., Lauridsen, C., Engberg, R.M., Jensen, S.K., 2004. Dietary antibiotic growth promoters enhance the bioavailability of alpha-tocopheryl acetate in broilers by altering lipid absorption. Journal of Nutrition 134, 1487-1492.

La Ragione, R.M., Narbad, A., Gasson, M.J., Woodward, M.J., 2004. *In vivo* characterization of *Lactobacillus johnsonii* FI9785 for use as defined competitive exclusion agent against bacterial pathogens in poultry. Letters in Applied Microbiology 38, 197-205.

Leske, K.L., C.J. Jevne, Coon, C.N., 1991. Extraction methods for removing soybean alpha galactosides and improving true metabolisable energy for poultry. Animal Feed Science and Technology 41, 73-78.

Lu, J. Idris, U., Harmon, B., Hofacre, C., Maurer, J.J., Lee, M.D., 2003. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. Applied and Environmental Microbiology 69, 6816-6824.

Maczulak, A.E., M.J. Wolin, Miller, T.L., 1993. Amounts of viable anaerobes, methanogens and bacterial fermentation products in faeces of rats fed high fiber or fiber-free diets. Applied and Environmental Microbiology 59, 657-662.

Madsen, K.L., Cornish, A., Soper, P., McKaigney, C., Jijon, H., Yachimec, C, Doyle, J., Jewell, L., De Simone, C., 2001. Probiotic bacteria enhance murine and human intestinal epithelial barrier function. Gastroenterology 121, 580-591.

Mariadason, J.M., Barkla, D.H., Gibson, P.R., 1997. Effect of short-chain fatty acids on paracellular permeability in Caco-2 intestinal epithelium model. American Journal of Physiology 272, G705-712.

Ng, S.G., Hart, A.L., Kamm, M.A., Stagg, A.J., Knight, S.C., 2009. Mechanism of action of probiotics: recent advances. Inflammatory Bowel Diseases 15: 300-310.

Niewold, T.A., 2007. The nonantibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. World Poultry Science Journal 86: 605-609.

Novak, M., Vetvicka, V., 2008. Beta-glucans, history, and the present: immunomodulatory aspects and mechanisms of action. Journal of Immunotoxicology 5, 47-57.

Nurmi, E., Rantala, M., 1973. New aspects of *Salmonella* infection in broiler production. Nature 241, 210-211.

Ofek, I., Mirelman, D., Sharon, N., 1977. Adherence of Escherichia coli to human mucosal cells mediated by mannose receptors. Nature (London) 265, 623-625.

Parks, C.W., Grimes, J.L., Ferket, P.R., Fairchild, A.S., 2001. The effect of mannan-oligosaccharides, Bambermycin, and Virginiamycin on performance of Large White male mate turkeys. Poultry Science 80, 718-723.

Patten, J.D., Waldroup, P.W., 1988. Use of organic acids in broiler diets. Poultry Science. 67, 1178-1182.

Patterson, J.A., Burkholder, K.M., 2003. Application of prebiotics and probiotics in poultry production. Poultry Science 82, 627-631.

Pedroso, A.A., Menten, J.F., Lambais, M.R., Racanicci, A.M., Longo, F.A., Sorbara, J.O., 2006. Intestinal bacterial community and growth performance of chickens fed diets containing antibiotics. Poultry Science 85: 747-752.

Penalver, P., Huerta, B., Borge, C., Astorga, R., Romero, R., Perea, A., 2005. Antimicrobial activity of five essential oils against origin strains of the Enterobacteriaceae family. APMIS 113, 1-6.

Peng, L., He, Z., Chen, W., Holzman, I.R., Lin, J., 2007. Effects of butyrate on intestinal barrier function in a Caco-2 cell monolayer model of intestinal barrier. Pediatry Research 61, 37-41.

Ramirez-Farias, C., Slezk, K., Fuller, Z., Duncan, A., Holtrop, G., Louis, P., 2009. Effect on inulin on the human gut microbiota : stimulation of Bifidobacterium adolescentis and Faecalibacterium prausnitzii. British Journal of Nutrition 101, 541-550.

Salanitro, J.P., Blake, I.G., Muirhead, P.A., Maglio, M., Goodman, J.R., 1978. Bacteria isolated from the duodenum, ileum and caecum of young chicks. Applied and Environmental Microbiology 35, 782-790.

Schauber, J., Svanholm, C., Termén, S., Iffland, K., Menzel, T., Scheppach, W., Melcher, R., Agerbeth, B., Lührs, H., Gudmundsson, G.H., 2003. Expression of the cathelicidin LL-37 is modulated by short-chain fatty acids in colonocytes: relevance of signalling pathways. Gut 52, 735-741.

Schepetkin, I.A., Quinn, M.T., 2006. Botanical polysaccharides: macrophage immunomodulation and therapeutic potential. International Immunopharmacology 6, 317-333.

Schols, H.A., Voragen, A.G., Colquhoun, I.J. 1994. Isolation and characterization of rhamnogalacturan oligomers, liberated during degradation of pectic hairy regions by rhamnogalacturonase. Carbohydrate Research 256, 97-111.

672 Sherman, P.M., Ossa, J.C., Johnson-Henry, K., 2009. Unravelling mechanisms of action of
673 probiotics. Nutrition Clinical Practice 21, 10-14.
674
675 Smits, C.H.M. Annison, G., 1996. Non-starch polysaccharides in broiler nutrition – towards a
676 physiologically valid approach to their determination. World Poultry Science Journal 52, 203-221.
677 Snyder, D.L., Wostmann, B.S., 1987. Growth rate of male germfree Wistar rats fed ad libitum or
678 restricted natural ingredient diet. Laboratory Animal Science 37, 320-325.
679
680 Spring, P., Wenk, C., Dawson, K.A., Newman, K.E., 2000. The effects of dietary mannan-
681 oligosaccharides on caecal parameters and the concentrations of enteric bacteria in the caeca of
682 Salmonella-challenged broiler chicks. Poultry Science 79, 205-211.
683
684 Teirlynck, E., Bjerrum, L., Eeckhaut, V., Huyghebaert, G., Pasmans, F., Haesebrouck, F., Dewulf,
685 J., Ducatelle, R., Van Immerseel, F., 2009. The cereal type in feed influences gut wall morphology
686 and intestinal immune cell infiltration in broiler chickens. British Journal of Nutrition in press.
687
688 Thompson, J.L., Hinton, M., 1997. Antibacterial activity of formic and propionic acids in the diet of
689 hens on Salmonella in the crop. British Poultry Science 38, 59-65.
690
691 Timbermont, L., 2009. A contribution to the pathogenesis and treatment of Clostridium perfringens
692 associated necrotic enteritis in broilers. PhD thesis, Faculty of Veterinary Medicine, Ghent
693 University.
694
695 Van Craeyveld, V., Holopainen, U., Selinheimo, E., Poutanen, K., Delcour, J., Courtin, C.M. 2009.
696 Extensive dry ball milling of wheat and rye bran leads to in situ production of arabinoxylan
697 oligosaccharides through nanoscale fragmentation. Journal of Agricultural Food Chemistry 57,
698 8467-8473.
699
700 Van Der Wielen, P.W., Biesterveld, S., Notermans, S., Hofstra, H., Urlings, B.A., Van Knapen, F.,
701 2000. Role of volatile fatty acids in development of the cecal microflora in broiler chickens during
702 growth. Applied and Environmental Microbiology 71, 2206-2207.
703
704 Van Der Wielen, P.W., Keuzenkap, D.A., Lipman, L.J., Van Knapen, F., Biesterveld, S., 2002.
705 Spatial and temporal variation of the intestinal bacterial community in commercially raised broiler
706 chickens during growth. Microbial Ecology 44, 286-293.
707
708 Van Immerseel, F., Russell, J.B., Flythe, M.D., Gantois, I., Timbermont, L., Pasmans, F.,
709 Haesebrouck, F., Ducatelle, R., 2006. The use of organic acids to combat Salmonella in poultry: a
710 mechanistic explanation of the efficacy. Avian Pathology 35, 182-188.
711
712 Waldroup, A.L., Skinner, J.T., Hierholzer, R.E., Waldroup, P.W., 1993. An evaluation of fructo-
713 oligosaccharide in diets for broiler chickens and effects on salmonellae contamination of carcasses.
714 Poultry Science 72, 643-650.
715
716 Wierup, M., 2001. The Swedish experience of the 1986 ban of antimicrobial growth promoters,
717 with special reference to animal health, disease prevention, productivity, and usage of
718 antimicrobials. Microbial Drug Resistance 7, 183-190.
719
720 Williams, D.L., Mueller, A., Browder, W., 1996. Glucan-based macrophage stimulators-A review
721 of their anti-infective potential. Clinical Immunotherapy 5, 392-399.
722

Wise M.G., Siragusa, G.R., 2007. Quantitative analysis of the intestinal bacterial community in one- to three-week-old commercially reared broiler chickens fed conventional or antibiotic-free vegetable-based diets. Journal of Applied Microbiology 102, 1138-1149.