Antibiotic Growth Promoters in Agriculture: History and Mode of Action

J. J. Dibner*[,1] and J. D. Richards*

*Novus International, Inc., St. Charles, Missouri 63304

ABSTRACT This report will review the history of antibiotic growth promoter (AGP) use in the animal industry, concerns about development of antimicrobial resistance, and response in the European Union and United States to these concerns. A brief description of the history of legislation regarding feed use of antimicrobials in Denmark and the experience of animal producers following the 1998 ban will serve to illustrate the consequences on animal performance and health of withdrawing the approval for this use. The biological basis for antibiotic effects on animal growth efficiency will consider effects on intestinal microbiota and effects on the host animal and will use the germ-free animal to illustrate effects of the conventional microflora. The probability that no single compound will replace all of the functions of antimicrobial growth promoters will be considered, and methods to consolidate and analyze the enlarging database will be discussed.

(*Key words*: antibiotic growth promoter, antimicrobial, poultry, nutrition)

2005 Poultry Science 84:634–643

INTRODUCTION

Antibiotic growth promotion in agricultural animal production has been practiced for about 50 yr in the United States and other countries. Early indications of a beneficial effect on production efficiency in poultry and swine were reported by Moore et al. (1946) and Jukes et al. (1950). One of the first reports of resistance in food animals was made by Starr and Reynolds (1951) after experimental feeding of streptomycin in turkeys. Other researchers (Barnes, 1958; Elliott and Barnes, 1959) have reported an association of resistance to tetracycline when growth-promoting levels of antibiotic are fed to chickens. Early concerns about the development of antibiotic resistance in human pathogens and recommendations to ban subtherapeutic use in animal feeds were discussed by Swann in a report to the British Parliament (1969). Indeed, evidence exists that antibiotic resistance genes can be and are transmitted from animal to human microbiota (Greko, 2001). Monitoring and identifying resistance mechanisms and their dissemination into the food chain were recently reviewed by Roe and Pillai (2003). Pathogenic bacteria resistant to a number of antimicrobial agents emerged worldwide in the 1980s (Aarestrup, 2003). As these were detected, several reports were published recommending a ban on antimicrobial use in food animals as a precautionary measure.

In the United States, recommendations to reduce or eliminate the use of antimicrobials in feed were made in 2 reports by the Institute of Medicine (1980, 1989), a Council for Agricultural Science and Technology report (1981), and a Committee on Drug Use in Food Animals report (1998). The reports did not present data proving that resistant microorganisms selected during the use of antibiotic growth promoters (AGP) in food animals cause antibiotic-resistant infections in humans. In fact the relationship is still under vigorous debate (Alpharma, 2004; Dawe, 2004, Phillips et al., 2004; Vaughn and Copeland, 2004). Recent meetings of the Poultry Science Association and Western Poultry Disease Conference (WPDC) included sessions on the issue, and reports from these and other proceedings discuss the significance of existing scientific evidence that antibiotic resistance in feed animals is associated with resistant infections in humans (Cervantes, 2004). The proceedings from the WPDC contain reports presenting scientific and political information from both sides of the debate (WPDC, 2004).

The World Health Organization (WHO) published a report on the medical impact of the use of antimicrobials in food animals suggesting a link between the two on an epidemiological basis (1997). This report (World Health Organization, 2000) recommends, on precautionary

Received for publication July 23, 2004.
Accepted for publication November 12, 2004.
[1]To whom correspondence should be addressed: Julia.Dibner@novusint.com.

Abbreviation Key: AGP = antibiotic growth promoter; DANMAP = Danish Integrated Antimicrobial Resistance Monitoring and Research Programme; EU = European Union; GI = gastro intestinal; SCFA = short chain fatty acids; WHO = World Health Organization; WPDC = Western Poultry Disease Conference.

grounds, that national governments adopt a proactive approach to reduce the need for antimicrobial use in animals and establish surveillance of antimicrobial usage and resistance. With respect to the use of antimicrobial growth promoters, WHO suggests that use of antimicrobial growth promoters that are in classes also used in humans be terminated or rapidly phased out, by legislation if necessary, unless and until risk assessments are carried out (World Health Organization, 2000). The organization also suggests that animal health management should be routinely practiced so as to avoid the prophylactic use of antimicrobials, and antimicrobial availability should be limited to therapeutic use by prescription. The recommendations are precautionary, based on the potential for a reservoir in food animals of an antibiotic resistant bacterial population (primarily enterococci) that could be transferred to humans.

ANTIMICROBIAL GROWTH PROMOTER USE IN DENMARK AND THE EUROPEAN UNION

Voluntary and Legislated Bans of Antimicrobial Growth Promoters

The first nation to eliminate the use of antimicrobials for growth promotion was Sweden in 1986 (Aarestrup, 2003). In 1993, there were reports of glycopeptide-resistant enterococci (GRE) isolated from food animals in England (Bates et al., 1993). This finding was unexpected because glycopeptides were not approved for use in animals to treat infections. Avoparcin, however, was in use as an antimicrobial growth promoter (Aarestrup, 2003). In response to the reports of GRE, a survey of avoparcin resistance was conducted using isolates from conventional and organic poultry farms (Aarestrup, 1995). No connection was made between the resistance in bacteria from food animals and infection in humans. Nevertheless, the findings led to the first ban on an antimicrobial growth promoter. Avoparcin was banned in Denmark in 1995. The avoparcin ban was in response to concerns that its use created an animal reservoir of GRE and that this was a potential risk to public health (World Health Organization, 2003). In 1997 the Commission of the European Union banned avoparcin in all European Union (EU) member states. In January 1998 Denmark banned the antimicrobial growth promoter virginiamycin, and in February 1998 Danish cattle and chicken producers voluntarily stopped use of all antimicrobial growth promoters as did producers of swine for finisher pigs (World Health Organization, 2003). In July and September 1999, other individual growth promoters were banned by the EU Commission because they belonged to classes of antimicrobials also used in humans (tylosin, spiramycin, bacitracin, and virginiamycin) or were considered unacceptable occupational toxicity risks (olaquindox and carbadox). In December 1999, the Danish swine industry voluntarily stopped the use of all remaining antimicrobial growth promoters in swine under 35 kg (World Health Organization, 2003). Thus, Denmark has restricted the use of antimicrobials to therapeutic use, by prescription only, since January 2000. Use of anticoccidials in the poultry industry is still permitted. The EU Commission plans to withdraw approval for the remaining AGP, including some ionophore antibiotics, in EU member nations in 2006 (Cervantes, 2004).

Actual Usage of Antimicrobials in Denmark Before and After January 2000

Denmark began surveillance of antimicrobial use in food animals in 1995 with the formation of the Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP, 2002). Prior to the ban (1994), total usage of antimicrobials in food animals was 205,686 kg compared with a total use of 94,200 in 2001 (World Health Organization, 2003; Angulo, 2004), a reduction of 54%. In terms of therapeutic use of specific antimicrobials, Table 1 shows the usage (kg of active compound) for treatment of food animals from 1994 through 2002 (DANMAP, 2002). Use has increased, before and after the AGP ban, although there is relatively little change between 2001 and 2002. The use of antimicrobials in aquaculture was not included in the survey before 2001(DANMAP, 2002). How much of the increase between 2000 and 2001 is due to that addition is not clear. The increase in antimicrobial use has included some (e.g., penicillins and macrolides) that are also used in human medicine (DANMAP, 2002). Thus it appears that loss of AGP has resulted in some increase in therapeutic use (Table 1; a 5% increase). It will be interesting to follow the data in future years. Certainly the WHO is continuing to pressure the industry to reduce consumption, encouraging management changes to reduce therapeutic use of antimicrobials (World Health Organization, 2000). Denmark may represent the best opportunity to gauge the effect of banning AGP because addition of new member states over the past 2 yr will make therapeutic antimicrobial usage for the EU very difficult to interpret, and historical data on use as AGP among new members are likely to be incomplete.

Consequences for Animal Productivity and Health

For the broiler industry in Denmark, productivity (kg of broilers produced/m^2 per grow out) has not been affected by the ban of AGP nor has livability (Emborg et al., 2002). Feed conversion, however (total kg of feed used per grow out/total kg of live weight per grow out), did increase by 0.016 kg/kg from November 1995 to May 1999 (1.78 to 1.796). There was very little additional change from 1999 to June 2002 (Emborg et al., 2002). It should be noted that feed efficiency went to highs of 1.83 immediately after the ban and to more than 1.84 in late 1999 (Emborg et al., 2002).

Based on mortality records, fatalities due to necrotic enteritis did not increase after the AGP ban. It should be noted, however, that the consumption of the ionophore

TABLE 1. Trends in the Therapeutic use of antimcrobial compounds[1] in food animals in Denmark (WHO, 2003)

Compound	1994	1996	1998	1999	2000	2001[2]	2002[2]
Tetracyclines	36,500	12,900	12,100	16,200	24,000	28,300	24,300
Penicillins, β-lactamase sensitive	9,400	7,200	14,300	14,700	15,100	16,000	16,900
Other penicillins, cephalosporins	4,400	5,800	6,700	6,600	7,300	8,700	9,800
Sulfonamides and trimethoprim	9,500	4,800	7,700	6,800	7,000	9,400	10,400
Sulfonamides	5,600	2,100	1,000	1,000	1,000	900	850
Macrolides, lincosamides, tiamulin	11,400	7,600	7,100	8,700	15,600	19,900	21,200
Aminoglycosides	8,600	7,100	7,800	7,500	10,400	9,600	9,200
Others	4,400	600	650	350	300	900	1,600
Total	89,900	48,000	57,300	61,900	80,700	93,700	94,300

[1]Only veterinary drugs are included, excluding drugs obviously used in pets (kg of active product).
[2]Does not include consumption in aquaculture before 2001.

anticoccidial salinomycin, which has activity against *Clostridium perfringens* (Watkins et al., 1997; Elwinger et al., 1998; Martel et al., 2004), has increased steadily in Denmark since the ban on AGP. Use of salinomycin in 1996 was 4,500 kg (active compound) and was 11,213 kg in 2002 (DANMAP, 2002). This increase may reflect attempts by producers to use the drug to control necrotic enteritis since the AGP ban in Denmark in 1999.

Finally, these relatively positive health and productivity results for poultry are in contrast to those for swine, in which withdrawal of AGP from weaner pigs is associated with a decline in average daily gain from 422 g in 1995 to 415 g/d in 2001 and an increase from 2.7 to 3.5% in mortality over the same period (Callesen, 2003).

ANTIMICROBIAL GROWTH PROMOTERS IN THE UNITED STATES

There has been relatively little regulatory activity regarding AGP use in the United States. Recently, the use of fluoroquinolones has been examined, and their use as therapeutic agents may be discontinued, based on their similarity to drugs used in humans to treat bacterial infections. The relationship of fluoroquinolone use and human health or food safety is still vigorously debated (Vaughn and Copeland, 2004). It is clear, however, that the practice of using AGP in general is under scrutiny in the United States (Angulo, 2004) and that consumer pressure is affecting commerce to remove AGP from animal feeds. For example, Internet web sites for McDonald's Corporation and for KFC both have statements claiming that they do not accept chicken meat grown using AGP (KFC, 2002; McDonald's Corporation, 2003). Even if no other regulations are forthcoming, the largest consumers of poultry have mandated their removal from much of the broiler feed in the United States. Producers in any country that seek export markets will be forced to give up AGP if they are to sell to the EU and many other markets.

FUTURE OF ANTIMICROBIAL GROWTH PROMOTERS WORLDWIDE

On a global level, a recent joint workshop was held involving the WHO, Food and Agriculture Organization of the United Nations (FAO), and the World Organization for Animal Health (OIE) on nonhuman antimicrobial usage and antimicrobial resistance (World Health Organization, 2004). The resulting report recommends implementation of the WHO global principles for the containment of antimicrobial resistance in animals intended for food (World Health Organization, 2004). These principles include the withdrawal from food animal production of AGP that are in classes also used to treat human disease unless and until a risk assessment is carried out (World Health Organization, 2000). In addition, the report recommends the implementation on a national level of risk assessment studies and establishment of surveillance programs to monitor AGP use and antimicrobial resistance in bacteria from food animals (World Health Organization, 2004). The use of risk assessment models in evaluating and regulating food animal antibiotics has been reviewed recently (Cox, 2004).

The reality that AGP use is being curtailed by market actions, if not legislative, has led to a new urgency in the search for replacements. Among the candidates for replacement, organic acids appear to have the most widespread acceptance at this time (Dibner and Buttin, 2002; Dibner, 2003). Any replacement for AGP would have to provide an improvement in feed efficiency that is economically viable. If the replacement does not have antimicrobial properties, other concerns, such as incidence of enteric diseases and airsacculitis, will have to be addressed with the continued use of ionophores, management changes, or both. A recent review of the major categories of replacement candidates and methods to select among them has been provided by Rosen (2004). The review points out the need for analysis of numerous candidates simultaneously rather than evaluating the accumulating database one study at a time. This would involve the inclusion of all properly controlled test data available using a multifactorial model (Rosen, 2004). The strength of the argument is that the relevant properties of AGP (i.e., improved efficiency, gain, and livability) would be the basis for selection, and that combinations of candidates could be identified. The mode of action of AGP, which is of theoretical interest and the subject of the rest of this report, is in fact incidental to the issue of rapidly identifying replacement combinations.

BIOLOGICAL BASIS FOR ANTIBIOTIC EFFECTS ON ANIMAL GROWTH EFFICIENCY

Introduction

Orally ingested antibiotics promote growth and efficiency of poultry and other animals. The effect can include gain but often is limited to feed efficiency effects only. The mechanism of action must be focused on the gut because some of these antibiotics are not absorbed. Following early demonstrations that oral antibiotics do not have growth-promoting effects in germ-free animals (Coates et al., 1955; Coates et al., 1963), studies of the mechanism for growth promotion have focused on interactions between the antibiotic and the gut microbiota. Thus, direct effects of AGP on the microflora can be used to explain decreased competition for nutrients and reduction in microbial metabolites that depress growth (Visek, 1978a; Anderson et al., 1999). Additional AGP effects that also occur in germ-free animals include reduction in gut size, including thinner intestinal villi and total gut wall (Coates et al., 1955). This may be due, in part, to the loss of mucosa cell proliferation in the absence of luminal short chain fatty acids derived from microbial fermentation (Frankel et al., 1994) The reduction in gut wall and villus lamina propria has been used to explain the enhanced nutrient digestibility observed with AGP (Jukes et al., 1956; Franti et al., 1972; Anderson et al., 1999).

Finally, a reduction in opportunistic pathogens and subclinical infection has also been linked to use of AGP. It should be noted that injection of bacterial metabolites such as lipopolysaccharides or immune mediators such as interleukin-1 can mimic the reduced efficiency of an animal with a conventional microflora and no antimicrobial in the diet (Roura et al., 1992), which illustrates the importance of the host response to the microflora as another factor limiting growth efficiency. The reduction in microflora, and its consequences, may be the underlying mechanism for beneficial effects of antibiotics.

Gastrointestinal Microflora

The gastrointestinal (GI) tract of vertebrate animals contains a species-diverse group of microflora, although bacteria, and particularly gram-positive bacteria, predominate (Savage, 1977; Mackie et al., 1999). As many as 500 bacterial species exist in the GI microflora, with numbers up to 10^{10} to 10^{12} bacterial cells/g of colonic content or feces (Moore and Holdeman, 1974; Savage, 1977; Lee, 1984; Jensen, 2001). These numbers are consistent with the estimation that bacterial cells outnumber host cells by 10:1 (Gaskins, 2001). The bacterial population influences a variety of immunological, physiological, nutritional, and protective processes of the GI tract and exerts profound effects on the overall health, development, and performance of monogastric animals. Indeed, experiments comparing conventionally reared versus sterile (germ-free) animals have demonstrated that commensal bacteria play important roles in organ, tissue, and immune system development, as well as providing a variety of nutritional compounds (Gaskins, 2001; Snel et al., 2002).

The benefits imparted by normal microflora come at a great cost to the animal, even under ideal conditions. The commensal bacteria compete with the host for nutrients, secrete toxic compounds, and induce an ongoing immune/inflammatory response in the GI tract. All of these costs negatively impact animal health and performance. Two important areas for future research are 1) to determine the optimal microflora for animal health and performance under commercial growth conditions (in other words, to discover the microflora that maximize the benefits while minimizing the costs) and 2) to develop dietary and other interventions to foster development of this microflora.

Early Colonization and Succession

The young animal is exposed to a succession of microbial populations in the gut. These population waves are remarkably similar in the GI tracts of chicks, piglets, calves, and humans (Mackie et al., 1999); exert profound influences over animal development and health; and impact growth performance. Prior to hatch or birth, the GI tract of poultry and swine is sterile (Kenworthy and Crabb, 1963; Kelly and King, 2001). Bacteria from the environment, the mother (in case of mammals), and the diet begin to colonize the GI tract almost immediately. By 5 to 6 hours postbirth, an animal's feces are populated with 10^9 to 10^{10} cfu/g of feces (Snel et al., 2002). Aerobic and facultative anaerobes including *Escherichia coli*, lactobacilli, and streptococci all colonize immediately after birth (Smith and Jones, 1963; Mackie et al., 1999). Numbers are low—between 10^2 and 10^5 cfu/mL of digesta—but these numbers rapidly increase. These species provide a reduced environment, which in turn allows for establishment of the obligate anaerobes that appear some time later, and that constitute the predominant species of the stable microflora, at least in the small intestine. These genera include the *Bacteroides*, *Bifidobacterium*, and *Clostridium*. Overall, numbers of each group increase rapidly as the animal grows. Pederson and Tannock (1989) reported that the number of lactobacilli in esophagus, stomach, duodenum, jejunum, and ileum of piglets increased 10-fold between d 1 and 10 after birth.

It is interesting to note that although the environment plays a significant role in the order of species colonization, animals appear to have powerful selection mechanisms to ensure a proper sequence of succession. For example, in a study comparing sterilely born piglets raised off their sows, to vaginally born piglets raised on the sows, both sets of piglets exhibited a similar progression of microbial colonization (Ducluzeau, 1985). *E. coli* and *Streptococcus* populations established rapidly, followed later by *Lactobacillus* and *Clostridium*, in both sets of piglets. *Clostridium* failed to become predominant in the sow-reared piglets even though they were among the dominant species in

the sow's feces and on the sow's teats. Whatever the mechanisms are that provide for this orderly succession, the end result generally is a stable, diverse population of species that serve to protect the animal from the establishment of pathogenic species of bacteria. On a gross level, the microflora species present are similar across many species (Gaskins, 2001). Nevertheless, significant animal to animal variations do occur (Zhu et al., 2002).

Composition. There have been many attempts to estimate the overall proportions of different bacterial species in the GI tract of animals. These estimates are controversial, and numbers reported depend heavily on the specific GI sites that are sampled and the measuring techniques employed (traditional culturing methods vs. newer molecular techniques, for instance). For example, the proportion of *E. coli* in the gut has been estimated to be as low as 1% (Jensen, 1999) and up to 22% (Jensen, 2001). Nevertheless, it is commonly reported that the major bacterial groups in the pig GI tract include (in order of prevalence, according to Gaskins): *Streptococcus*, *Lactobacillus*, *Eubacterium*, *Fusobacterium*, *Bacteroides*, *Peptostreptococcus*, *Bifidobacterium*, *Selenomonas*, *Clostridium*, *Butyrivibrio*, and *Escherichia* (Moore et al., 1987; Stewart, 1997; Gaskins, 2001; Jensen, 2001; van der Klis and Jansman, 2002).

Different species are preferentially localized to different areas of the GI tract, and not all areas are as heavily colonized as others. Compared with the large intestine, for example, the stomach and proximal small intestine in the pig contain relatively few bacteria (10^3 to 10^5 cfu/g of digesta) because of the low pH and fast rate of digesta passage (Moughan et al., 1992). Here, acid-tolerant *Lactobacilli* and *Streptococci* predominate (Fewins et al., 1957; Gaskins, 2001). The ileum contains a far more diverse microflora, with greater numbers of cells (10^8 to 10^9 cfu/g). Due to slow rates of digesta passage, the large intestine (cecum and colon) contains yet even more bacterial cells (10^{10} to 10^{12} cfu/g of digesta), more than 99% of which are strict anaerobes (Moore and Holdeman, 1974; Savage, 1977; Gaskins, 2001).

In poultry, *Enterococci* and *Lactobacilli* are the dominant species in the crop, duodenum, and ileum during the first week of life, whereas coliforms, *Enterococci*, and *Lactobacilli* are present in high numbers in the ceca (Barnes et al., 1972; Mead and Adams, 1975; van der Wielen et al., 2000; Snel et al., 2002). After the first week, a highly complex group of mostly obligate anaerobes begins to take over the ceca, whereas lactobacilli take over the crop, duodenum, and ileum. After 2 to 3 wk, the intestinal microflora are established and stable (Snel et al., 2002).

Benefits and Costs Associated with Microflora

Introduction. It is clear that the microflora provide real benefits to the animal. For example, the microflora provide both nutrition and protection to the animal, in the form of fermentation products and prevention of colonization by pathogens, respectively. However, these benefits come at a cost. The GI microflora compete with the host for other nutrients, stimulate rapid turnover of absorptive epithelial cells, require an increased rate of mucus secretion by intestinal goblet cells, and stimulate immune system development and inflammatory responses. All of these effects come at the expense of animal growth performance. For example, GI tissues in the pig represent only about 5% of the body weight, but they require 15 to 35% of whole-body oxygen consumption and protein turnover due to relatively high rates of epithelial cell turnover and metabolism. Furthermore, 90% of the total protein synthesized by the GI tract is lost due to mucus secretion and epithelial cell shedding (Gaskins, 2001).

Benefits. The first major benefit provided by normal microbiota is resistance to colonization by pathogenic and other nonindigenous microbes, a phenomenon also known as competitive exclusion (van der Waaij et al., 1971; Lloyd et al., 1977; Rolfe, 1997; Gaskins, 2001; Kelly and King, 2001; Snel et al., 2002). Many studies have demonstrated that germ-free animals are far more susceptible to colonization by pathogens than are conventionally grown animals (Gordon et al., 1966; Koopman et al., 1984). The specific mechanism(s) by which this protection occurs have yet to be demonstrated, but many hypotheses have been proposed. Most believe the resident flora suppresses colonization by secreting antimicrobial compounds such as organic acids, by direct stimulation of the immune system, and by competing for nutrients and attachment to the mucosal surfaces (Rolfe, 1997; Kelly and King, 2001).

A second benefit is that the normal microflora stimulate development of intestinal host defenses, including the mucus layer; the epithelial monolayer; and the lamina propria, with its system of immune cells that underlie the epithelium (McCracken and Gaskins, 1999; Kelly and King, 2001). The mucus layer segregates both normal and pathogenic microbes away from the animal tissues, the epithelium provides a barrier to entry into the animal tissues when the mucus layer has been crossed, and the underlying network of immune cells provides antibodies, cytotoxic and helper T cells, and phagocytic cells. These immune cells combat not only pathogenic bacteria and their toxins but also the overgrowth of or inappropriate attachment by the normal microflora. Evidence here is from studies of germ-free animals, which exhibit delayed lymphocyte and other immune cell development in the lamina propria and far fewer IgA-producing cells when compared to conventionally reared animals (Gordon and Pesti, 1971; Berg and Savage, 1975; Umesaki et al., 1993; Rothkotter et al., 1994; Umesaki et al., 1999). For example, the development of antibody diversity in poultry is inhibited by germ-free growth conditions (Schaffner et al., 1974; Ekino et al., 1980). Indeed, the majority of evidence supports the notion that the intestinal immune system develops in parallel with the development of the normal microflora. Introduction of even a single species of commensal bacteria into germ-free animals can stimulate the development of the secretory IgA system (McCracken and Gaskins, 1999).

It should be noted, however, that while the microflora-induced development of the intestinal immune system may be key to the long-term health of the animal, there is inherent inefficiency when immune stimulation is maintained at a chronic level as appears to be the case in conventional versus germ-free animals (Gordon et al, 1963). The typical adult pig, for example, secretes several grams of IgA each day, approximately 50% of which is specific to antigens from the very same resident microflora. Thus, microflora-specific IgA secretion that is not directed toward growth can cost the animal several hundred grams of protein over a lifetime.

A third benefit is the microflora-secreted nutrients that become available for use by the host. These include short-chain fatty acids, amino acids as well as vitamins B and K (Savage, 1986; Wostmann, 1996; Snel et al., 2002). Short chain fatty acids (SCFA), such as acetate, butyrate and propionate are highly prevalent anions in the pig colon produced by anaerobic species that ferment dietary fiber (Kelly and King, 2001; Sakata and Inagaki, 2001). Likewise, the commensal bacteria in broiler chickens also generate lactate, acetate, propionate and butyrate (Barnes et al., 1979; van der Wielen et al., 2000). These fatty acids contribute significantly to the energy supply of the animal. Furthermore, the undissociated forms of SCFA play important roles in reducing the numbers of "undesirable" bacterial species in the cecum (van der Wielen et al., 2000; Snel et al., 2002). SCFA also stimulate gut epithelial cell proliferation and villus size, thereby increasing the absorptive surface area (Galfi and Bokori, 1990; Sakata and Inagaki, 2001). The extent to which microbial-derived amino acids contribute to requirements is less clear.

Costs. Despite these many benefits, the microflora imposes a variety of costs to the animal as well, in addition to the immunological disadvantages mentioned above. These costs include competition for nutrients and the production of toxic amino acid catabolites, decreased fat digestibility, and the requirement for increased mucus secretion and gut epithelial cell turnover. These and other bacterial-induced effects exact a large toll on animal health and performance. It has even been proposed that the reduction in amino acid catabolites and the prevention of bile catabolism are among the primary mechanisms by which antibiotics improve animal performance (Visek, 1978a,b; Feighner and Dashkevicz, 1987, 1988; Gaskins et al., 2002).

It is generally accepted that many bacterial species compete with the host for nutrients (Furuse and Okumura, 1994). Experiments have demonstrated that as much as 6% of the net energy in the pig diet is lost to the microflora, for example (Vervaeke et al., 1979). Bacteria also compete with the host for uptake of amino acids, thereby reducing nitrogen utilization (Furuse and Yokota, 1985). These amino acids can be incorporated into bacterial protein (Salter and Coates, 1974). Alternatively, certain bacteria ferment amino acids, producing toxic catabolites which can impact intestinal cell turnover and growth performance of the animal (Russell, 1983; Macfarlane and Macfarlane, 1995; Gaskins, 2001). Examples of these catabolites include ammonia, a variety of amines, phenols and indoles. All negatively impact animal health and performance. Ammonia is produced by amino acid deamination and urea hydrolysis. Evidence exists that high concentrations of ammonia depress growth (Pond and Yen, 1987; Veldman and Van der Aar, 1997). This is at least partly due to an increase in gut epithelial cell turnover in high ammonia conditions (Visek, 1978b). Toxic amines are produced by decarboxylation of amino acids. A number of different bacterial species mediate these reactions, including *Bacteroides*, *Clostridium*, *Enterobacterium*, *Lactobacillus*, and *Streptococcus*. The resulting products include histamine, cadaverine and many others (Gaskins, 2001). Increased amine production has been linked to diarrhea in weanling pigs (Porter and Kenworthy, 1969). Finally, the production of phenols and indoles, via the breakdown of aromatic amino acids, is mediated by *Bacteroides*, *Lactobacillus*, *Clostridium*, and *Bifidobacterium* (Gaskins, 2001). These compounds can have negative impacts on growth performance (Yokoyama et al., 1982) and flavor characteristics of meat (Lundstrom et al., 1988).

The microflora also decreases fat digestibility. Bile acids and their salts are required for proper fat digestion and absorption. Once they are secreted into the gut, they are subjected to catabolism by a variety of bacterial species but primarily by *Lactobacillus* (Baron and Hylemon, 1997). This catabolism reduces lipid absorption (Eyssen, 1973) and produces toxic degradation products that inhibit growth performance (Baron and Hylemon, 1997).

Finally, the resident microflora necessitates great increases in mucus secretion and gut epithelial cell turnover. Whereas one major function of the mucus layer is simply to lubricate the GI tract, it also serves to prevent the microflora from attaching to and invading the intestinal epithelial cells of the host. Because many bacterial species enzymatically digest away the mucus layer, the host must constantly secrete more (Gaskins, 2001). Furthermore, the mucin-secreting goblet cells and the absorptive enterocytes on the intestinal villi have a short lifespan. In fact, the gut epithelium has the fastest rate of renewal of any tissue in the body (Imondi and Bird, 1966). This high cell turnover is accompanied by an extremely high rate of metabolism and protein synthesis, resulting in 23 to 36% of the whole body energy expenditure (Summers, 1991; Cant et al., 1996). Remarkably, the turnover rate, and therefore the energy and amino acids required for cell turnover, is increased by the presence of commensal microflora (Abrams et al., 1963; Lesher et al., 1964). It is not clear whether gut epithelium lifespan is shortened by microbial metabolites or some other factor or influence brought in by the microflora. The effect may be secondary to toxic effects of microbial metabolites such as ammonia, produced by the action of bacterial urease in the intestinal lumen (Visek, 1978b). Nevertheless, these factors are a major hindrance to growth performance due to loss of the protein in endogenous secretions and a high expenditure of metabolic energy.

Manipulating the Microflora

With respect to animal production, an important goal is to determine the optimal microflora for the animal (maximum benefits with minimum costs) and then be able to manipulate the microflora through diet, supplements, etc. to obtain the desired microflora. Many products, including antibiotics, organic acids, probiotics, prebiotics, trace minerals, enzymes, herbs and spices, and others are sold with the goal of altering the microflora for the benefit of animal health and production. However, much work still needs to be done with respect to the first step: not just identifying an optimal microflora, but also developing quantitation methods.

Where law has not restricted it, the use of antibiotics is the most common dietary intervention to modulate the gut microflora. The performance benefits of antibiotics have been demonstrated for all major livestock species. For example, a meta-analysis of more than 1,000 growth experiments performed in swine over a 25-yr period demonstrated that antibiotics improved growth rate in starter pigs (7 to 25 kg) by an average of 16.4% and feed efficiency by 6.9% (Cromwell, 2002). There were significant improvements for grower and finisher pigs as well. Although the exact mechanism(s) by which antibiotics promote growth have not been demonstrated, it is presumed that their effects lie in the reduction of the overall numbers and/or the numbers of species of gut bacteria (Visek, 1978a; Jensen, 1998; Close, 2000; Gaskins et al., 2002; Collier et al., 2003). This notion is supported by the observation that antibiotics do not promote the growth of germ-free animals (Coates et al., 1963). As of 1999, it was estimated that 90% of pig starter diets, 70% of grower diets, and 50% of finisher diets in the US contained some form of antibiotics (Dewey et al., 1999). Antibiotic usage in the broiler industry is also widespread, although it has declined from the mid-1990s; in the United States, approximately 60% of all poultry diets contain growth-promoting antibiotics (Chapman and Johnson, 2002). This represents a decrease from 1995, in which 93% of starter, 97% of grower, and 86% of finisher diets contained these drugs.

One recent experiment to investigate the effects of antibiotics on the microflora was performed by Gaskins and colleagues (Collier et al., 2003). In this paper, 4-wk-old barrows were ileally cannulated and placed on a no-antibiotic diet, diets with tylosin, or a weekly rotation of antibiotics. The effects of the different treatments were analyzed using two methods from molecular biology: PCR-denaturing gradient gel electrophoresis (DGGE) and quantitative PCR. The results of these experiments were that, relative to the no antibiotic controls, antibiotic treatments reduced species diversity and total numbers of bacteria, including lactobacilli.

SUMMARY AND CONCLUSIONS

It seems inevitable that use of AGP will decline in the future. Where legislation is not already in place, consumer pressure is building to make the practice of using antimicrobials economically impractical because of market limitations and export restrictions. This review has considered the history of AGP use in the EU and the series of regulations that have been adopted. Economic consequences for the poultry industry have been relatively minor but are tied to the continued use of anticoccidial ionophores, which also have antimicrobial (i.e., growth promoting) effects. Use of germ-free animals to model the effects of AGP suggests that most of the benefits of antimicrobials derive from effects on the intestinal microflora. Finding replacements for AGP will likely involve the use of multiple products in the diet, each with some of the benefits of AGP, and management changes will play a key role in maintaining animal productivity in their absence. It is unlikely that a single replacement will be found that will prove to be economically viable.

REFERENCES

Aarestrup, F. M. 1995. Occurrence of glycopeptide resistance among *Enterococcus faecium* isolates from conventional and ecological poultry farms. Microb. Drug Resist. 1:255–257.

Aarestrup, F. M. 2003. Effects of termination of AGP use on antimicrobial resistance in food animals. Pages 6–11 in Working papers for the WHO international review panels evaluation. Document WHO/CDS/CPE/ZFK/2003.1a. World Health Organization, Geneva, Switzerland.

Abrams, G. D., H. Bauer, and H. Sprinz. 1963. Influence of the normal flora on mucosal morphology and cellular renewal in the ileum: A comparison of germ-free and conventional mice. Laboratory Invest. 12:355–364.

Alpharma. 2004. The ultimate lesson we may learn from Denmark. Pages 1–4 in For the Record: Straight talk about antibiotic use in food-animal production. http://www.alpharma.com/ahd/For_The_Record/html. Accessed June 2004.

Anderson, D. B., V. J. McCracken, R. I. Aminov, J. M. Simpson, R. I. Mackie, M. W. A. Vestegen, and H. R. Gaskins. 1999. Gut microbiology and growth-promoting antibiotics in swine. Pig News Inf. 20:115N–122N.

Angulo, F. J. 2004. Impacts of antimicrobial growth promoter termination in Denmark. Pages 16–19 in Proceedings of the 53rd Western Poultry Disease Conference, Sacramento, CA.

Barnes, E. M. 1958. The effect of antibiotic supplements on the faecal streptococci (Lancefield group D) of poultry. Br. Vet. J. 114:333–344.

Barnes, E. M., C. S. Impey, and B. J. H. Stevens. 1979. Factors affecting the incidence and anti-salmonella activity of the anaerobic caecal flora of the young chick. J. Hyg. 82:263–283.

Barnes, E. M., G. C. Mead, D. A. Barnum, and E. G. Harry. 1972. The intestinal flora of the chicken in the period 2 to 6 weeks of age, with particular reference to the anaerobic bacteria. Br. Poult. Sci. 13:311–326.

Baron, S. F., and P. B. Hylemon. 1997. Biotransformation of bile acids, cholesterol, and steroid hormones. Pages 470–510 in Gastrointestinal Microbiology. R. I. Mackie, B. A. White and R. E. Isaacson, ed. Chapman & Hall, New York.

Bates, J., J. Z. Jordens, and J. B. Selkon. 1993. Evidence for an animal origin of vancomycin resistant enterococci. Lancet 342:490–491.

Berg, R. D., and D. C. Savage. 1975. Immune responses of specific pathogen-free and gnotobiotic mice to antigens of indigenous and nonindigenous microorganisms. Infect. Immun. 11:320–329.

Callesen, J. 2003. Effects of termination of AGP use on pig welfare and productivity. Pages 43–46 in Working papers for the WHO international review panels evaluation. Document

WHO/CDS/CPE/ZFK/2003.1a. World Health Organization, Geneva, Switzerland.
Cant, J. P., B. W. McBride, and W. J. Croom, Jr. 1996. The regulation of intestinal metabolism and its impact on whole animal energetics. J. Anim. Sci. 74:2541–2553.
Cervantes, H. 2004. Why responsible antibiotic use enhances animal and human health. Pages 201–210 in Proceedings of the 2004 Midwest Poultry Federation Convention, St. Paul, MN.
Chapman, H. D., and Z. B. Johnson. 2002. Use of antibiotics and roxarsone in broiler chickens in the USA: Analysis for the years 1995 to 2000. Poult. Sci. 81:356–364.
Close, W. H. 2000. Producing pigs without antibiotic growth promoters. Adv. Pork. Prod. 11:47–56.
Coates, M. E., M. K. Davies, and S. K. Kon. 1955. The effect of antibiotics on the intestine of the chick. Br. J. Nutr. 9:110–119.
Coates, M. E., R. Fuller, G. F. Harrison, M. Lev, and S. F. Suffolk. 1963. Comparison of the growth of chicks in the Gustafsson germ-free apparatus and in a conventional environment, with and without dietary supplements of penicillin. Br. J. Nutr. 17:141–151.
Collier, C. T., M. R. Smiricky-Tjardes, D. M. Albin, J. E. Wubben, V. M. Gabert, B. Deplancke, D. Bane, D. B. Anderson, and H. R. Gaskins. 2003. Molecular ecological analysis of porcine ileal microbiota responses to antimicrobial growth promoters. J. Anim. Sci. 81:3035–3045.
Committee on Drug Use in Food Animals Panel on Animal Health, Food Safety, and Public Health. 1998. Use of Drugs in Food Animals: Benefits and Risks. National Academy Press, Washington, DC.
Council for Agricultural Science and Technology. 1981. Antibiotics in Animal Feeds. Report 88. CAST, Ames, IA.
Cox, T. 2004. Use of risk assessment models in regulating food animal antibiotics. Pages 10–16 in Proceedings of the 53rd Western Poultry Disease Conference, Sacramento, CA.
Cromwell, G. L. 2002. Why and how antibiotics are used in swine production. Anim. Biotechnol. 13:7–27.
Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP). 2002. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark. ISSN 1600–2032.
Dawe, J. F. 2004. The relationship between poultry health and food safety. Pages 24–27 in Proceedings of the 53rd Western Poultry Disease Conference, Sacramento, CA.
Dewey, C. E., B. D. Cox, B. E. Straw, E. J. Bush, and S. Hurd. 1999. Use of antimicrobials in swine feeds in the United States. Swine Health Prod. 7:19–25.
Dibner, J. J., and P. Buttin. 2002. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. J. Appl. Poult. Res. 11:453–463.
Dibner, J. 2003. Alimet feed supplement: Value beyond methionine. Feedstuffs 44:12–16.
Ducluzeau, R. 1985. Implantation and development of the gut microflora in the newborn piglet. Pig News Inf. 6:415–418.
Ekino, S., Y. Nawa, K. Kanaka, K. Matsuno, H. Fugi, and M. Kotani. 1980. Suppression of immune response by isolation of the bursa of Fabricius from environmental stimuli. Aust. J. Exp. Biol. Med. Sci. 58:289–296.
Elliott, S. D., and E. M. Barnes. 1959. Changes in serological type and antibiotic resistance on Lancefield group D streptococci in chickens receiving dietary chlortetracycline. J. Gen. Microbiol. 20:426–433.
Elwinger, K., E. Engstrom, B. Berndston, O. Fossum, and L. Waldenstedt. 1998. Effect of antibiotic growth promoters and anticoccidials on growth of *Clostridium perfringens* in the caeca and on performance of broiler chickens. Acta Vet. Scand. 39:433–441.
Emborg, H. D., A. K. Ersboll, O. E. Heuer, and H. C. Wegener. 2002. Effects of termination of antimicrobial growth promoter use for broiler health and productivity. Pages 38–42 in Working papers for the WHO international review panel's evaluation. Document WHO/CDS/CPE/ZFK/2003.1a. World Health Organization, Geneva, Switzerland.
Eyssen, H. 1973. Role of gut microflora in metabolism of lipids and sterols. Proc. Nutr. Soc. 32:59–63.
Feighner, S. D., and M. P. Dashkevicz. 1987. Subtherapeutic levels of antibiotics in poultry feeds and their effects on weight gain, feed efficiency, and bacterial cholytaurine hydrolase activity. Appl. Environ. Microbiol. 53:331–336.
Feighner, S. D., and M. P. Dashkevicz. 1988. Effect of dietary carbohydrates on bacterial cholytaurine hydrolase in poultry intestinal homogenates. Appl. Environ. Microbiol. 54:337–342.
Fewins, B. G., L. G. M. Newland, and C. A. E. Briggs. 1957. The normal intestinal flora of the pig. III. Qualitative studies of lactobacilli and streptococci. J. Appl. Bacteriol. 20:234–242.
Frankel, W. L., W. Zhang, A. Singh, D. M. Klurfeld, S. Don, T. Sakata, I. Modlin, and J. L. Rombeau. 1994. Mediation of the trophic effects of short-chain fatty acids on the rat jejunum and colon. Gastroenterology 106:375–380.
Franti, C. E., L. M. Julian, H. E. Adler, and A. D. Wiggins. 1972. Antibiotic growth promotion: Effects of zinc bacitracin and oxytetracycline on digestive circulatory, and excretory systems of New Hampshire cockerels. Poult. Sci. 51:1137–1145.
Furuse, M., and J. Okumura. 1994. Nutritional and physiological characteristics in germ-free chickens. Comp. Biochem. Physiol. 109A:547–556.
Furuse, M., and H. Yokota. 1985. Effect of the gut microflora on chick growth and utilization of protein and energy at different concentrations of dietary protein. Br. Poult. Sci. 26:97–104.
Galfi, P., and J. Bokori. 1990. Feeding trial in pigs with a diet containing sodium n-butyrate. Acta Vet. Hung. 38:3–17.
Gaskins, H. R. 2001. Intestinal bacteria and their influence on swine growth. Pages 585–608 in Swine Nutrition. 2nd ed. A. J. Lewis and L. L. Southern, ed. CRC Press, Boca Raton, FL.
Gaskins, H. R., C. T. Collier, and D. B. Anderson. 2002. Antibiotics as growth promotants: Mode of action. Anim. Biotechnol. 13:29–42.
Gordon, H. A., E. Bruckner-Kardoss, T. E. Staley, M. Wagner, and B. S. Wostmann. 1966. Characteristics of the germfree rat. Acta Anat. 64:367.
Gordon, H. A., B. S. Wostmann, and E. Bruckner-Kardoss. 1963. Effects of microbial flora on cardiac output and other elements of blood circulation. Proc. Soc. Exp. Biol. Med. 114:301–304.
Gordon, H. A., and L. Pesti. 1971. The gnotobiotic animal as a tool in the study of host microbial relationships. Bacteriol. Rev. 35:390–421.
Greko, C. 2001. Safety aspects on non-use of antimicrobials as growth promoters. Pages 219–230 in Gut Environment of Pigs. A. Piva, K. E. Bach Knudsen and J. E. Lindberg, ed. Nottingham University Press, Nottingham, UK.
Imondi, A. R., and F. H. Bird. 1966. The turnover rate of intestinal epithelium in the chick. Poult. Sci. 45:142–147.
Institute of Medicine. 1980. The Effects on Human Health of Antimicrobials in Animal Feeds. National Academy Press, Washington, DC.
Institute of Medicine. 1989. Human Health Risks with the Subtherapeutic Use of Penicillin or Tetracyclines in Animal Feed. National Academy Press, Washington, DC.
Jensen, B. B. 1998. The impact of feed additives on the microbial ecology of the gut in young pigs. J. Anim. Feed Sci. 7:45–64.
Jensen, B. B. 1999. Impact of feed composition and processing on the gastrointestinal ecosystem in pigs. Pages 43–56 in Nutrition and Gastrointestinal Physiology—Today and Tomorrow. A. J. M. Jansman and J. Huisman, ed. Wageningen Academic Publishers, Wageningen, The Netherlands.
Jensen, B. B. 2001. Possible ways of modifying type and amount of products from microbial fermentation in the gut. Pages 181–200 in Gut Environment of Pigs. A. Piva, K. E. Bach

Knudsen and J. E. Lindberg, ed. Nottingham University Press, Nottingham, UK.
Jukes, T. H., E. L. R. Stokstad, R. R. Taylor, T. J. Combs, H. M. Edwards and G. B. Meadows. 1950. Growth promoting effect of aureomycin on pigs. Arch. Biochem. 26:324–330.
Jukes, T. H., D. C. Hill and H. D. Branion. 1956. Effect of feeding antibiotics on the intestinal tract of the chick. Poult. Sci. 35:716–723.
Kelly, D., and T. P. King. 2001. Luminal bacteria: Regulation of gut function and immunity. Pages 113–131 in Gut Environment of Pigs. A. Piva, K. E. Bach Knudsen, and J. E. Lindberg, ed. Nottingham University Press, Nottingham, UK.
Kenworthy, R., and W. E. Crabb. 1963. The intestinal flora of young pigs with reference to early weaning and *Escherischia coli* scours. J. Comp. Pathol. 73:215–228.
KFC. 2002. Get the facts about KFC kitchen fresh chicken. KFC, Louisville, KY. www.kfc.com/about/facts.htm. Accessed May 2004.
Koopman, J. P., H. M. Kennis, J. W. Mullink, R. A. Prins, A. M. Stadhouders, H. De Boer and M. P. Hectors. 1984. 'Normalization' of germfree mice with anaerobically cultured caecal flora of 'normal' mice. Lab. Anim. 18:188–194.
Lee, A. 1984. Neglected niches: The microbial ecology of the gastrointestinal tract. Pages 115–162 in Advances in Microbal Ecology. K. Marshall, ed. Plenum Press, New York.
Lesher, S., H. E. Walburg, and G. A. Sacher. 1964. Generation cycle in the duodenal crypt cells of germ-free and conventional mice. Nature 202:884–886.
Lloyd, A. B., R. B. Cummings, and R. D. Kent. 1977. Prevention of *Salmonella typhimurium* infection in poultry by pretreatment of chickens and poults with intestinal extracts. Aust. Vet. J. 53:82–87.
Lundstrom, K., B. Malmfors, G. Malmfors, S. Stern, H. Petersson, A. B. Mortensen, and S. E. Sorenson. 1988. Skatole, androstenone and taint in boars fed two different diets. Livest. Prod. Sci. 18:55.
Macfarlane, S. and G. T. Macfarlane. 1995. Proteolysis and amino acid fermentation. Pages 75–100 in Human Colonic Bacteria: Role in Nutrition, Physiology and Pathology. G. R. Gibson and G. T. Macfarlane, ed. CRC Press, Boca Raton, FL.
Mackie, R. I., A. Sghir, and H. R. Gaskins. 1999. Developmental microbial ecology of the neonatal gastrointestinal tract. Am. J. Clin. Nutr. 69:1035S.
Martel, A., L. A. Devriese, K. Cauwerts, K. De Gussem, A. Decostere, and F. Haesebrouck. 2004. Susceptibility of *Clostridium perfringens* strains from broiler chickens to antibiotics and anticoccidials. Avian Pathol. 33:3–7.
McCracken, V. J., and H. R. Gaskins. 1999. Probiotics and the immune system. Pages 85–111 in Probiotics: A Critical Review. G. W. Tannock, ed. Horizon Scientific Press, Norfolk, UK.
McDonald's Corporation. 2003. McDonald's global policy on antibiotic use in food animals. www.mcdonalds.com/corp/values/socialrespons.html. Accessed Feb. 2005.
Mead, G. C., and B. W. Adams. 1975. Some observations on the caecal microflora of the chick during the first two weeks of life. Br. Poult. Sci. 16:169–176.
Moore, P. R., A. Evenson, T. D. Luckey, E. McCoy, E. A. Elvehjem, and E. B. Hart. 1946. Use of sulphasuccidine, streptothricin and streptomycin in nutrition studies with the chick. J. Biol. Chem. 165:437–441.
Moore, W. E. and L. V. Holdeman. 1974. Human faecal flora: The normal flora of 20 Japanese-Hawaiians. Appl. Micriobiol. 27:961–979.
Moore, W. E. C., L. V. H. Moore, E. P. Cato, T. D. Wilkins and E. T. Kornegay. 1987. Effect of high-fiber and high-oil diets on the fecal flora of swine. Appl. Environ. Microbiol. 53:1638–1644.
Moughan, P. J., M. J. Birtles, P. J. Cranwell, W. C. Smith, and M. Pedrazza. 1992. The piglet as a model animal for studying aspects of digestion and absorption in milk-fed human infants. Pages 40–113 in Nutritional Triggers for Health and in Disease. A. P. Simopoulos, ed. Karger, Basel, Switzerland.
Pederson, K., and G. W. Tannock. 1989. Colonization of the porcine gastrointestinal tract by Lactobacillus. Appl. Environ. Microbiol. 55:279–283.
Phillips, I., M. Casewell, T. Cox, B. DeGroot, C. Friis, R. Jones, C. Nightingale, R. Preston, and J. Waddell. 2004. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. J. Antimicrob. Chemother. 53:28–52.
Pond, W. G., and J. T. Yen. 1987. Effect of supplemental carbadox, an antibiotic combination, or clinoptilolite on weight gain and organ weights of growing swine fed maize or rye as the grain sources. Nutr. Rep. Int. 35:801–809.
Porter, P., and R. Kenworthy. 1969. A study of intestinal and urinary amines in pigs in relation to weaning. Res. Vet. Sci. 10:440–447.
Roe, M. T., and S. D. Pillai. 2003. Monitoring and identifying antibiotic resistance mechanisms in bacteria. Poult. Sci. 82:622–626.
Rolfe, R. 1997. Colonization resistance. Pages 501-536 in Gastrointestinal Microbiology. Vol. 2. R. I. Mackie, B. A. White and R. E. Isaacson, ed. Chapman & Hall, New York.
Rosen, G. D. 2004. Optimizing the replacement of pronutrient antibiotics in poultry nutrition. Pages 93–101 in Proceedings of Alltech's 20th Annual International Symposium. Alltech, Lexington, KY.
Rothkotter, H. J., T. Kirchhoff, and R. Pabst. 1994. Lymphoid and non-lymphoid cells in the epithelium and lamina propria of intestinal mucosa of pigs. Gut 35:1582–1589.
Roura, E., J. Homedes, and K. C. Klasing. 1992. Prevention of immunologic stress contributes to the growth-permitting ability of dietary antibiotics in chicks. J. Nutr. 122:2382–2390.
Russell, J. B. 1983. Fermentation of peptides by *Bacteroides ruminicola* B14. Appl. Environ. Microbiol. 45:1566–1574.
Sakata, T., and A. Inagaki. 2001. Organic acid production in the large intestine: Implication for epithelial cell proliferation and cell death. Pages 85–94 in Gut Environment of Pigs. A. Piva, K. E. Bach Knudsen, and J. E. Lindberg, ed. Nottingham University Press, Nottingham, UK.
Salter, D. N., and M. E. Coates. 1974. The utilization of protein and excretion of uric acid in germ-free and conventional chicks. Br. J. Nutr. 31:307–318.
Savage, D. C. 1977. Microbial ecology of the gastrointestinal tract. Annu. Rev. Microbiol. 31:107–133.
Savage, D. C. 1986. Gatrointestinal microflora in mammalian nutrition. Annu. Rev. Nutr. 6:155–178.
Schaffner, T., J. Mueller, M. W. Hess, H. Cottier, B. Sordat, and C. Ropke. 1974. The bursa of Fabricius: A central organ providing for contact between the lymphoid system and intestinal content. Cell. Immun. 13:304–312.
Smith, H. W., and J. E. T. Jones. 1963. Observation on the alimentary tract and its bacterial flora in healthy and diseased pigs. J. Pathol. Bacteriol. 86:387.
Snel, J., H. J. M. Harmssen, P. W. J. J. van der Wielen, and B. A. Williams. 2002. Dietary strategies to influence the gastrointestinal microflora of young animals, and its potential to improve intestinal health. Pages 37–69 in Nutrition and Health of the Gastrointestinal Tract. M. C. Blok, H. A. Vahl, L. de Lange, A. E. van de Braak, G. Hemke, and M. Hessing, ed. Wageningen Academic Publishers, Wageningen, The Netherlands.
Starr, M. P., and D. M. Reynolds. 1951. Streptomycin resistance of coliform bacteria from turkeys fed streptomycin. Pages 15–34 in Proceedings of the 51st General Meeting, Society of American Bacteriology, Chicago, IL.
Stewart, C. S. 1997. Microorganisms in hindgut fermentors. Pages in Gastrointestinal Microbiology. Vol. 2. R. I. Mackie, B. A. White and R. E. Isaacson, ed. Chapman and Hall, New York.

Summers, M. 1991. Energy metabolism in the broiler chick. Ph.D. Thesis. University of Guelph, Ontario, Canada.
Swann, M. M. 1969. Report of Joint Committee on the Use of Antibiotics in Animal Husbandry and Veterinary Medicine. HMSO, London.
Umesaki, Y., H. Setoyama, S. Matsumoto, A. Imaoka, and K. Itoh. 1999. Differential roles of segmented filamentous bacteria and clostridia in development of the intestinal immune system. Infect. Immun. 67:3504–3511.
Umesaki, Y., H. Setoyama, S. Matsumoto, and Y. Okada. 1993. Expansion of alpha beta T-cell receptor-bearing intestinal intraepithelial lymphocytes after microbial colonization in germ-free mice and its independence from thymus. Immunol. 79:32–37.
van der Klis, J. D., and A. J. M. Jansman. 2002. Optimising nutrient digestion, absorption and gut barrier function in monogastrics: Reality or illusion? Pages 15–36 in Nutrition and Health of the Gastrointestinal Tract. M. C. Blok, H. A. Vahl, L. de Lange, A. E. van de Braak, G. Hemke, and M. Hessing, ed. Wageningen Academic Publishers, Wageningen, The Netherlands.
van der Waaij, D., J. M. Berghuis-de Vries, and J. E. C. Lekkerkerk-van der Wees. 1971. Colonization resistance of the digestive tract in conventional and antibiotic-treated mice. J. Hyg. 69:405–411.
van der Wielen, P. W. J. J., S. Biesterveld, S. Notermans, H. Hofstra, B. A. P. Urlings, and F. van Knapen. 2000. Role of volatile fatty acids in development of the caecal microflora in broiler chickens during growth. Appl. Environ. Microbiol. 66:2536–2540.
Vaughn, M. B., and D. Copeland. 2004. Is there human health harm following fluoroquinolone use in poultry? Pages 27–29 in Proceedings of the 53rd Western Poultry Disease Conference, Sacramento, CA.
Veldman, A., and P. J. Van der Aar. 1997. Effects of dietary inclusion of a natural clinoptilolite (mannelite) on piglet performance. Agribiol. Res. 50:289–294.
Vervaeke, I. J., J. A. Decuypere, N. A. Dierick, and H. K. Henderickx. 1979. Quantitative in vitro evaluation of the energy metabolism influenced by virginiamycin and spiramycin used as growth promoters in pig nutrition. J. Anim. Sci. 79:846–856.
Visek, W. J. 1978a. The mode of growth promotion by antibiotics. J. Anim. Sci. 46:1447–1469.
Visek, W. J. 1978b. Diet and cell growth modulation by ammonia. Am. J. Clin. Nutr. 31(Suppl. 10):S216–S220.
Watkins, K. L., T. Shryock, R. N. Dearth and Y. M. Saif. 1997. The *in vitro* antibiotic susceptibility of *Clostridium perfringens* from commercial turkey and broiler chicken origin. Vet. Microbiol. 54:195–200.
Western Poultry Disease Conference. 2004. Pages 1–32 in Proceedings of the Western Poultry Disease Conference. American Association of Avian Pathologists, Athens, GA.
World Health Organization. 1997. Pages 1–39 in The medical impact of the use of antimicrobials in food animals: Report of a WHO meeting, Berlin, Germany.
World Health Organization. 2000. WHO Global Principles for the Containment of Antimicrobial Resistance in Animals Intended for Food. Pages 1–23 in Document WHO/CDS/CSR/APH/2000.4. WHO, Geneva, Switzerland.
World Health Organization. 2003. Impacts of antimicrobial growth promoter termination in Denmark. Pages 1–57 in Document WHO/CDS/CPE/ZFK/2003.1. WHO, Foulum, Denmark.
World Health Organization. 2004. Proceedings of the Joint FAO/OIE/WHO expert workshop on non-human antimicrobial usage and antimicrobial resistance: Scientific assessment. Pages 1–71 in Document WHO/CDS/DIP/ZFK/04.20. World Health Organization, Geneva, Switzerland.
Wostmann, B. S. 1996. Germ-free and Gnotobiotic Animal Models: Background and Applications. CRC Press, Boca Raton, FL.
Yokoyama, M. T., C. Tabori, E. R. Miller, and M. G. Hogberg. 1982. The effects of antibiotics in the weanling pig diet on growth and excretions of volatile phenolic and aromatic bacterial metabolites. Am. J. Clin. Nutr. 35:1417–1424.
Zhu, X. Y., T. Zhong, Y. Pandya, and R. D. Joerger. 2002. 16S rRNA-based analysis of microbiota from the cecum of broiler chickens. Appl. Environ. Microbiol. 68:124–137.