Antibiotic Growth Promoters in Poultry, and their Potential Alternatives

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Growth promoters can be classified into four groups; those that increase growth and also increase feed consumption, those that increase growth without altering feed consumption, those that do not alter growth but decrease feed consumption (generally referred to as the feed consumption ratio; FCR=kg feed consumed/kg body weight), and those that increase growth and decrease feed consumption. Obviously, a growth promoter that increases feed consumption would not be a commercially viable product. Furthermore, a growth promoter that adversely alters meat quality and/or taste also would be unacceptable.

Commercial breeders of broiler chickens have been selecting for growth since the early 1950s, but they have apparently reached a point where broilers have attained approximately 95% of their genetic growth potential (Siegel and Dunnington, 1987). Nutritionists also have contributed extensively to improved growth and to improved feed conversion, but they, too, have reached a plateau from which modification of feed ingredients has not been commercially advantageous. In several cases, they have succeeded to significantly improve growth, but availability of the new ingredients is insufficient and/or too expensive.

Administering antibiotics to poultry, cattle and pigs in their feed or drinking water has had a major impact on the commercial production of meat for human consumption since the early 1950s (Jukes, 1972). Growth enhancement with antibiotics (sulphonamides) was first observed by Moore et al. (1946). The discovery by Jukes and his colleagues at Lederle Laboratories (Jukes, 1985) that Aureomycin stimulated significant growth in chickens, cattle and pigs was the foundation of antibiotic growth promotion in animals for more than 60 years. Many factors have contributed to a low cost for poultry meat to the consumer, but none has contributed as much to financial profit for producers and low cost for consumers as have the antibiotic growth promoters (AGP).

Therapeutic use of antibiotics is effective against intestinal and other infections of food animals, and they are effective against colibacillosis, coryza, fowl cholera, mycoplasmosis and Salmonella infections in chickens. Therapeutic use results in weight gain as health improves, but low doses of antibiotics also stimulate weight gain in healthy animals fed nutritionally complete feed (Jukes, 1977). Although the AGP do not change the number of intestinal bacteria, Jukes speculated that the antibiotics might suppress mildly harmful bacteria in the intestines.

Luckey (1956) provided evidence from germfree chickens fed AGP that there may be another, undefined mechanism that stimulates an increase in growth. This was contested by Forbes and Park (1959) and by Coates et al. (1963), who did not find increased growth in germfree chickens fed low doses of penicillin. Experiments with germfree chickens could be performed only with a small number of chickens because of limitation of the germfree chambers. This prevented rigorous, statistical analysis, leaving the issue of growth promotion in these studies unresolved, but it is unlikely that AGP enhance growth of germfree chickens.

One would expect producers of the antibiotics had invested some of their profit in research to identify mechanism(s) of action in promoting growth, and such work may have been performed but not

published. We found only one report published by a manufacturer (Merck, Sharp and Dohme). It focused on the effect of antibiotics on the transforming potentials and specific activities of cholytaurine hydrolase of bacteria in the small intestine of young chickens that correlated with growth performance (Feighner and Dashkevicz, 1987).

As early as the 1950s, concern was being expressed that continued use of antibiotics to promote growth of poultry and other food animals might result in antimicrobial resistance of pathogenic bacteria in humans. It gained momentum in the late 1960s and soon developed into advocacy for government withdrawal of approval for promoting growth with antibiotics on the premise that the food animal industry, with its large use of antibiotics, is a major source of antibiotic-resistant bacterial infections in humans. The AGP had been used extensively worldwide for several decades without causing a problem with resistant bacteria in food animals, which makes one wonder why they would be responsible for infection with resistant bacteria in humans when they had not caused a problem in the intense production of food animals. Nevertheless, it was easier to fault the animal industry than the medical profession's promiscuous use of antibiotics, often at their patients' insistence. Demands of the advocatory groups became so persistent and unrelenting that they could not be ignored.

Starr and Reynolds's report of resistant bacteria in turkeys after they had been fed streptomycin (1951) may have been the first report of resistant bacteria in food animals fed an antibiotic. The bacteria had not caused disease in the turkeys, but the authors mentioned its possibility and also the possibility of spread of resistant Salmonella from poultry to humans. Resistant bacteria in poultry have been characterised and both horizontal transmission and vertical transmission of some of them, especially *Escherichia coli*, from breeder flocks to poultry houses documented (Dierikx et al., 2013; Kemmett et al., 2013). These transferred, resistant strains can cause infection in young broiler chicks (Kemmett et al., 2014). Colibacillosis in young chicks also is caused by antibiotic-susceptible strains, so the frequency of infections with resistant strains is not known. It is reasonable to believe the incidence of infections with resistant strains has increased but when and by how much? Was there a significant increase in the early 1980s when resistance in bacteria increased considerably (Impacts of Antibiotic-Resistant Bacteria, 1995; Levy, 2001), and what has happened since feeding AGP was discontinued? We expect the industry now has a greater problem with resistant infections but it cannot be quantified.

Numerous papers have asserted the AGP are a major source of antimicrobial-resistant, bacterial infections in humans, but careful scrutiny of these papers reveals that most of the conclusions are inductive, not deductive, i.e. it is guilt by association and not evidence based. A report by E. S. Anderson (1968) is often mentioned as early evidence that feeding AGP to animals leads to resistant pathogens that pass from animal to human, causing disease, in this case *Salmonella* Typhimurium from cattle to humans. Although he could not determine how frequently antibiotic-resistant Salmonella passed from cattle to humans, Anderson was convinced they did. Given the methods available to him at that time, it was a reasonable conclusion. A recent paper by Mather et al. (2013) puts this in doubt. They conducted whole-genome sequencing on more than 200 isolates of *Salmonella* Typhimurium DT104 from the Scottish Salmonella Reference Laboratory. The samples were from both humans and livestock, collected over 20 years from the same geographical region. Distribution of different multi-drug resistant profiles between the two groups also was determined. Consequently, the characterised isolates could be used to evaluate their transfer between the two groups, human and animal, and it was found to be limited. Thus, the widely held view that most of the resistant Salmonella infecting humans had their origin in livestock could not be confirmed. Human types tend to infect humans, and animal types tend to infect animals. Now that this has been demonstrated for Salmonella, it implies that the same pattern might exist for most, if not all, infectious bacteria. Unfortunately, large collections of isolates from humans and animals living in the same region are not available for the other bacteria. Humans and animals are infected with many of the same bacteria but it is not known how often they pass from one group to the other. The new Salmonella

data indicate it should not be assumed that they frequently do, except from contaminated food.

Unquestionably, people may be directly infected with bacteria, some of them serious pathogens, from various foods, including poultry meat and eggs, but many of them are not antimicrobial-resistant bacteria. Several case reports are clearly examples of direct transfer from infected animal(s) to the patient (Fey et al., 2000; Huijsdens et al., 2006), and several reports strongly implicate food from animals as the source of infections in people who consumed the food (Holmberg et al., 1984; Mølbak et al., 1999). The report of Huijdens et al. involved *Staphylococcus aureus*, and the others involved Salmonella. A currently ongoing outbreak of multidrug-resistant *Salmonella* Heidelberg infections has been linked to poultry meat from Foster Farms in California (Centers for Disease Control and Prevention, 2013). More than 500 cases have been identified, which is unexpectedly high if the meat had been properly stored and transported, and if the affected people had properly stored, washed and cooked it.

Silbergeld et al. (2008) have summarised the extensive literature calling for prohibition of the use of AGP by the food animal industry. The scientific rationale for the claim that it is a major source of antimicrobial-resistant bacteria in human infections was detailed. They presented the various ways genetic resistance to antibiotics can be transmitted among bacteria, emphasised the presence of reservoirs of resistant bacteria in the vicinity of facilities where animals are fed antibiotics, and pointed out that people living in the same vicinity carry a large number of resistant bacteria, but the presence of infectious disease caused by these bacteria was limited. The authors acknowledged that while an abundance of data implies that the use of antibiotics in animals contributes to antimicrobial-resistant infections in humans, it might not be possible to determine an accurate risk for agricultural antibiotics in the incidence of resistant human infections. Nevertheless, the concern that antibiotic use in food animals might provide a source for human infections should be taken seriously, as the proposed molecular basis for it is widely distributed. The feared crisis could be eminent, but will banning the use of AGP prevent it? Probably not. Antibiotic use is regulated only for food animals, and the abundant use of antibiotics by human and veterinary pet practitioners remains unregulated. People are far more intimate with each other and their pets than they are with farm animals.

A recent report on surveillance of antimicrobial resistance (WHO Antimicrobial Resistance: Global Report on Surveillance, 2014) forewarns that a crisis in antimicrobial resistance is eminent, and the antibiotic era is at risk if remedial action is not undertaken to delay the crisis while governments and drug companies cooperate in searching for new antibiotics. It called for reduction in the use of antibiotics in human and veterinary medicine. Concerning food animals, the magnitude of transmission of resistant pathogens to humans is unknown and needs to be addressed through better data obtained by improved surveillance, and it needs to be harmonized with human data (similar to the recent Scottish Salmonella report – authors' comment). Use of antibiotics in food animals should be reduced, but therapeutic use will have to be continued to protect the food chain. Regulation of human and companion animal use was not mentioned.

Feeding AGP now is prohibited in the EU, and 25 manufacturers of antibiotics have voluntarily withdrawn them from the market for non-therapeutic use in the US. Many countries regulate, but do not prohibit, their use for growth promotion in animals. Permission may be obtained in the EU to use certain, specified antibiotics to treat these infections, except Salmonella. Although numerous reviews by government-established bodies in the EU and in the US, as well as international, resistant-bacteria surveillance programs, have not found scientific evidence to support a ban on AGP, the EU chose to ban them on basis of *the precautionary principle* (Dibner and Richards, 2005; Cervantes, 2006).

The consequences of not feeding AGP to broilers are not known because of a lack of sufficient, available data. Perdue Farms, Inc. conducted an extensive study of about 7 million broilers in two geographic locations, the Delmarva Peninsula (DMV) and North Carolina (NC), where birds fed with and without

antibiotics were compared (Engster et al, 2002). Results were different between the two locations. Absence of antibiotics in the feed resulted in reduction of livability by 0.2% (DMV) and 0.14% (NC), an average decrease in body weight of 0.03 lb (DMV) and 0.04 lb (NC), an average increase in FCR of 0.016 (DMV) and 0.012 (NC). Body weights were less uniform without antibiotics (uniformity is an important criterion for the industry). Feed conversion was not affected during the first year but FCR consistently increased in the unmedicated chickens thereafter. Bray et al. (2009) also found that sequential flocks of chickens reared without antibiotics for one year performed as well as chickens fed antibiotics, but Sun et al. (2005) and Martins da Costa et al. (2011) found that chickens fed antibiotics performed better than unmedicated chickens. These three studies were for one year or less.

Graham, Boland and Silbergeld (2007) used the data published by Perdue Farms to estimate the financial cost associated with withdrawal of antibiotics from feed. They rightly referred to the many improvements in the husbandry of commercial poultry production over the decades antibiotics have been added to poultry feed, and this might make the practice unnecessary. While the Perdue data did not include all the data the authors preferred to have, it was concluded the available data were sufficient to show there was no financial justification to continue feeding antibiotics. Poultry companies, who have access to their own data, are not convinced, and they actively seek alternatives for antibiotics. These companies maintain close attention to their costs and profits, and they surely would discontinue an unnecessary expense.

Are there equivalent replacements for AGP? Presently there is none, but there are potential alternatives.

Inhibitors of bile salt hydrolase are potential replacements for antibiotics (Lin, 2014). Feighner and Dashkevicz reported (1987) that six AGP reduce cholytaurine hydrolase activity in the intestinal ileum of chickens. Polymyxin B, an antibiotic that does not promote growth, did not reduce hydrolase activity. Digestion, emulsification and absorption of fats and lipids in the small intestine are aided by conjugated bile salts secreted from the liver into the small intestine. Once in the intestine, the bile salts are deconjugated and dehydroxylated by the intestinal microflora, in particular by cholytaurine hydrolase that the bacteria produce, and this results in reduction of the amount of fats and lipids that are absorbed into the body. This reduces the amount of energy available to a chicken for growth. The mechanism(s) by which AGP reduce cholytaurine hydrolase activity is/are not known, and knowing it/them should identify targets for drug development. This is actively being investigated (Smith et al., 2014) but it will be some years before a potential replacement can be identified, developed, and approved by regulatory agencies.

Many alternatives to AGP, such as pre- and probiotics, hormones, ionophores, methane inhibitors and other additives in animal diets, have been explored for a decade. These practices not only impose an extra financial burden on animal producers but they also leave residues in animal products, which may cause health concerns (Sharma et al., 2008). With the restricted use or outright ban on certain feed additives, new strategies of improving and protecting the health of farm animals must be explored. Additionally, useful additives should ensure optimum animal performance and increase nutrient availability. This goal can be approached by good housing or climate conditions, better feeding strategies with the best possible combination of the pronutrients available, including pro- or prebiotics, organic acids, dietary fibre, highly available nutrients, herbs, spices or botanicals (Rosen, 1996). Feeding prebiotics and probiotics is a safe practice that has been implemented for years, but researchers have not reached a concrete conclusion about their benefit. Feeding yeast, whether it is alive or dead, has not shown any conclusive evidence that supplementation is beneficial at all times (Yoon and Stern, 1996; Erasmus et al, 2005) and most of the research in this area has been conducted under *in vitro* conditions (Chaucheyras et al., 1995; Lynch and Martin, 2002; Lila et al., 2004; Rossi et al., 2004).

Many specific alternatives that have been proposed and marketed are less regulated than drugs. Huyghe-

baert, Ducatelle and Van Immerseel (2011) have reviewed the prominent ones. Exogenous enzymes are added to feed to modify and counteract the negative effects of non-starch polysaccharides in feedstuffs and improve the physical character of intestinal contents. Organic acids, and their salts, are added to drinking water or feed to promote various antibacterial activities. Butyric acid decreases the incidence of necrotic enteritis caused by clostridia, provides energy to intestinal epithelial cells, and has anti-inflammatory effects. Probiotics are live bacteria, such as lactobacilli, that are administered orally to restore or enhance the number of beneficial bacteria in the gut. Prebiotics are non-living, non-digestible feed ingredients that can promote growth and increase activity in the gut of beneficial bacteria, such as lactobacilli and Bifidobacteria. Herbs and etheric oils are promoted as having various beneficial effects on intestinal digestion. The results with many of these additives are often inconsistent, which results in lack of confidence in their usefulness. As regulations governing these products are not as demanding as they are for drugs, much work needs to be done to define mechanisms of action and consistency to establish confidence in their performance. Although these non-pharmaceutical agents are physiologically beneficial for chickens, their best use may be prevention and treatment of gastrointestinal infections (Alali et al, 2013).

Zagreb Biotek d.o.o. has been investigating growth promotion of broilers with recombinant glycoproteins produced in yeast transformed with artificial genes. GP1 was not stable. GP2 has been stable for more than two years at -20ºC and -80ºC. It is added to drinking water. Weight gain is not affected, but the FCR of treated chickens is significantly less than in untreated chickens ($p<0.05$) in conventional feeding trials. The birds appeared to be healthy and no physiological changes were observed. Uniformity of the treated chickens was better than it was among the untreated chickens. Immune responses to vaccination were not affected by treatment. Conventional tests for meat quality (based on pH value, colour and water holding capacity) and sensory traits did not detect any changes that would adversely affect acceptance by consumers. Considering the cost of feed and market prices at the time each feeding trial was performed, poultry producers would have saved an average of €0.22 per bird. This did not include the cost of GP2, which cannot presently be determined. Work with GP2 is continuing, and Zagreb Biotek will soon begin testing another candidate, GP3.

It is obvious that a variety of different compounds can stimulate growth promotion. Apparently, antibiotics employ differing modes of action, as some of them increase weight gain, whereas others reduce FCR, and some of them do both. Some of the non-pharmaceuticals also vary in the way they promote growth. Glycoproteins and the non-pharmaceutical additives are very different in structure from the AGP, and it is difficult to believe they would employ the same mechanism(s). There is much research to be done, and it is very likely growth promoters will be found/developed that produce results equivalent to the AGP, but without the stigma of increasing antimicrobial-resistant bacteria.

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