Rational development of new additives

Microflora manipulation without AGPs

WHAT SHOULD AN EFFECTIVE FEED ADDITIVE DO? ESTABLISHING THE GOALS OF NEW ADDITIVES SHOULD BE THE FIRST STEP IN THEIR DEVELOPMENT. THEN IT'S A CASE OF FILLING IN THE GAPS. BUT IS THIS EASIER SAID THAN DONE? SARAH MELLOR WENT TO NOORDWIJK AAN ZEE IN THE NETHERLANDS IN SEARCH OF SOME EXPERT OPINION.

In the blustery coastal setting of Noordwijk aan Zee in the Netherlands at the end of January, some 200 plus experts and interested parties from 19 different countries participated in an intensive two-day debate about the implications of the European Union's impending ban on the use of antibiotic growth promoters (AGP's) in livestock feed. While the rationale of implementing this legislation occupied the first day's discussion time (To ban or not to ban? *Feed Mix*, Volume 13 Number 2, page10), the consequences from a scientific and product-development point of view were perhaps the more inspiring and productive element for many delegates.

GONE BUT NOT FORGOTTEN

AGP's have occupied a vital place in livestock production for more than 50 years. Their performance enhancing characteristics helped shape the modern production systems that satisfied the western world's craving for meat, milk and eggs at the levels we expect today; bringing animal agriculture into the modern industrial era.

It has often been said that we never worked out quite how AGP's exerted their growth promoting effects, but nevertheless their effects on the intestinal microflora were pivotal- the lack of benefits conferred under germ-free conditions (and the fact that antimicrobials kill bacteria) more than suggest that AGP's effects were exerted via a change in the balance of the intestinal microflora. The modern obsession with a mode of action with new feed additives may also have prompted some researchers to look again at AGP's for comparison, but in many parts of the world they still play an important role.

MOLECULAR BASIS FOR AGP EFFECTS

Margie Lee's research at the University of Georgia's Poultry Diagnostic and Research Center in the US has led her to use molecular techniques to characterise complex microbial ecosystems and study the effects of feed additives and feed formulations thereof. Lee and her group have shown how the bacterial community structure and the distribution of bacterial species in the chicken ileum responds to AGPs and different diet formulations.

Comparing ileal bacterial communities from birds fed a variety of AGP's showed that, once the diversity of the microflora composition stabilised after 14 days of age so that only a few abundant species remained, there were significant differences between treatments (different AGPs) and control birds fed a corn-soy diet. With age, an abundance of *Escherichia coli* at 14 days gave way to a predominance of *Clostridium irregularis* in older birds (21-28 days of age) in AGP treated birds, indicating a suppression of some bacterial populations by the AGP treatments. These differed from the control group, whose ileal microflora was composed mainly of lactobacilli. The total bacterial population of each group did not differ significantly from the controls. That significant differences were found between the AGP and control groups, indicating that the antibiotics were selecting for a uniquely different ileal bacterial community and suggesting, said Lee, that the microbial population of the intestine is very sensitive to feed additives may seem obvious, but it was not until research groups began to apply molecular tools that the real effects of feed and feed additives on the intestinal microflora began to be explored. In Lee's study for example, feeding poultry a mix of virginiamycin and bacitracin may not have enhanced the populations of bacteria that are commonly accepted to be 'beneficial', such as *Lactobacillus acidophilus*. Instead, she speculated that the antibiotics and monensin might have selected for populations of as yet uncharacterised intestinal symbionts that have other effects on mucosal health and feed conversion.

MICROBIAL MODULATION

Juha Apajalahti of Alimetrics in Finland reiterated the effects on the microflora outlined by Lee and added a few extra. Reducing pathogens where there is a high environmental burden and other stress factors, antibiotics may also help prevent development of clinical disease, which would lead to suppressed growth and increased mortality, he said. Further to their nutritional and health benefits, AGP's have also been reported to improve meat quality by reducing the numbers of bacteria whose metabolites can negatively impact carcass quality while having no impact on host health, such as skatole producers in the pig colon.

Removing AGP's could have other effects than feed efficiency and mortality, added Apajalahti. Modern management and breeding were developed against a backdrop of AGP use, so the 'normal' intestinal microflora we see today consists of antibiotic tolerant strains. Removing AGP's will inevitably change the selection pressure on the microflora to favour bacterial species that prefer an antibiotic environment. The outcome, he said is unpredictable and may include previously unknown bacterial species and genera (not least because we now have better identification and characterisation techniques)- of whose health implications we are currently unaware.

"IT IS HARDLY SURPRISING THAT THE RESPONSES REPORTED IN THE LITERATURE ARE SO VARIED" MIKE BEDFORD, ZYMETRICS.

WHAT DO WE NEED?

New microbial modulators will be developed in a rapidly changing microbial environment. While Apajalahti outlined some measures he deems necessary for such products to be successful (updating our knowledge of the relevant members of the bacterial community; developing rapid assays for quantitative analysis of bacteria and developing relevant, practical laboratory simulation tools to mimic the microbial competition in the intestinal tract), Rob Havenaar and his colleagues at TNO Quality of Life in the Netherlands have been using two gastrointestinal models dubbed TIM-1 (stomach and small intestine) and TIM-2 (large intestine) to test a number of feed additives already available on the market. These computer-controlled models simulate the peristaltic movements, secretions and large intestinal fermentation activity of pigs, calves, dogs and humans over a realistic time scale. The system has been validated against human and animal studies for proteins, minerals, vitamins and bioactive food components, the formation of toxic compounds and mycotoxin binding, he said. Furthermore, when used in combination with cultured intestinal cell lines, mucosal transport and cell metabolism can be taken into account.

So far, the group have used the model to select robust strains of probiotic bacteria; to study the effects of organic acids on the intestinal microflora; of prebiotics and dietary fibres on nutrient digestibility in the small intestine and fermentation in the large intestine, including volatile fatty acid production.

IT'S NOT ALL ANTIMICROBIALS

Zymetrics' Mike Bedford gave an eloquent illustration of the possible negative effects of bacterial fermentation in the intestine and hence the potential to confer benefits by reducing bacterial numbers and improving nutrient availability. Although this is a very suitable model for visualising the positive effects of AGP use, given the knock-on effects of changes in the enteric flora in terms of digestive enzyme secretion, gut motility, mucin quantity and quality, rate of enterocyte turnover and immune effects, it is hardly surprising, he said, that the responses reported in the literature are so varied. The net result of AGP use commercially is an improvement in the ration's nutritive value, by improving nutrient availability and/or utilisation. In other words, he said, birds have to work less to get the energy out of their diet. Given such a clear mechanistic picture, it would seem rational, said Bedford, that provided all the activities of an AGP are duplicated, it should be possible to replace them via alternative mechanisms without loss of performance. The problem we still have, Bedford continued, is that we still don't know which microorganisms need to be controlled or encouraged.

Enzymes are the classical agents used to increase the nutritive value of feedstuffs. Through their action on the cell walls of cereal grains or by releasing phosphorus from phytate, their use may mitigate some of the circumstances that lead to disease. But it's not all down to improving diet digestibility, Bedford warned- the host microflora participate actively in this process. For example, degradation of plant cell walls releases oligomeric carbohydrates- obvious substrates for some bacteria to ferment further down the tract. It seems sensible that there is some overlap between the manipulation of the bacterial microflora exerted by AGP use and that caused by enzymes, meaning that synergy between the two is not inevitable.

Commercial experience has shown that removal of AGP's from diets did result in reduced performance. Furthermore, feed mills that were producing diets responsive to enzyme use were mostly already doing so and in those that weren't, adding enzymes didn't solve the problem. Put simply, said Bedford, AGP's aren't so diet dependent as enzymes are.

While enzymes are clearly of great value in AGP-free diets, Bedford concluded, it is not realistic to expect that they, or any other non-antimicrobial additive will be able to replace all of the activities of an AGP.

WHAT DOES THIS MEAN TO PRODUCTION?

Gordon Rosen is a practising and very active pronutritionist. For those already familiar with his work, Rosen has taken meta-analysis to a whole new level. *Holo*-analysis has created practical feeding models constructed out of all the available data that Rosen collects from the literature on the animal, management or dietary factors that may (or may not) impact on the ultimate performance responses. The process begins with mining the literature for negatively controlled nutritional response tests.

So far he has studied AGP's, non-starch polysaccharides and phytase use in pigs and broilers, but his experience has also helped him develop a useful rule of thumb for anyone trying to find their way through the huge array of different pronutrient products now available on the market.

His seven question test is as follows (responses given in parentheses might be considered acceptable):

-> 1. How many properly controlled feeding tests do you have on the efficacy of Product X? (≥30)

-> 2. How many of the tests have no negative controls? (5)

-> 3. Is a bibliography available for points 1 and 2 above? (yes)

-> 4. How many times out of ten does the product improve liveweight gain and feed conversion? (≥7, equivalent to the data available on AGP's)

-> 5. What are the coefficients of variation in the gain and feed conversion responses? (100-200%)

-> 6. What dosage of Product X will maximise return on my investment and why? (x ppm)

-> 7.Can you supply me with a model to predict responses to product X under my conditions? (yes)<-