

◀Review▶

# The Chicken Gut Microflora and Probiotic Supplements

Roy Fuller

Russet House, Ryeish Green,  
Reading RG7 1ES, United Kingdom

**Key words** : probiotics, microflora, intestine, chicken

---

## Introduction

Chickens are an important food animal but can also be responsible for public health problems such as *Salmonella* and *Campylobacter* infections. It is, therefore, important to maximise the growth of the bird without compromising its ability to resist disease.

Fundamental to the understanding and achievement of this aim is a knowledge of the gut microflora and the ways in which it affects the host animal. Recent studies have shown that the deficiencies in the gut microflora produced by modern rearing techniques can be reversed by administration of live microbial feed supplements which have come to be known as “probiotics”.

These supplements reconstitute the natural flora of the bird and it is, therefore, essential to use strains of bacteria capable of surviving in and/or colonising the gut. One important colonisation factor is the ability to attach to the intestinal epithelium. This allows establishment in the gut in large numbers where the organisms can elaborate the factors responsible for the probiotic response.

Probiotic supplements have been developed for a wide variety of different farm and pet animals ; they are particularly relevant to the optimal growth of commercially reared chickens where there is separation of the newly hatched chicken from its mother hen and the opportunity for transfer of microorganisms is reduced.

## The Gut Microflora

The chicken resembles the ruminant in having at the anterior end of its gastrointestinal tract a sac, called the crop, in which bacteria can grow before being subjected to the acid environment of the stomach. The microflora of the crop consists of large numbers of lactobacilli and smaller numbers of coliforms and streptococci (Fuller, 1997) ; the lactobacilli remain dominant throughout the small intestine. It is only in the caeca where different nutritional conditions exist and residence time is longer that the strict anaerobes become the dominant components of the microflora. The periodic release of caecal contents into the rectum similarly enriches the microbial flora of the

site.

The dominance of the lactobacilli in the crop is maintained by their ability to adhere to the crop epithelial cells. When the food moves on from the crop to the gizzard, large numbers of lactobacilli are left behind attached to the crop epithelium. These are available for inoculation of the incoming food. Under *in vitro* conditions  $10^7$  lactobacilli/ml are required to suppress the growth of *Escherichia coli* (Fuller, 1977) indicating that the count of  $10^9$ /g of lactobacilli found in the crop will be able to inhibit the growth of *E. coli* and other organisms ingested with the feed.

In the fasting chicken, the lactobacillus count in the crop is low. Overnight, when the bird stops feeding, the number of lactobacilli attached to the crop wall drops from  $10^9$  to  $10^6$ . However, this is still sufficient to give the lactobacilli a numerical advantage over other organisms present in the feed. The decrease in lactobacilli following fasting is also correlated with the growth of *Salmonella enteritidis* in the crop and the migration to the spleen and liver (Durant *et al.* 1999).

That the coliforms are being suppressed by the lactobacilli can be confirmed in two ways. Firstly, if chickens are fed high levels of penicillin the lactobacillus count falls and the attached lactobacilli disappear whereas the coliform count increases. (Fuller, 1973). Secondly, if germfree chickens are monoassociated with *E. coli* the count in the crop is  $10^8$ /g but if an adhering strain of lactobacillus is present the count falls to  $10^5$ /g (Fuller, 1978).

The decrease in the count of *E. coli* is associated with a fall in pH from pH 6.0 to pH 4.5 and this is due mainly to the production of lactic acid by the lactobacilli. Tests under *in vitro* conditions confirm that although lactic acid at pH 4.5 prevents the growth of many other bacteria, it still allows the growth of some strains of lactobacilli (Fuller, 1977).

### Mechanism of Adhesion

The lactobacillus flora attached to the crop wall is an important factor in the control of the composition of the chicken gut flora and attempts have been made to describe the mechanisms by which lactobacilli become associated with the crop epithelium. The squamous epithelial cells lining on the crop must have their surface receptors which are specific for the attachment of lactobacilli. In this way the chicken can select out those lactobacilli which are characteristic of its gut microflora.

The presence of this attached lactobacillus flora can be confirmed in Gram-stained sections and by electron microscopy. Such studies reveal that the lactobacilli are entirely superficial and there is no evidence that they are penetrating into the epithelial cells or becoming invasive in any way (Brooker and Fuller, 1975).

Attachment of the lactobacilli ensures that certain strains of lactobacilli become dominant in the crop. The host specificity of this effect can be demonstrated by *in vitro* tests in which lactobacilli derived from various host species are tested for attachment to chicken crop epithelial cells. When this is done, only lactobacilli from the chicken or related avian species will adhere (Fuller, 1973).

The adhering lactobacilli grow well in the chicken diet indicating that they are not

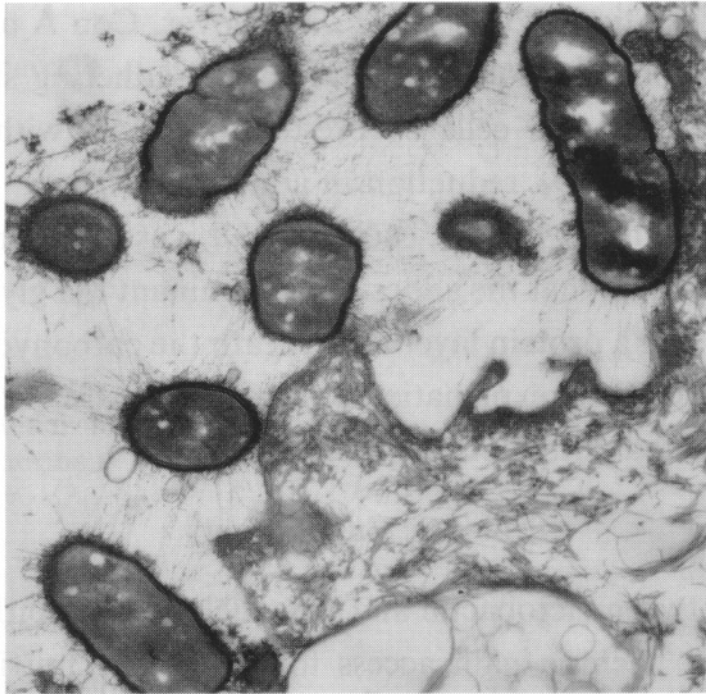


Fig. 1. Lactobacilli attached to chicken crop squamous epithelium. Treated with ruthenium red. (Brooker and Fuller, 1975)

obtaining any nutrients from the epithelial cells.

The lactobacillus flora of the crop is very stable and persistent ; providing the chicks have access to a suitable strain of lactobacillus, attachment occurs within hours of hatching and is not affected by changes in diet or rearing conditions.

Electron micrographs of material stained with ruthenium red, colloidal iron, Alcian blue-lanthanum nitrate or periodic acid - thiosemicarbazide - silver proteinate (PA-TSC-Ag) reveal an extracellular layer on the lactobacillus cell which is not seen in conventional Gram-stained preparations (Fig. 1 ; Brooker and Fuller, 1975). The staining characteristics of this microcapsule indicate that it is composed of material rich in acidic carbohydrate. The morphology of this microcapsule varied according to the staining technique employed. With ruthenium red fibrillar structures extending from the bacterial to the epithelial cells were seen. The significance of these fibrils is not clear ; they may be artifacts produced by the methods used for preparation of the material for electron microscopy. The presence of an extracellular layer staining with ruthenium red was confirmed by Overdahl and Zottola (1991). They found such a layer in 10/17 of the strains of *L. acidophilus* which they examined.

Ruthenium red staining also revealed a layer of acidic carbohydrate on the surface of the crop epithelial cells. This layer was also seen on the crop cells of germfree chickens and was, therefore, not of bacterial origin. The primary site of attachment is where the two layers of acidic carbohydrate meet. The fibrils are not essential for attachment because *in vitro* grown lactobacilli which, in the stationary phase of growth have no fibrils, will still adhere to crop cells.

The carbohydrate nature of the adhesion determinant on the lactobacillus cell is supported by chemical studies performed in *in vitro* tests (Fuller, 1975). Adhesion was completely eliminated by exposure to sodium periodate at pH 4.5 for 10 min. Adhesion was reduced by Concanavalin A treatment indicating that a mannose moiety was

important in the manifestation of the effect. Moreover, Con A agglutinated 3 strains of adhering lactobacilli but did not agglutinate 3 non-adhering strains. Attempts to block receptors on the epithelial cells by pre-treating them with mannan were only partially successful giving a 21% reduction in adhesion.

The adhesion determinant on the bacterial cell was also sensitive to proteolytic enzymes. This may indicate that the adhesion determinant is a glycoprotein or that the proteases were hydrolysing a protein layer underneath the carbohydrate and releasing it. The latter seems the more likely explanation because the treatment with pepsin released material which precipitated with Con A (Fuller, 1975).

### Probiotic Supplements

The association between lactobacilli and the chicken crop epithelium is an example of symbiosis where the bacteria gain access to the dietary nutrients and favourable conditions of growth and the chicken is protected against the potential adverse effects of microorganisms ingested with the feed. The attachment of bacteria to the epithelium ensures that specific types of lactobacilli become dominant in the crop producing a low pH which prevents the growth of many other bacteria in the anterior regions of the intestinal tract. The lactobacilli are, therefore, of central importance in determining the composition of the gut microflora and are for this reason commonly used in microbial feed supplements called probiotics. These have been defined as :

*“A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance”.* (Fuller, 1989)

In the wild the chicken would receive a complete gut flora from its mother's faeces and would consequently be protected against infection. However, commercially reared chickens are hatched in incubators which are clean and do not usually contain organisms commonly found in the chicken gut. The chicken is an extreme example of a young animal which is deprived of contact with its mother or other adults and which is, therefore, likely to benefit from supplementation with microbial preparations designed to restore the protective gut microflora. (Fig. 2)

This was first illustrated by Nurmi and his colleagues (1973) when they showed that dosing day-old chicks with faeces from adult chickens improved their resistance to colonisation of the gut by *Salmonella enteritidis*. Since these original observations this so called “competitive exclusion” effect has been shown to be active against other species of *Salmonella* and against *Campylobacter*. The use of raw gut contents as a feed supplement is beset with the problem of pathogen transfer. This has been minimised by obtaining caecal contents from specific pathogen-free flocks and culturing under *in vitro* conditions to eliminate protozoa and viruses.

Many attempts have been made to identify the organisms responsible for the protective effect. Defined active mixtures have been obtained but these have contained 10–50 different strains of bacteria and have not given as much protection as the raw caecal material. Also, the mixtures are complex and contain some anaerobes which are difficult to maintain making these defined mixtures relatively unattractive as commercial supplements (see Barrow, 1992).

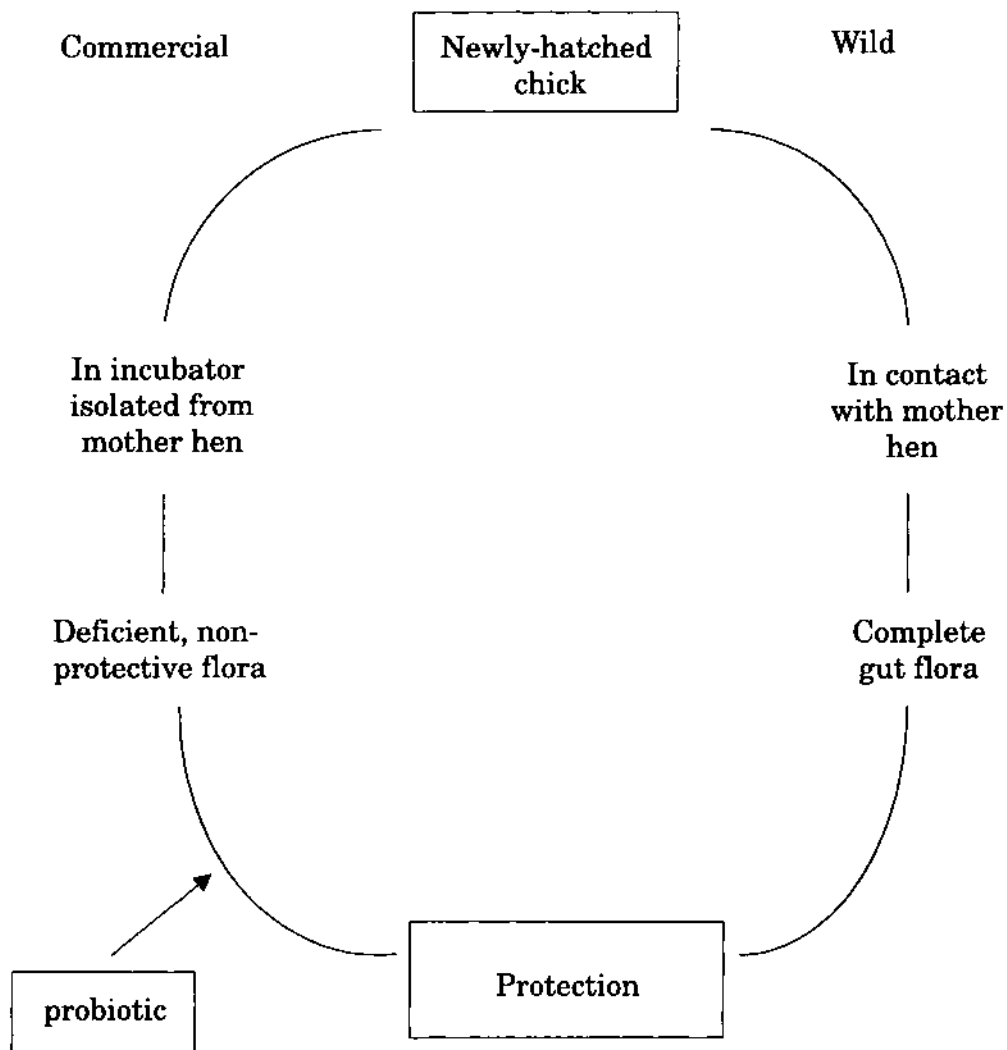


Fig. 2 Schematic representation of the probiotic concept

The other approach to probiotic supplementation has been generated by the work of Metchnikoff (1907) at the beginning of the last century. This had led to the development of microbial supplements based mainly on lactic acid bacteria (LAB). These comprise lactobacilli, streptococci, enterococci and the closely related bifidobacteria although these are not strictly speaking LAB. The central role of lactobacilli in controlling the composition of the chicken gut flora has recommended this type of probiotic to those involved in research and development of chicken microbial feed supplements.

There are currently many different chicken probiotics containing LAB ; they may contain only one strain such as *Lactobacillus reuteri* (e.g. GAIA feed, Bio Gaia Biologics Inc.) or as in the case of Protexin (Probiotics International Ltd.) as many as seven strains (*L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. plantarum*, *L. rhamnosus*, *Enterococcus faecium*, *Streptococcus thermophilus* and *Bifidobacterium bifidum*). They may come in the form of powders, liquid suspensions or sprays. The latter have been very useful in the dosing of day old chicks in boxes used for transport. This ensures early establishment of the protective flora. Probiotic supplementation can then be maintained by administration of powders or liquid suspensions in the feed or water. Another method which produces early gut colonisation is the so-called *in ovo* method where the probiotic is delivered to the shell membrane of the air cell after 18 days' incubation.

Many different species of LAB have been used in chicken probiotics. With the exception of the yoghurt starter strains (*L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*) they are all intestinal isolates.

As well as LAB chicken probiotics may also contain *Bacillus* sp., *Aspergillus* sp., *Candida* sp., *Pediococcus* sp., *Clostridium* sp. and *Saccharomyces* sp. Some of these organisms are not gut isolates and will not grow well in the gut. Continuous administration ensures that large numbers of metabolising organisms are present throughout the gut and overcomes the inability to colonise the gut.

The selection of strains for use in probiotic preparations has in the past been made on a largely empirical basis but as knowledge of the factors affecting colonisation of the gut has increased, attention has been paid to such features as ability to attach to the gut epithelium and resistance to antibacterial factors such as HCl in the proventriculus and gizzard, bile acids in the small intestine and short chain fatty acids in the caeca. The production of antibacterial substances such as bacteriocins has also been considered by some to be a desirable attribute but since most of these are peptides it seems likely that they will be rapidly degraded in the gut by host and microbial proteases. However, the advocates of bacteriocins suggest that intimate contact between producer and target cell would allow the bacteriocins to be active under in vivo conditions. It remains to be proven.

The use of probiotic preparations in laboratory and field trials has been shown to be effective. The results are sometimes variable but positive effects have been obtained with respect to :

- increased growth rate (Mohan *et al.*, 1996)
- improved feed utilisation (Nahason *et al.*, 1994)
- improved resistance to infection (Zani *et al.*, 1998)
- improved egg production (Nahason *et al.*, 1994).

The probiotic effect is a complex interaction between an ingested viable agent and more than 400 different types of bacteria that form the chicken intestinal microflora. The host animal being dosed is also a variable feature and will be changing physiologically with age, diet and environment. The various factors which can affect the probiotic response have been discussed in more detail previously (Fuller, 1997). Unfortunately, in many cases insufficient information is available to enable an accurate assessment of the result obtained to be made. For example, the viable count of the preparation being used is seldom given when the results of field trials are published. The viability of the probiotic is essential for the manifestation of its effects and a low viable count would account for a negative result. Viability and several other factors may explain apparently inconsistent results and careful consideration of these factors should be made when analysing the results of probiotic trials.

### The Future

There is incontrovertible evidence that the gut microflora is involved in the chicken's resistance to infectious disease and this forms the basis for the probiotic approach. The microbial supplements currently available aim to enhance resistance to

disease and to increase growth rate. At present, the way in which these two improvements in the well-being of the chicken are achieved is not clear. The lack of information on the mode of action of probiotics makes their rational development difficult. It should be possible to identify the metabolite(s) responsible for the probiotic effects. This would enable us to identify potentially active probiotic strains in the laboratory and would eliminate some of the expensive and time-consuming field trials that are now necessary.

At the moment the one feature which is essential for the manifestation of the beneficial effects is the ability to survive in the gut in large numbers. The gut flora contains strains which are constantly being replaced and those which persist in the gut for long periods (McCartney *et al.*, 1996). The characteristics which determine prolonged survival in the gut should be identified and used to select strains which need only minimal administration. Whether or not the organisms can actually multiply in the gut, the ability to attach to the epithelium will maximise the residence time in the gut. Consequently this feature has been used in the selection of gut organisms for probiotic supplements.

If we had knowledge of the metabolite(s) responsible for the probiotic effect, it might be possible to combine, by genetic manipulation, the ability to adhere and the ability to produce the key substances. The use of molecular methods for selection of probiotic organisms had been discussed by Klaenhammer and Kullen (1999).

One of the problems in the past has been the difficulty of monitoring the survival and growth of probiotic organisms in the gut. Methods based on culturing and phenotypical identification have not been satisfactory because they cannot always distinguish between the probiotic organisms and the naturally occurring indigenous strains. Currently under development are genera - and species-specific gene probes which will enable us to identify and enumerate various components of the gut flora without recourse to culture techniques (Tannock *et al.*, 2000).

The concern about the development of resistance which follows the use of antibiotic feed supplements has stimulated the search for an alternative growth promoter. Probiotics used properly can fulfil the same role as antibiotics in the farm animal feeds and they will be used more and more as the number of antibiotics allowed for use as growth promotants is reduced. At present in the European Community only four antibiotics can be used and these are likely to be discontinued in the near future. There is, therefore, a rapidly expanding market for the use of probiotics in farm animal feeds. The chicken, because of its disruptive early life in an incubator, is likely to benefit from manipulation of its gut microflora in a way that re-establishes the conditions which exist in the wild. The use of probiotics is an example of reconstitution rather than addition and as such is a way of restoring the chicken to its natural state.

### **Acknowledgements**

I am indebted to Ms. Rosemary Brown (Probiotics International Ltd.) for typing the manuscript.

### References

- Barrow PA. Probiotics for chickens. In : Probiotics. The Scientific Basis (Fuller R ed.) pp. 225-257. Chapman & Hall. London. 1992.
- Brooker BE and Fuller R. Adhesion of lactobacilli to the chicken crop epithelium. *Journal of Ultrastructure Research*, 552 : 21-31. 1975.
- Durant JA., Corrier DE, Byrd JA, Stanker LH and Ricke SC. Feed deprivation affects crop environment and modulates *Salmonella enteritidis* colonization and invasion of leghorn hens. *Applied and Environmental Microbiology*, 65 : 1919-1923, 1999.
- Fuller R. Ecological studies on the lactobacillus flora associated with the crop epithelium of the fowl. *Journal of Applied Bacteriology*, 36 : 131-139. 1973.
- Fuller R. Nature of the determinant responsible for the adhesion of lactobacilli to chicken crop epithelial cells. *Journal of General Microbiology*, 876 : 245-250. 1975.
- Fuller R. The importance of lactobacilli in maintaining normal microbial balance in the crop. *British Poultry Science*, 18 : 85-94. 1977.
- Fuller R. Epithelial attachment and other factors controlling the colonisation of the intestine of the gnotobiotic chicken by lactobacilli. *Journal of Applied Bacteriology*, 45 : 389-395. 1978.
- Fuller R. Probiotics in man and animals. *Journal of Applied Bacteriology*, 66 : 365-378, 1989.
- Fuller R. Introduction. In : Probiotics 2 : Applications and Practical Aspects (Fuller R. ed). pp 1-9 Chapman & Hall. London. 1997.
- Klaenhammer TR and Kullen MJ. Selection and design of probiotics. *International Journal of Food Microbiology*, 50 : 45-57. 1999.
- McCartney AL, Wang W and Tannock GW. Molecular analysis of the composition of the bifidobacterial and lactobacillus microflora of humans. *Applied and Environmental Microbiology*, 62 : 4608-4613, 1996.
- Metchnikoff E. *The Prolongation of Life*. Heinemann. London 1907.
- Mohan B, Kadirvel R, Natarajan A and Bhaskaran M. Effect of probiotic supplementation on growth, nitrogen utilisation and serum cholesterol in broilers. *British Poultry Sciences*, 37 : 395-401. 1996.
- Nahason SN, Nakaue HS and Mirosh LW. Production variables and nutrient retention in single comb White Leghorn laying pullets fed diets supplemented with direct fed microbials. *Poultry Science*, 73 : 1699-1711. 1994.
- Nurmi E and Rantala M. New aspects of *Salmonella* infection in broiler production. *Nature*. London, 241 : 210-211. 1973.
- Overdahl BJ and Zottola EA. Relationship between bile tolerance and the presence of a ruthenium red staining layer on strains of *Lactobacillus acidophilus*. *Journal of Dairy Science*, 74 : 1196-2000. 1991.
- Tannock GW, Munro K, Harmsen HJM, Welling GW, Smart J and Gopal PK. Analysis of the fecal microflora of human subjects consuming a probiotic product containing *Lactobacillus rhamnosus* DR20. *Applied and Environmental Microbiology*, 66 : 2578-2588. 2000.