



Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvj

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

ARTICLE INFO

Article history:

Accepted 2 March 2010

Keywords:

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

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 profitability in intensive husbandry, but 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 are briefly described in this review.

© 2010 Elsevier Ltd. All rights reserved.

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, most were given 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. 1831/2003¹). 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, 1992²).

In other countries, such as the USA, consumer pressure is pushing the poultry industry to rear animals without AGPs (Dibner and Richards, 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 often referred to as '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', and 'feed passage syndrome'. 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 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

* Corresponding author. Tel.: +32 92522619; fax: +32 92522601.

E-mail address: gerard.huyghebaert@ilvo.vlaanderen.be (G. Huyghebaert).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>.

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 Dashkevich, 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 are added to broiler feed (Pedroso et al., 2006; Wise and Siragusa, 2007). Indeed, sub-MIC concentrations do 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) hypothesised 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 con-

tains 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., 2009) suggesting that the concentrations of soluble NSPs in wheat are inversely correlated with their metabolisable energy (ME_N)-values in broiler chickens (Annisson, 1991).

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

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

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 Annisson, 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 Annisson, 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, 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 micro-organisms 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 antimicrobial 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, such as 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 (Dalmaso 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; Schaubert 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) 'colonising' species, such as *Lactobacillus* and *Enterococcus* spp., and (2) free flowing 'non-colonising' 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 micro-organisms.

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 have 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; Kalliomäki 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, H₂O₂, intermediary metabolites with antimicrobial activity), interaction with receptor sites, stimulation of the immune system and some others (Madsen et al., 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' micro-organisms, 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 *Escherichia 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, 2008). 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, 2008). 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 gastro-intestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria (Patterson and Burkholder, 2003). This combination would thus combine substrate and bacteria.

Herbs and etheric oils

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

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

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, and (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-mannan-oligosaccharides, yeast cell walls rich in

³ See: http://www.echamp.eu/fileadmin/user_upload/Regulation/Directive_2001-82-EC_-_Consolidated_Version_.pdf.

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 0767/2009⁴ 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 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 European Food Safety Authority (EFSA) 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 (SCFAH), 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 is quite variable, ranging from (€/ton⁹): 2 to 3 for enzymes, 3 to 12 for organic acids, 4 to 7 for probiotics, 9 to 17 for immunostimulants, 2 to 15 for oligosaccharides, and 3 to 25 for herbs and etheric oils (in comparison with 1–2 and 2–4 for feed antibiotics and anticoccidials, respectively, depending on dose and type of product). The net economic effect will depend on several factors including the effects on performance levels and the cost of any technologies adopted to compensate for the termination of AGPs, and may be offset by the benefits of increased consumer confidence.

Unlike with pigs, termination of AGPs in European poultry has not resulted in increases in therapeutic use of antimicrobials largely because of the continuing use of ionophores for the prophylaxis of coccidiosis (Grave et al., 2006). Indeed both chemical and ionophore anticoccidials are almost universally used in the broiler industry. Without these drugs (e.g. during the withdrawal period),

birds will become infested and damage to the intestinal epithelial cells will provide further opportunities for 'subclinical' necrotic enteritis and performance problems. Ionophore anticoccidials also have an additional antibacterial potential. In article 11 of the European Council regulation 1831/2003, the European Union states that the use of anticoccidials as feed additives should be phased out by December 2012. However, in 2008, the European Commission submitted a report on the use of these substances as feed additives and existing alternatives to the Council and the European Parliament (COM/2008/0233¹⁰). In this report, the European Commission clearly recommends maintaining the current legislation and allowing the use of ionophore anticoccidials as feed additives because of the lack of alternatives and to preserve the economic viability of the poultry industry.

AGP replacers seem to be adequate when other control measures that beneficially affect gut health are also applied. The question remains whether the current AGP replacers have enough 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, 2005).

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 or feed additives. Enzymes, acids, pre- 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 therefore an urgent need to describe the mechanisms 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.
- Annisson, 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.
- Barbosa, L.N., Rall, V.L., Fernandes, A.A., Ushimaru, P.I., da Sliva Probst, I., Fernandes Jr., A., 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.
- ¹⁰ See: [http://www.ipex.eu/ipex/webdav/site/myjahiasite/groups/CentralSupport/public/2008/COM_2008_0233/COM_COM\(2008\)0233_EN.pdf](http://www.ipex.eu/ipex/webdav/site/myjahiasite/groups/CentralSupport/public/2008/COM_2008_0233/COM_COM(2008)0233_EN.pdf).

⁴ See: <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:229:0001:0028:EN:PDF>.

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>.

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

- 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., 2002. Non-starch polysaccharides: effect on nutritive value. In: McNab, J.M., Boorman, K.N. (Eds.), *Poultry Feedstuffs*. CABI Publishing, New York, US.
- 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.
- 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., Dashkevich, M.P., 1987. Sub-therapeutic levels of antibiotics in poultry feeds and their effects on weight gain, feed efficiency, and bacterial cholytaurine hydrolase activity. *Applied and Environmental Microbiology* 53, 331–336.
- Fernandez, F., Hinton, M., Van Gils, B., 2002. Dietary mannan-oligosaccharides 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 and 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 prebiotics. *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.
- 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- κ B 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* F19785 for use as defined competitive exclusion agent against bacterial pathogens in poultry. *Letters in Applied Microbiology* 38, 197–205.
- Leske, K.L., Jevne, C.J., 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., Wolin, M.J., 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–G712.
- 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., Větvicka, 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. *Pediatrics 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.
- Rosen, G., 2005. Setting and meeting standards for the efficient replacement of pronutrients antibiotics in poultry and pig nutrition. In: *Proceedings of Antimicrobial Growth Promoters: Worldwide Ban on the Horizon*, Noordwijk aan Zee, The Netherlands, p. 66.
- 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 rhamnagalacturon oligomers, liberated during degradation of pectic hairy regions by rhamnagalacturonase. *Carbohydrate Research* 256, 97–111.
- Sherman, P.M., Ossa, J.C., Johnson-Henry, K., 2009. Unravelling mechanisms of action of probiotics. *Nutrition Clinical Practice* 21, 10–14.
- Smits, C.H.M., Annison, G., 1996. Non-starch polysaccharides in broiler nutrition – towards a physiologically valid approach to their determination. *World Poultry Science Journal* 52, 203–221.
- Snyder, D.L., Wostmann, B.S., 1987. Growth rate of male germfree Wistar rats fed ad libitum or restricted natural ingredient diet. *Laboratory Animal Science* 37, 320–325.
- Spring, P., Wenk, C., Dawson, K.A., Newman, K.E., 2000. The effects of dietary mannan-oligosaccharides on caecal parameters and the concentrations of enteric bacteria in the caeca of *Salmonella*-challenged broiler chicks. *Poultry Science* 79, 205–211.
- Teirlinck, E., Bjerrum, L., Eeckhaut, V., Huyghebaert, G., Pasmans, F., Haesebrouck, F., Dewulf, J., Ducatelle, R., Van Immerseel, F., 2009. The cereal type in feed

- influences gut wall morphology and intestinal immune cell infiltration in broiler chickens. *British Journal of Nutrition* 102, 1453–1461.
- Thompson, J.L., Hinton, M., 1997. Antibacterial activity of formic and propionic acids in the diet of hens on *Salmonella* in the crop. *British Poultry Science* 38, 59–65.
- Timbermont, L., 2009. A contribution to the pathogenesis and treatment of *Clostridium perfringens* associated necrotic enteritis in broilers. PhD thesis, Faculty of Veterinary Medicine, Ghent University.
- Van Craeyveld, V., Holopainen, U., Selinheimo, E., Poutanen, K., Delcour, J., Courtin, C.M., 2009. Extensive dry ball milling of wheat and rye bran leads to in situ production of arabinoxylan oligosaccharides through nanoscale fragmentation. *Journal of Agricultural Food Chemistry* 57, 8467–8473.
- Van Der Wielen, P.W., Biesterveld, S., Notermans, S., Hofstra, H., Urlings, B.A., Van Knapen, F., 2000. Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. *Applied and Environmental Microbiology* 71, 2206–2207.
- Van Der Wielen, P.W., Keuzenkap, D.A., Lipman, L.J., Van Knapen, F., Biesterveld, S., 2002. Spatial and temporal variation of the intestinal bacterial community in commercially raised broiler chickens during growth. *Microbial Ecology* 44, 286–293.
- Van Immerseel, F., Russell, J.B., Flythe, M.D., Gantois, I., Timbermont, L., Pasmans, F., Haesebrouck, F., Ducatelle, R., 2006. The use of organic acids to combat *Salmonella* in poultry: a mechanistic explanation of the efficacy. *Avian Pathology* 35, 182–188.
- Verdonk, J.M.A.J., Shim, S.B., van Leeuwen, P., Verstegen, W.A., 2005. Application of inulin-type fructans in animal feed and pet food. *British Journal of Nutrition* 93 (Suppl. 1), S125–S138.
- Waldroup, A.L., Skinner, J.T., Hierholzer, R.E., Waldroup, P.W., 1993. An evaluation of fructo-oligosaccharide in diets for broiler chickens and effects on *Salmonellae* contamination of carcasses. *Poultry Science* 72, 643–650.
- Wierup, M., 2001. The Swedish experience of the 1986 ban of antimicrobial growth promoters, with special reference to animal health, disease prevention, productivity, and usage of antimicrobials. *Microbial Drug Resistance* 7, 183–190.
- Williams, D.L., Mueller, A., Browder, W., 1996. Glucan-based macrophage stimulators – a review of their anti-infective potential. *Clinical Immunotherapy* 5, 392–399.
- 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.