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FEEDING POULTRY IN THE POST-ANTIBIOTIC ERA

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INTRODUCTION

Antibiotics have been used in animal agriculture since shortly after their discovery. Concerns of antimicrobial resistance have existed for nearly as long, but recent concerns regarding the prevalence of antibiotic-resistant infections in humans have raised the controversy to new heights. The use of antibiotic growth promotants (AGPs) has been the focal point for agriculture. Certainly, antibiotic resistance is on the rise and there is much good research that supports this. Investigations which attempt to show the actual causes and sources of this resistance are beginning to appear in greater numbers and we would be foolish to ignore them. This paper gives a brief update of resistance issues and controversies and then provides some discussion on possible strategies for poultry production in the "post-antibiotic" era.

RESISTANCE IS REAL

Antimicrobial resistance can arise is several ways. *De novo* resistance arises as a result of single or multiple genetic mutations. This is a phenomenon to which all cells are susceptible but which increases in the face of a selection pressure. Further, resistance may be acquired through the horizontal flow of genetic material. Conjugative plasmids may carry resistance elements between different genera of bacteria. However, we are also beginning to realize that multiple resistance elements can be, and are, transferred between completely different species of micro-organisms. Integrons, distinct families of DNA elements, are involved in the capture and recombination of DNA "cassettes", which normally encode for a single resistance mechanism. Multiple cassette insertions can, and do, occur and this confers the property of multi-drug resistance. *S. typhimurium* DT104 is a well-know example of this phenomenon (1).

The elucidation of these "new" resistance-transfer mechanisms has important implications for agriculture. Obviously, the food animal population is a potential reservoir of resistance and there is a very real possibility of entrance of this resistance into the human population via the food supply. In the past, we (those of us in animal agriculture) have relied on arguments that support the use of antibiotics which are not used directly in human medicine. This thinking may be flawed, or at least over-simplified: not only are many antibiotic compounds chemically related in terms of their functional groups and modes of action, but the phenomenon of multi-drug-resistance acquisition through integron/cassette mechanisms tends to suggest that resistance for one class of compound may facilitate the acquisition of resistance to other compounds as well. The controversy surrounding the use of virginiamycin in food animals and its relationship to quinupristin/dalfopristin resistance in *E. faecium* (2) is an example of this – an example that is astutely used by the media and the medical community to strengthen the case against agricultural use of streptogramins despite the fact that the quinupristin/dalfopristin combination is, arguably, a very poor human therapeutic.

Perhaps the most glaring example of the agriculture/antibiotic resistance relationship is that of the rise and prevalence of vancomycin resistant enterococci (VRE). Vancomycin is a very important human therapeutic. Resistance to this relatively new compound can be found on a global scale, but the epidemiology of VRE in Europe differs markedly from that found in the United States. Although the hospital-associated prevalence of VRE is on the rise in the U.S., virtually no VRE has been found in the gut flora of healthy humans living outside of the hospital environment. In Europe, on the other hand, VRE rates are much higher in the general population and are readily isolated from sewage, animal waste, meat products and the feces of healthy persons (1). This prevalence of isolates in Europe has been linked to the approved and widespread use of avoparcin, a related glycopeptide antibiotic, in the feed of food-producing animals. As a result, the use of avoparcin as a growth promotant was discontinued in the European Union in 1996 and subsequent investigations have shown a precipitous decline in VRE isolates in meat products as well as in the gut flora of healthy persons. Avoparcin never received approval for use in feed in the U.S. (or in Canada). This serves as rather compelling evidence to suggest that the link between agricultural use and the prevalence of resistant human isolates is real, and indeed, the European avoparcin ban has become the engine of change that has driven much policy direction since that time.

ADAPTATIONS TO REDUCTIONS IN ANTIBIOTIC USE

The experiences of other countries yield important information about how we might best cope with a restricted use of antibiotics and what changes we might expect. In some European countries, growth promoter bans were followed by an overall *increase* in total antibiotic consumption (3). Presumably this was a result of the greater need to treat clinical disease outbreaks which otherwise would have been suppressed by growth promotant use. This fact is sometimes held up as an example of the fallacy of such a ban. However, it can be argued that the therapeutic use of antibiotics, although leading to higher use rates, applies them in ways which may actually minimize the possibility of development of resistance.

Many European countries have implemented programs which severely curtail the use of antibiotics in animal agriculture, particularly for growth promotion. In 1986, Sweden banned the use of antibiotic growth promoters. It is interesting to note that this action was largely driven by the farm lobby as a means of improving consumer confidence in animal food products. The Danish authorities issued bans on a number of antibiotics (avoparcin, virginiamycin, bacitracin, spiramycin and tylosin) for use in animals, in the late 1990s. In early 1998, the various food animal industries in Denmark agreed to voluntarily discontinue the use of all antibiotic growth promoters by the end of 1999. Concurrent with these changes, regulations were implemented to the effect that veterinarians could not profit from the sale of therapeutic antibiotics to livestock and poultry producers, and a comprehensive surveillance program for antimicrobial resistance was initiated (4). Recent follow-up data show striking changes in antimicrobial usage patterns as well as in the occurrence of resistance isolates (Table 1).

Table 1: Change in Rates of Resistance in Specific Organisms Isolated from Broilers and Pigs in Denmark Subsequent to a Decrease in Antimicrobial Use**.** Adapted from Aarestrup *et al*., (4)

Type	**Isolate**	**Peak Rate, % (year)**	**Rate, % (2000)**
Broiler	glycopeptide res. *E. faecium*	73% (1995)	6%
Pig	glycopeptide res. *E. faecium*	20% (1997)	6%
Broiler	erythromycin res. *E. faecium*	76% (1997)	13%
Pig	erythromycin res. *E. faecium*	90% (1997)	47%
Pig	erythromycin res. *E. faecalis*	90% (1997)	28%
Broiler	virginiamycin res. *E. faecium*	66% (1997)	34%
Broiler	avilamycin res. *E. faecium*	77% (1996)	5%

Emborg *et al*., (5) conducted a retrospective study of Danish broiler production subsequent to a voluntary ban on AGP use in that industry. They found that the kilograms of broiler produced per square meter of housing did not change, nor did the rate of mortality. The most significant correlate to total percent mortality was age at slaughter, as one would expect. Feed conversion deteriorated by only somewhat less than 1%. These researchers noted that anecdotal evidence was present to suggest that feed manufacturers had made changes to feed to accommodate the withdrawal of AGPs, but no mention of producer profitability in the face of these changes is presented. In Sweden, similar results were seen. For finishing pig, layer, turkey and beef production, no adverse effects were noted with a ban on AGP use. However, broiler chicken and baby pig production was affected negatively and it took approximately four years subsequent to the ban to develop alternative production strategies that could overcome this (3).

The European experience is largely seen as an overwhelming success and the European Commission has proposed an extension to regulations that will virtually eliminate all feed-borne antibiotics by 2006. The intention is that these regulations will extend to imports (6).

STRATEGIES TO DEAL WITH THE LOSS OF AGPs

It is impossible to discuss feeding approaches without some mention of related husbandry factors that are well known to influence the animal response to feeds. There are three basic strategies that can be used to cope with the loss of AGPs. These are: 1) pathogen reduction; 2) augmentation of the immune response; and, 3) nutritional strategies and/or additives that either improve performance in their own right, or help to directly modify the gut microbial flora.

Pathogen Reduction: This has always been important but has taken on a new urgency. Barn sanitation, pest control, environmental quality, including litter management and biosecurity are important prerequisites to minimizing the need for antimicrobial therapy. Good litter management means lower pathogen exposure. Nipple drinker systems have kept water clean, reduced water spillage and given drier litter, which reduces pathogen load. Water and air quality improvements are also beneficial. Most producers can do better and should embrace new technologies that provide these benefits.

Augmentation of the Immune Response: Much is coming to light regarding how to make the most of the bird's inherent immune response. Increasing reliance on vaccination and development of improved vaccine delivery may provide opportunities to minimize feed-borne medications. At the same time, we are learning more about how to control the negative effects on performance that the immune response confers. It seems to be the systemic, acute phase response of the animal to disease challenge that confers significant nutrient requirement and therefore detracts from productive efficiency (7). Conjugated linoleic acid is one compound that has been investigated for its apparent abilities to alleviate the immune-associated anorexic response (8).

Nutritional Strategies and Additives: Currently approved antibiotic compounds have characteristics that help to explain their position as the additives of choice for growth promotion. In general, they are extremely effective at remarkably low doses and they are relatively cheap, thus yielding significant return-on-investment. Although food-product residues can be a concern, many AGPs have relatively low toxicity for higher animals. Notwithstanding the concerns about antibiotic resistance, there are few alternatives available today that can meet the benefits of the AGPs that they purport to replace. Following is a brief description of some of the many nutritional and additive approaches that show promise.

The link between diet and the incidence of enteric disease in birds is well known. Diets based on wheat, barley or rye, for example, seem to confer greater susceptibility to necrotic enteritis (9). The provision of substrate to the lower gastrointestinal tract increases the risk of bacterial overgrowth. Thus, any approach that improves digestibility is typically of benefit to the bird.

Enzymes have shown great utility by increasing digestibility, decreasing intestinal viscosity and inactivating antinutritional factors. By removing fermentable substrates from the ileum, ileal populations of microflora are reduced (10). The relative contribution of an enzyme preparation is greater when the quality of the cereal source is lower, a fact which will be difficult to exploit in most of the U.S. where corn/soy predominates. In factorial studies, use of enzymes alone resulted in an average of 5.9% improvement in feed conversion ratio compared to controls while AGPs yielded an average improvement of 3.3%. This data was obtained using European-type diets (high cereal grain). Once AGPs are removed, the relative response to enzymes will increase but may still not reach that obtained by AGPs.

While exogenous enzyme addition appears to limit microbial growth in the ileum, the opposite may be true in the cecal environment. Here, the products of enzymatic breakdown may provide fermentable substrates to the cecal flora. Increases in volatile fatty acid (VFA) production and changes in the VFA profile serve to favour the beneficial organisms (*Bifidobacteria*, for example) and suppress populations of deleterious organisms (*Campylobacter, Salmonella, Clostridium*).

Betaine, an osmolyte, has been used to improve the moisture characteristics of manure and to alleviate the effects of heat stress in birds. One concern over the loss of AGP availability is an expected increase in necrotic enteritis (NE) caused by *Clostridium perfringens*. NE is often associated with coccidiosis challenge. When fed with ionophore anticoccidials, betaine appears to enhance the ability of the ionophore to lower lesion scores (Figure 1).

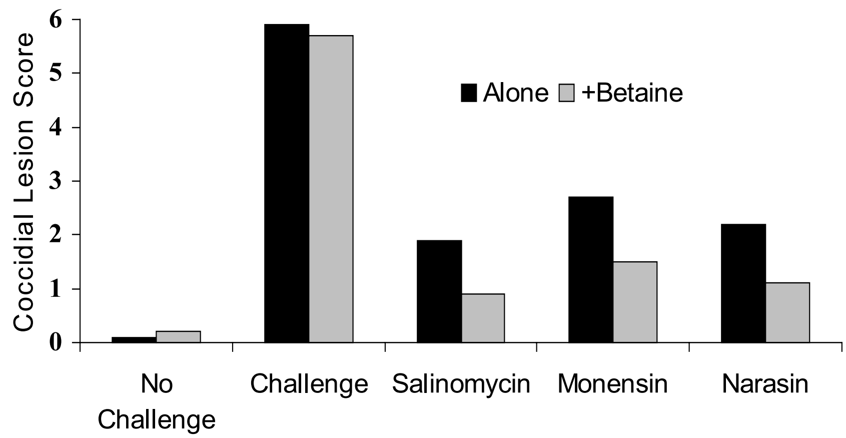

Figure 1: Interaction between betaine and selected ionophore anticoccidials in broilers when fed as factorial combinations. Birds were challenged with *E. acervulina, E. maxima* and *E. tenella*. Adapted from Bedford, (10). Differences within each ionophore are significant ($P<0.05$).

While AGPs act primarily by inhibiting the growth of Gram-positive bacterial populations, **mannanoligosaccharides** (MOS) and other oligosaccharides have been shown to act as "decoy" attachment sites for gram-negative pathogens. For pathogenesis to occur, bacterial cells must colonize the surface of the host enterocyte through specific fimbrionic attachments. Oligosaccharide preparations of yeast cell wall have been shown to block fimbrionic receptors and prevent colonization (11). In addition, MOS may also improve the structural integrity of the gastrointestinal tract, through mechanisms not yet clearly understood. Studies comparing the use of MOS with two commonly-used AGPs, virginiamycin and bambermycins have shown that feeding MOS may be an alternative and effective strategy (Table 2).

Table 2: Effect of mannanoligosaccharides (MOS), bambermycins (BAM) and virginiamycin (VIR) on 20 week production variables of heavy tom turkeys. Adapted from Parks *et al*., (11). Means within a column having different superscripts are significantly different (P<0.05).

Treatment	Weight, kg	Feed:Gain	Mortality, %	Cull Rate, %
Control	17.5[b]	2.55	3.13	3.13
MOS	17.9[a]	2.54	2.56	2.20
BAM	17.8[a]	2.57	1.88	4.38
VIR	17.8[a]	2.51	2.50	5.63

Probiotics, live, defined cultures of micro-organisms have also been fed to poultry in an attempt to improve performance. Gram positive bacteria of the *Lactobacillus, Enterococcus, Pediococcus* and *Bacillus* types are used, as are fungi of the *Saccharomyces* (yeast) genus. Such cultures are often fed as an adjunct to antibiotic therapy as a means of re-introducing a beneficial flora to the GI of affected animals. More recently, an extension of the "competitive exclusion" principle has lead to more routine administration although the factors affecting colonization of a given probiotic culture are not clear. Difficulties arise in defining the specific culture to be used and in application, since the heat-treatment conferred by pelleting is obviously deleterious to live-cell products. Although probiotics may be beneficial in enhancing GI environment, grow-out data is often lacking in significant improvements. Most of the proposed mechanisms of action remain in the hypothetical realm and much work remains to be done to refine the application of this approach (12).

Herbs, spices and various plant extracts have received increasing attention as possible AGP replacements. There is evidence to suggest that some of these components have appetite-stimulating properties (menthol from peppermint), anti-bacterial effects (carvacrol from oregano) or may provide anti-oxidant functions (cinnamaldehyde from cinnamon) (13). To be effective on a practical scale, it is likely that these compounds will need to be provided in more concentrated form than they are found in their natural source. These materials are often positioned as being "all natural", which may be true but which may be misleading from a safety point of view. Many antibiotics are "all natural" compounds in concentrated form; it is likely that those herbal extracts that prove most effective in modifying GI microbial ecology will also face increasing pressure for regulatory approval.

Organic acids are also known to have strong antibacterial effects. Use of acidifiers in baby pig feeds has proven beneficial, and organic acids have been used as *salmonella*-control agents in feed and in water supplies for livestock and poultry. Success of acidifiers in baby pig nutrition is typically due to high buffering capacity of feeds coupled with limited ability for hydrochloric acid production in the piglet. Typically, blends of organic acids representing an array of pKa optima are more effective than single acids alone. Table 3 shows the effects of two essential oil products and an organic acid treatment compared to control and bambermycin treatments for broilers.

Table3: Effect of antibiotic growth promoter (bambermycins), two essential oil products (Extracts A and B) and an organic acid on growth and feed conversion of broiler chicks from 5 to 28 days of age. Means within a column sharing like superscripts are not significantly different ($P<0.05$). Adapted from Langhout, (13).

Treatment	Weight Gain, g	Feed:Gain
Control	1393	1.66^{a}
10 ppm bambermycins	1401	1.61^{c}
Extract A	1389	1.65^{ab}
Extract B	1382	1.63^{bc}
Organic acid	1389	1.64^{abc}

One promising alternative to antibiotics involves the feeding of **specific antibodies** to neutralize pathogenic organisms. In simple terms, hens are exposed to particular antigens and their systems stimulated to produce immunoglobulins. The immune proteins are then harvested from eggs and processed for inclusion into feed. This approach has met with some success in the swine industry. There may be problems with mode of delivery since heat treatment is obviously detrimental to the functionality of protein, as is the digestive process of the animal.

Bacteriophages are viruses which infect bacterial cells and may destroy them by lysis. They can be very specific to certain pathogens. This idea is not new, but has been stalled perhaps because of the prevalence and success of antibiotics in fighting bacterial disease. Recent work with poultry has suggested that the bacteriophage may be a useful replacement for antibiotics (14). In fact, researchers at the National Institutes of Health have successfully used bacteriophage to combat experimentally induced VRE infection in mice (15).

CONCLUSIONS

Limited data has been presented to support the efficacy of AGP alternatives. Few of these alternatives will match the efficacy of growth promotion that we have come to appreciate, but it is likely that several of these strategies, applied in tandem, will serve to bring animal performance to within reach of previous levels. Perhaps the most daunting impediment will be that of cost; most of the above approaches are relatively expensive to implement on their own, let alone in combination. More integrated operations, prevalent in the U.S., may have greater opportunity than those engaged in commercial sales.

Whether or not resistant human infections are a result of agricultural use or misuse/overuse on the part of the medical community is purely academic. It has been noted that rational arguments do not sway frightened politicians (16). The reality that we face as poultry producers is that the consuming public does not like the idea of routinely feeding antibiotics to poultry, for whatever reason, and so it is not inconceivable that such practice will be severely curtailed at some point in the future. If we wish to maintain the availability of antibiotics for the treatment of disease in food-producing animals, then perhaps it's time to embrace the concepts of "prudent use", implement alternative production strategies, and strive to minimize our dependence on antibiotics in general.

SYNOPSIS

1. The threat of antibiotic resistance is real.
2. We need to learn the lessons presented by other countries.
3. Management factors other than feeds and feeding can also be helpful in the absence of AGPs. These include genetics, vaccination, and environmental management issues.
4. There is a host of feed additive opportunities presently being made available that show promise; the trick will be sorting out those that are effective from those that are not. Cost will also be a factor.
5. Antibiotic resistance and AGP use in agriculture is a public perception issue. We need to embrace alternative technologies that can help to reduce our dependency on AGPs so as to ensure their continued availability for therapeutic application.

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