Microbial activity in the alimentary tract of birds

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Nearly all the work on the avian gut microflora has been done using the domestic chicken (Gallus domesticus). There has been some work on the turkey and quail, which have dietary habits similar to the chicken, and there are also a few reports on the pheasant, grouse and goose with respect to fibre digestion. There is virtually nothing known about the gut microflora of carnivorous birds. The greater part of this paper will, therefore, deal with information from the chicken.

Composition of the gut microflora

In the chicken the two main sites of bacterial activity are the crop and caecum (Smith, 1965). The crop is an expanded part of the oesophagus anterior to the acid stomach (the gizzard) where the low pH is responsible for the suppression and inhibition of the bacteria as they pass through from the crop to the small intestine. Broadly speaking it can be said that the flora of the anterior gut from crop to terminal ileum are composed of facultative anaerobes, whereas the caecum contains facultative and strict anaerobes with the latter dominant. This difference in the bacterial populations of the crop and caecum is reflected in the organic acids present. In the crop the main acid is lactic whereas in the caecum it is acetic with smaller amounts of lactic, butyric and propionic acids (Moore, 1969).

This general statement applies only to herbivorous birds. In most carnivorous birds the crop and caecum are either absent or poorly developed and the transit time of food is short. These adaptations which keep down body-weight allow predatory birds to move at high speed and may have the effect of simplifying the gut flora (Metchnikoff, 1907).

Twenty-nine different genera have been isolated from the avian gut. Each of these genera would be represented by three to four species and each species by three to four different metabolic types so that there may be over 200 different types in the gut of the chicken, and the more you look the more you will find. In the mouse, where more work has been done, over 400 different types have been described (Moore & Holdemann, 1975).

Factors affecting the gut microflora

The chick differs from the mammal in that it does not have a period of intimate contact with the mother during which it can acquire an adult flora. In the wild the chicken would peck round the mother hen and acquire its flora in this way. However, chickens reared under commercial conditions are totally divorced from any direct contact with the mother and their only contact is indirectly via the egg,
which is not a good source of bacteria. Thus the environment in which the egg hatches is one factor which determines which bacteria colonize the gut.

Smith (1965) followed the development of the flora from hatching to day 20. Coliforms, streptococci and clostridia rapidly colonized the gut by day 1 but lactobacilli were not found until day 3. Bacteroides were not recovered until day 5. The colonization by lactobacilli can be delayed even longer by hatching eggs in a clean environment not previously used for chicks (Bare & Wiseman, 1964). Conversely, if newly-hatched chicks are dosed with lactobacilli, lactobacilli will be established by the end of the first day (Fuller, 1973). The delayed colonization by bacteroides appears to be dependent on the development of suitable environmental conditions in the gut and they are therefore not easily established before day 5–6.

Interbacterial antagonism can also influence colonization. In the chicken the number of lactobacilli in the crop increased as the numbers of Escherichia coli decreased suggesting that the one was responsible for the other. In gnotobiotic chickens the numbers of E. coli established alone is higher than when a chicken lactobacillus is also present (Fuller, 1978). The lactobacilli suppress the E. coli by producing lactic acid which lowers the pH to about 4.5, a pH at which lactobacilli can grow but E. coli and most other bacteria cannot (Fuller, 1977).

Another factor which is known to be important in determining colonization is peristalsis. The contents of the gastrointestinal tract are continually being passed towards the vent. Therefore, in order for an organism to colonize the gut it must either multiply at a rate faster than the rate at which it is being removed by peristalsis or else it must attach to the epithelial surface. It is now known that some organisms solve this problem by attaching. Lactobacilli in the chicken crop are an example (Fuller & Turvey, 1971). Electron micrographs (Plate 1) show that this attached lactobacillus flora forms almost a complete cover of the crop epithelium (Brooker & Fuller, 1975). It is a very specific phenomenon; only lactobacilli isolated from birds will attach to crop epithelial cells (Fuller, 1973).

Attachment occurs via the bacterial microcapsule (Plate 2). The staining reactions (ruthenium red, colloidal iron, Alcian blue-lanthanum nitrate and periodic acid-thiosemicarbazide silver proteinate) indicate that this capsule is rich in carbohydrate (Brooker & Fuller, 1975). Inhibition of adhesion by periodate and concanavalin A tend to confirm this (Fuller, 1975). The way in which this system selects specific strains of lactobacilli for growth in the chick gut suggests that it is of some use to the bird and this active lactobacillus fermentation helps control the composition of the flora.

Bacteria attached to or associated with the gut epithelium are also seen in the small intestine and caecum (Fuller & Turvey, 1971). The filamentous organism attached to the small intestine is seen in several other hosts but has never been isolated. In the caecum there is a large population of varied morphological types trapped in the mucous layer.

Thus there is in the gut a large population of different metabolic and morphological types. The number of bacterial cells in the gut is greater than the number of eukaryotic cells composing the body of the host. This large number of
cells, many of which are actively metabolizing, may be important in certain nutritional contexts and could be regarded as another tissue when criteria of this sort are being considered.

Another factor which has less effect than one might at first expect is diet. The characteristic flora which establishes itself in the gut is very stable and only very drastic changes of diet will affect it. For example, raw navy beans (*Phaseolus vulgaris*) have an immunosuppressive effect on the quail and allow the normally harmless *E. coli* of the gut to become invasive and kill the host (Jayne-Williams, 1976). This sort of effect does illustrate the harmful potential of the flora.

An example of a natural dietary antibacterial effect is provided by the penguin. This bird lives off algae containing large amounts of acrylic acid. The high levels which accumulate in the gut are antibacterial and a very restricted flora develops. For example, there are no coliforms or enterococci in the gut of penguins (Sieburth, 1959).

**Effect of nutrition and growth of the chicken**

A wide variety of different metabolic activities due to bacteria can be demonstrated in the chicken gut. For example, bacterial enzymes acting on cellulose (Hegde *et al.* 1982), starch (Ivorec-Szylit, 1971), disaccharides (Siddons & Coates, 1972), urea (Harbers *et al.* 1963), uric acid (Barnes & Impey, 1974; Suomalainen & Arhimo, 1945), amino acids (Fujita, 1968) and bile acids (Cole & Fuller, 1984) are all present in the gut and as a result of bacterial metabolic activity nutritionally-active compounds such as vitamins (Coates *et al.* 1968) and volatile fatty acids (Moore, 1969) are produced.

However, although these metabolic activities appear to be potentially useful, there is no evidence that they are of any benefit to the chicken. In fact the net effect of the flora is harmful. This can be demonstrated by comparing the growth of germ-free and conventional chicks. When this is done the conventional birds grow more slowly than the germ-free birds (Coates *et al.* 1963) and we infer that there are in the gut organisms which depress the growth of the chicks. This is confirmed by the finding that dietary antibiotics stimulate the growth of conventional but not germ-free chicks (Coates *et al.* 1963). The inclusion of antibiotics and other antibacterial agents in diets has now become common commercial practice.

Although there is no benefit derived from the flora of birds on complete commercial diets, this may be an artefact of domestication. Perhaps in the wild, where the diets are suboptimal, the flora contributes more. Certainly the grouse, which lives on a diet rich in fibre, relies on the bacteria in the gut to digest the fibre (Gasaway, 1976). While most of the fibre digestion probably occurs in the caecum, there is some evidence that fibre digestion occurs anterior to the caecum. In the chicken on a commercial diet there is little evidence of fibre digestion but in chicks on high-fibre diets it can occur (Hegde *et al.* 1982). Cellulolytic bacteria have never been isolated in large numbers from the chicken (Barnes *et al.* 1972) and strangely enough even the goose, which grazes on grass, has no cellulolytic bacteria in the caecum (Mattocks, 1971).
Mechanism of growth depression

The reduced growth of conventional compared with germ-free birds is, in part, due to *Streptococcus faecium* (Fuller et al. 1979). A large collection of chick intestinal bacteria was re-established in germ-free chickens and the effect on growth observed. Only *S. faecium* caused growth depression and this was prevented by dietary penicillin. By analogy with the suggested mechanism of contaminated small bowel syndrome in the human infant (Gracey et al. 1969) we proposed the following mechanism: *S. faecium* adheres to the duodenal epithelium, grows up to large numbers, deconjugates bile salts and causes nutrient malabsorption.

There was certainly evidence for the attachment of *S. faecium* to duodenal cells. In a series of experiments in which chicks were given penicillin and their body-weights recorded it was found that growth response (which is a measure of growth depression) was related to the appearance of high counts of *S. faecium* in the duodenum (Houghton et al. 1981). Comparison of duodenal counts with crop counts showed that when growth response occurred the count in the duodenum exceeded the count in the crop (Table 1). Growth in a region of fast-moving contents suggested that *S. faecium* was countering the effect of peristalsis by attaching to the epithelium. This could be demonstrated (Fuller et al. 1981) both by culture of washed epithelium and by electron microscopy (Plate 3).

The next stage in the proposed mechanism (the deconjugation of bile salts) has been demonstrated in vitro (Cole & Fuller, 1984). *S. faecium* deconjugates both taurocholic acid and taurochenodeoxycholic acid which are the two main bile acids in the chicken. Early work by Eyssen & DeSomer (1967) had shown that growth depression was accompanied by lipid malabsorption. Our results confirmed that more lipid was excreted by conventional than by germ-free chicks but the differences were small (Cole et al. 1981). Even the conventional birds retained 75% of the lipid ingested and the nutritional significance of the differences seen is difficult to assess. Another reason why this was not a very convincing explanation of growth depression was that although growth depression was maximal during the first week, lipid malabsorption showed no difference between first and second weeks.

It has been suggested that the increased excretion of lipid seen in conventional

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<tr>
<th>Growth response to penicillin (% of control)</th>
<th>Viable count* of <em>S. faecium</em> in</th>
<th>Crop</th>
<th>Duodenum</th>
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<tr>
<td>6.4</td>
<td>4.0</td>
<td>5.8</td>
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<tr>
<td>1.3</td>
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*Log_{10} colony-forming units/g wet weight.

Table 1. Growth response of chicks with and without *Streptococcus faecium* growing in the duodenum
Microbial action in the non-ruminal GI tract

animals is due to the formation of insoluble calcium soaps (Demarne et al. 1979). This results from more Ca being made available in conventional animals because deconjugation of bile salts also adversely affects Ca absorption. Certainly it has been shown in the chick that retention of Ca is greater in the germ-free state (Edwards & Boyd, 1963). Absorption tests on intestinal segments of anaesthetized birds showed no malabsorption of labelled glucose (Coates et al. 1981), methionine (Yokota & Coates, 1982) or oleate (Scharrer & Riedel, 1972). The effect of the gut flora on the chicken was not, therefore, a general malabsorption effect.

The two key characteristics of growth-depressing organisms according to our proposed mechanism are (a) attachment to duodenal epithelium enabling the organism to grow up to large numbers in the anterior small intestine, and (b) ability to deconjugate the two main chicken bile acids. A survey of bacteria isolated from the chicken gut showed that although S. faecium strain SY1 (which had been used for all of our work) did attach and did deconjugate, other isolates possessed these two characteristics without being able to depress growth (Cole & Fuller, 1984). We must, therefore, postulate that some other characteristic of S. faecium is involved.

One other potentially-adverse effect of S. faecium was observed during the course of the work (Fuller et al. 1981). For in vitro studies on adhesion we used duodenal brush-borders, the preparation of which involves washing and centrifugation. When these washings were added back to the in vitro system they increased the number of S. faecium cells which adhered. This enhancement of adhesion can be prevented by addition of soya-bean trypsin inhibitor and can be reproduced using trypsin. In our in vitro tests it was not possible to demonstrate inactivation of the trypsin but the possibility remains that in vivo, where larger numbers of bacteria will be involved, the removal of trypsin from the lumen and contact with the food substrates may have a harmful effect on digestion of protein.

In the past, growth depression of gnotobiotic chickens has been obtained with organisms classified as S. faecalis (Huhtanen & Pensack, 1965; Eyssen & DeSommer, 1967). However, the characteristics used to identify these organisms could apply equally to S. faecium and it is suggested that the bacteria used were, in fact, S. faecium not S. faecalis. At the moment, although the role of S. faecium in antibiotic relieved growth depression has been established, the way in which it produces growth depression is still only partially understood.

S. faecium is not the only growth-depressing agent which can be demonstrated. A bacteria-free filtrate of droppings will also depress the growth of chickens. This filtrate appears to contain a virus although none has ever been isolated or seen by electron microscopy. This growth depression is, of course, not reversed by antibiotics. Thus there is potential for further growth stimulation if a way of reversing the ‘filtrate effect’ can be found.

Concluding remarks

In summary, it can be said that the intestinal microflora of the bird is a complex mixture of many different types of bacteria under the control of the host.
and is also affected by interbacterial antagonisms. These various factors cause a characteristic flora to develop with an array of metabolic consequences which may affect the nutrition and growth of the bird.

REFERENCES

EXPLANATION OF PLATES

Plate 1. Scanning electron micrograph showing lactobacilli attached to the crop epithelial surface (Brooker & Fuller, 1975).

Plate 2. Transmission electron micrograph of lactobacilli attached to crop epithelium of gnotobiotic chick. Note extensions of bacterial microcapsule between bacteria and epithelial cell and between bacterial cells. Stained with ruthenium red (Brooker & Fuller, 1975).

Plate 3. Scanning electron micrograph of *Streptococcus faecium* attached to duodenal villous surface (Fuller *et al.* 1981).