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# Improve bird performance by feeding its microflora

**The chicken and the microflora in its intestines are one. The ingredients in the feed influence the behaviour of the microflora and as a result the digestion of the feed. To find feed formulas on which the chicken can perform on an optimal level, the 'feeding' of the intestinal microflora should be considered. Here lies a challenge for the nutritionist of the next millennium.**

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The microfloral populations of both the small and large intestines in chicken can reach densities of almost $10^9$ and $10^{11}$ per gram of ileal and caecal contents respectively. Such extraordinary concentrations of metabolically active organisms will evidently influence not only the availability of substrate for the host, but also the relative "well being" of the host and hence its ability to grow rapidly. Nutrition as a discipline should therefore consider the bird and its microflora as intrinsically linked and dependent upon the ingredients and nutrient densities we supply. The latest views on nutrition suggests that in the future feed compounders should be as concerned with the impact their dietary ingredients/density have on the microfloral species distribution and density as they are today on meeting the requirements of the bird.

Microflora have an impact on the nutrition of the bird. Their presence increases the apparent energy extraction from the diet, yet at the same time reduce the growth rate and efficiency of such conventional chicks with their germ free counterparts. This way the presence of the microflora dramatically alters the nutritional status of birds fed an identical diet.

For example, caecal flora not only constitute a large proportion of the total faecal amino acid pool, but also actively convert some amino acids into others. As a result, negative amino acid digestibility's can occur between the ileum and faeces, which makes it difficult to evaluate the digestion process.

## Antibiotics affect growth rate

The use of antibiotics has consistently been shown to improve growth rates and efficiencies of birds. Suppression of the microfloral populations can improve the growth rate of the host, presumably through reduction in sub-clinical infections and reduced competition in the intestines. The response to addition of antibiotics very much depends upon the type of diet being fed, with rye based diets for example responding more favourably than corn based diets.

Probiotics benefit the performance of the bird. When these live organisms are fed it is suggested that alteration of the microfloral status can significantly impact on the nutrient utilisation of the bird. Whilst it is clear that microflora does impact on the nutrition of the bird, there is very little acknowledgement that this is the case in classical nutrition. Conversely there is little evidence recognising the impact that nutrition of the bird can have on its microflora.

**Table 1. Relative abundance % of bacterial DNA collected from the caeca of broiler chickens fed wheat based diets with or without 2,500U xylanase/kg feed.**

| *Genera* | *Control* | *Enzyme supplemented* |
|---|---|---|
| ***Lactobacillus & Enterococcus*** | **26** | **26** |
| ***Clostridium*** | **14** | **11** |
| ***Escherichia & Salmonella*** | **11** | **6** |
| ***Peptostreptococcus*** | **10** | **11** |
| ***Bacteroides*** | **10** | **16** |
| ***Propionibacterium*** | **10** | **11** |
| ***Campylobacter*** | **9** | **6** |
| ***Eubacterium*** | **8** | **10** |
| ***Bifidobacterium*** | **2** | **4** |

## Tight relationship between feed and disease

The relation between some diseases and certain feed ingredients has driven much of the interest on effects of nutrition on the microbial status of the bird. It is recognised that there is an influence of the major cereal used in broiler diets and the incidence of necrotic enteritis, for example. Such evidence shows that ingested feed has an impact on the microbial community inhabiting the small and large intestine, which in turn influences the nutrient assimilation rate of the bird.

The lack of reliable, repeatable techniques has probably hampered efforts

**Table 2. Influence of percentage of wheat substituting for rye on the relative abundance of bacterial DNA (as % of total) in the caeca of broiler chickens.**

| *Genus* | *100% wheat* | *66% wheat* | *33% wheat* | *0% wheat* |
|---|---|---|---|---|
| *Enterococcus* | 17 | 36 | 39 | 43 |
| *Lactobacillus* | 49 | 35 | 16 | 6 |
| *Escherichia* | 8 | 7 | 27 | 25 |
| *Salmonella* | 3 | 2 | 4 | 8 |
| *Clostridium* | 12 | 13 | 8 | 10 |
| *Campylobacter* | 10 | 7 | 7 | 8 |

**Table 4. Influence of avoparcin or xylanase on the concentration of volatile fatty acids measured in the caeca of broilers fed wheat based diets (mM.)**

| | *Acetic acid* | *Propionate* | *Butyrate* | *Total VFA* |
|---|---|---|---|---|
| *Control* | 41 (53%) | 11 (14) | 27 (35) | 78 (100) |
| *Xylanase* | 50 (45) | 45 (40) | 17 (15) | 112 (100) |
| *Avoparcin* | 23 (48) | 14 (29) | 11 (23) | 48 (100) |

to link shifts in floral populations with particular dietary events. Most studies using plating techniques confer a degree of inaccuracy in enumeration, because there will be countless species which will not be found since the conditions for their growth are not catered for.

Extracted microflora are very sensitive to exposure to air for even very short periods. As a result there can be several log reductions in species numbers between the caeca and the test-tube. Therefore DNA- based techniques are helpful to get the exact outcome.

## Advantage of DNA-based techniques

Recent work in our laboratory has focused on the impact of the use of dietary xylanase (2,500 u/kg feed) on the microfloral species distribution in the caeca of broiler chickens fed wheat-based diets. Such enzymes have consistently been shown to improve the nutrient status of birds offered wheat and in particular rye-based diets. It is suggested that part of this response to enzyme utilisation is through alteration of the microfloral populations in the gut. With DNA-techniques we found a consistent alteration in the rnicrofloral profiles as shown in *Table 1*.

The addition of enzyme increased the number of the species of *Peptostreptococcus, Bacteroides, Propionibacterium, Eubacterium* and *Bifibobacterium* and decreased the number of the species of *Clostridium, Enterobacteriacaea* and *Campylobacter.*

LAB as a group is unaffected, but we have seen earlier that enterococci are reduced and lactobacilli increased by inclusion of this specific xylanase (derived from *Trichoderma*) in a wheat based diet. Unfortunately, whilst many differences approached significance, only the *Bacteroides* effect was statistically significant ($p<0.05$).

In our studies to date clostridia have been relatively abundant ranging from 15 to 40 percent of the total microbial population. We have found that the majority of the clostridia are the species *Clostridium clostridiiforme, C. celerecrescens, C. innocuum, C. leptum*, and *G. thermocellum.*

To our knowledge these species have not yet been isolated from the broiler ceacum. This clearly illustrates the limitations of traditional plating techniques - if you do not know the requirements of the bacteria living in the caeca then you will not be able to isolate and thus enumerate them.

## Desirable and unwanted strains

Discoveries as identified earlier are likely to be commonplace for several years to come, as far as bird health and performance are concerned. Due to endless interactions between species it is unlikely that the presence or absence of any one species controls the presence or absence of a disease condition. For example, *C. perfringens* is regularly found in the caeca, but it is only when its numbers explode that a disease outbreak is likely to occur. Supply of substrate and presence of inhibitors, competition and the immune system keep its numbers in check.

Since many species of bacteria rely on other bacteria for their substrate, it is often not an event, but a series of events which leads to a disease outbreak or reduction in growth rate. One bacterial species may digest starch and produce lactate as its end product, which is used by another to produce propionate, which is used by another to produce another product and so the process can go on and on. A pathogen or in fact a beneficial species could be at the end of such a chain. As a result we should consider how a particular set of nutrients will influence not only the pathogens and "beneficial bacteria" directly, but also their "substrate suppliers" and competitors.

## Links between nutrition and microflora

The links between provider and utiliser of a particular metabolite may be distant. We suspect, for example, that a large part of the lactate produced in the ileum is metabolised in the caecum. When one considers that there are over 400 species of bacteria that have been identified in the caeca, almost endless combinations of species distributions can occur.

The presence of an enzyme has a dramatic effect on caecal populations. We recently analysed caecal samples from birds used on trials in which diets based on various blends of wheat and rye were used. As expected, increasing the inclusion level

**Table 3. Influence of Avopacin or xylanase on the total numbers (all numbers are x $10^{10}$) of bacteria resident in the entire ileum**

| *Species* | *Control* | *Avoparcin* | *Xylanase* |
|---|---|---|---|
| *Lactobacilli* | 2.3 | 0.2 | 0.8 |
| *Coliforms* | 2.0 | 0.2 | 0.8 |
| *Enterococci* | 8.0 | 1.0 | 3.1 |
| *TOTAL* | 12.3 | 1.4 | 4.7 |

of rye at the expense of wheat severely limited performance of the chicks and increased the incidence of mortality. The population of microflora in the caeca is described in *Table 2.*

As can be seen, exchange of one cereal grain for another has a dramatic effect on the percent distribution of virtually all species. Interestingly the species of bacteria which tend to be associated with a probiotic effect (lactobacilli) tend to decrease with increasing rye concentration. This is in line with observation of greater disease challenge/problems which are encountered with rye compared with wheat based diets.

Increasing the concentration of rye in the diet will decrease the rate of digestion through increasing intestinal viscosity. Therefore as a consequence, more starch, protein and fat reach the terminal ileum undigested which provides substrate for a series of bacterial species that may otherwise not prosper, and in turn the products of their metabolism will provide further substrates for another series of bacterial species and so on.

Another consequence of increased viscosity is decreased mixing which provides a more anaerobic environment. As a result there is greater potential for obligate anaerobes to invade the ileum and not only compete for resources but precipitate disease. A mass change in microfloral populations is evident from *Table 2.* Clearly this is not simply a mild alteration in 1 or 2 species, but a mass movement towards bacteria of very different metabolic capabilities.

### The effect of a growth promoter

When investigating the effects on the ileal and caecal microflora as a composite unit, it might suggest we should view the intestinal microflora as a whole. A test was carried out to compare the effect of a growth promoter, avoparcin, and a xylanase in a wheat based diet. Then the ileal contents were collected and analysed for total numbers and metabolic activity.

Clearly the enzyme has improved the digestibility of the ration as far as the chick is concerned, which results in there being less substrate (starch, protein and fat) available for the bacterial community. As a result use of enzyme reduced ileal populations significantly. Such a significant reduction in bacterial numbers not only results in less competition for nutrients but also reduces the likelihood of a critical threshold being crossed and an immune response being mounted. Mounting immune responses to perceived challenges is extremely detrimental to the growth rate of the bird due to the anorexic effect of the whole process.

The response to avoparcin is more dramatic, but this is as would be expected given the fact it directly inhibits the growth of bacteria. Moreover it gives a clear indication that perhaps the benefit of such product is derived through reduced competition for substrates and reduced immune responses to subclinical disease for the reasons described above.

The xylanase is not only improving digestibility of the feed-borne nutrients through reducing viscosity, it is also providing a substrate for a certain class of bacteria. In addition to drastically reducing total numbers of bacteria in the ileum, the use of the enzyme, through provision of this novel substrate, altered the metabolic capability of the remaining bacteria through selection for xylose-utilisers.

It is interesting to note the influence of the xylanase and antibiotic on the caecal populations in this study. *Table 4* shows that the fermentation profiles were different between treatments, with the presence of the xylanase significantly increasing total and in particular propionic acid concentration, principally, we suppose, through favouring propionibacteria and bifidobacteria at the expense of clostridia (data not shown).

On utilisation of the xylanase, both *Salmonella* and *Campylobacter* numbers were reduced *(Table 1)*, as a result no doubt in part through elevated propionate concentrations. Thus not only the nutrition and health of the broiler can be influenced by diet, but also the health of the consumer.

It is interesting to note the significant reduction in all volatile fatty acids (VFA) concentrations on use of Avoparcin. Again, it appears that the growth promoter is influencing performance through reduction in total microbial numbers which will lessen the chance of an immune response. ❑

## Conclusions

**It can be concluded that the ingredients fed to the bird will significantly impact on the nutrients available for the microflora in the terminal ileum and caeca. As a result, seemingly equal diets in calories and nitrogen content may actually yield very large differences in performance. Knowledge of the factors which control the development of a beneficial versus a malevolent floral population is the key to successful intensive livestock production in the next millennium. ❑**

Key words:
Microflora, AGP, broiler, xylanase