PROBIOTICS FOR CONTROL OF *Salmonella* IN CHICKEN

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Introduction

Salmonella has been a major cause of food-borne illness in humans. Frequently, poultry products were identified as the source of the *Salmonella.* Antibiotics are used to prevent or combat these infections both in poultry and in man. The increasing frequency of antibiotic resistant pathogens threatens the reliability of such treatments. This article outlines the basis and status of microflora manipulation by probiotics as an alternative to antibiotic therapy to control *Salmonella* as well as other pathogens.

The microflora (flora) and its role

In mammalian and avian species, the digestive flora is usually a complex mixture of microbial populations variously colonizing areas of the gastro-intestinal (GI) tract. More than one hundred different organisms are known to exist in the flora of the chicken's GI tract. Many have been identified and cultured, but some have resisted cultivation efforts and have been identified, only now, by molecular techniques. Much of this research involving the new types was performed at the Food Research Program, AAFC by Dr. Joshua Gong.

The numbers of microbes in the contents of the chicken's cecum or cloaca are usually a billion or more per gram ($\geq 10^9$ g^{-1}). This flora is important for the control of ingested pathogens, especially food-borne pathogens like *Salmonella.* Laboratory animals with a developed flora require inoculation with approximately 100,000 times as many pathogenic bacteria than germ-free animals (no flora) to show the same pathogenic response. This indicates the full impact of the GI flora. The impact of flora improvements will not be quite as large; however, they can be of major benefit to host health. The flora confers these benefits by: competing with ingested, foreign organisms in a process known as "competitive exclusion" within the GI tract; producing volatile fatty acids and other metabolic compounds such as hydrogen sulphide and hydrogen peroxide which deter colonization by some pathogens; and stimulating or modulating the host's immune system to resist foreign microbes.

A healthy chick prior to hatching has no flora in the GI tract. In nature, the chick obtains organisms for the GI flora from the mother hen, other older chickens and environmental sources. The GI tract is colonized in a process called "ecological succession". Initially, the predominant microbes are facultative anaerobes such as coliforms, streptococci and, possibly, clostridia. These are succeeded in turn by lactobacilli and, eventually, obligate anaerobes (cannot tolerate oxygen). The flora in the cecum does not become stable until 4-6 weeks after hatching. Once established, the flora is quite stable for a prolonged period provided environmental, nutritional and physiological conditions remain stable. The composition of the flora varies not only among regions of the host's digestive tract but also among chickens and among species of hosts.

In commercial poultry production, flora development is compromised by production practices and by the use of antibiotics. The newly hatched chick, having no contact with chickens that already possess a GI flora, can only obtain bacteria from environmental sources. Moreover, cleaning and disinfection of hatching and rearing facilities to control poultry pathogens reduces the availability of organisms from the environment. Recent information on materials like oligosaccharides, β-glucans and soluble fibres common in various cereal grains or insect exoskeletons, suggests that they act as nutrients for some of the flora. In nature, chicks would have access to a variety of feedstuffs; however, poultry diets are comprised of a limited number of major ingredients and are fortified to meet the chicken's nutrient requirements. A diet designed for maintenance of the digestive flora has been given little attention. Most significantly, chick diets often contain prophylactic levels of antibiotics to prevent bacterial infections. These antibiotics tend to be detrimental to at least some organisms of the GI flora. Consequently, the development of the GI flora in chicks reared in commercial environments is usually suboptimal.

Flora manipulation

Manipulation of the GI flora to improve its resistance to food-borne and other pathogens is expected to provide an alternative to the use of prophylactic antibiotics for suppression of microbial pathogens such as *Salmonella* or *Campylobacter*. This would be much easier if: the composition of an ideal flora was known; the flora composition evaluation could be done economically without sacrificing the chicken; and all microbial components of the flora could be cultured.

Probiotics are pure or mixed cultures of live organisms which affect the host's health beneficially by improving the properties of the indigenous flora. Lactic acid bacteria, lactobacilli and bifidobacteria, have proven to be effective probiotics in human health.

The purpose of using probiotics is to augment or complement existing flora of the GI tract. To be successful probiotics must: i) complement the components of the flora; ii) survive delivery to, and become colonized in, the relevant region of the lower GI tract; iii) and survive in the intestinal environment (adequate nutrients, pH, etc.).

Prebiotics are growth factors which enhance the flora by stimulating growth or activity of specific beneficial bacteria to improve host health. Many prebiotics are complex carbohydrates such as oligosaccharides; however, other forms such as peptides and lactose derivatives also qualify. The degradation of prebiotics into simple sugars allows them to become nutrients for specific organisms of the flora.

There is some confusion in applied circles due to failure to distinguish between probiotics and prebiotics. In this instance the term, "probiotics", is used improperly and includes probiotics and prebiotics as well as synbiotics, i.e. the combination of the two.

The application of probiotics is not always successful. Several factors account for this. Little benefit is expected from provision of organisms already present in the flora. Our inability to determine which probiotics are capable of improving a flora is a problem. This is due to a lack of knowledge of: the microbial composition of the ideal flora; the microbial composition of the flora of the live host; and the ideal host diet to maintain the flora. Moreover, the current cost of flora population analysis is prohibitive. Another problem is that of maintaining probiotic organisms and delivering them to the lower GI tract alive. Probiotic organisms must pass through the stomach to get to the intestines. The stomach tends to be very hostile to probiotic organisms due to its high acidity.

Probiotics have failed statistically to reduce *Salmonella* in some tests. Possible explanations for this include: small sample size and failure to rule out results that were obtained by chance; possible contamination of the control birds (i.e. no probiotic) with litter from the probiotic-treated birds which would transfer probiotic organisms; failure to provide probiotics in a live form; and feeding rations that did not support colonization by probiotic organisms.

Currently the application of probiotics to newly hatched chicks seems warranted to enhance flora development. As chickens age, the opportunities to acquire the organisms for the flora from the environment multiply, and the benefits of probiotic treatments are reduced. If more were known about which specific organisms of the flora were eliminated by antibiotics, appropriate probiotics should be administered after antibiotic therapy to repair any damage to the flora by the antibiotic. This treatment would reduce secondary bacterial infections.

For more information about probiotics see either *Probiotics, the scientific basis* by R. Fuller or *Pro-*

biotics: a critical review by G. W. Tannock. These references also contain extensive information about the microflora of the GI tract.

Regulation of probiotics

Based on the results from some studies and on observations of flora functions, there should be major health benefits from the administration of probiotics to chickens having a deficient flora. To meet Health Canada regulations, a product must meet its health claims. However, as mentioned above, this poses a difficulty because positive results from probiotics are not always observed. Lack of information about what constitutes an adequate flora and means of evaluating its adequacy presents another difficulty, which is to determine which specific probiotic organisms are truly complementary. As a result the Bureau of Veterinary Drugs has approved few probiotics. Consequently probiotics must be marketed as GRAS (generally regarded as safe) and this restricts the health claims that might be made.

Application and challenges

Probiotics may be classified as defined (i.e. the specific organisms have been identified), or non-defined. Often, non-defined probiotics become better defined with further testing and use. Initially, raw or non-defined gut cultures were tested. Non-defined cultures would be very detrimental if they were to contain poultry pathogens. Research performed at Health Canada by Drs. Stavric, Blanchfield and Gleeson during the 1970s and 1980s indicated that it was difficult to develop a defined probiotic mixture that was as effective as non-defined material.

In Finland, Nurmi tested non-defined cultures from the gut of a chicken for their ability as a probiotic to control *Salmonella*. The Nurmi principle, plus other control measures such as testing for, and rejection of, imported feed ingredients containing *Salmonella,* has been most effective in controlling *Salmonella* in certain Scandinavian countries for over two decades.

The USDA laboratories have been successful in developing probiotics for chickens. At Athens, GA, a non-defined probiotic called Mucosal Starter Culture has been developed. This product was derived from the mucus lining the ceca of healthy spent hens, and has been shown to reduce numbers of *Salmonella* and *Campylobacter* in the gut. At College Station, TX, cecal bacteria selected for their ability to produce propionic acid were obtained from a healthy chicken and were cultured in fermentors. This product, Preempt, has been defined and contains 29 distinct bacterial types. Scientific research has demonstrated that both products are effective not only against *Salmonella* but also against other food-borne pathogens including *E. coli, Listeria*, and *Campylobacter*. Research at the Food Research Program, AAFC, by Dr. Jim Chambers demonstrated that Preempt reduced *Salmonella* colonization in challenged chickens by about 95%.

Other probiotics are available. Aviguard, marketed by Bayer, has many bacterial types and has been shown to be effective. Primalac, marketed by Star-Labs, contains four different bacteria and has scientific reports of successfully reducing *Salmonella* colonization of broilers. There are other probiotics with three or fewer organisms with which some success has been reported.

Use by broiler producers

Currently, in Canada, there is pressure to reduce the use of antibiotics in broiler production. The fast food trade is requesting chicken that has been reared without antibiotics. Consequently producers are interested in probiotics if they can be depended upon. However, a serious concern of producers is necrotic enteritis caused by *Clostridium perfringens* in conjunction with coccidiosis. Growers are reluctant to give up prophylactic antibiotics unless this is controlled. This reluctance would be alleviated by probiotics that would control *Clostridium perfringens*.

In the USA some probiotics have been federally approved, but adoption of them has been disappointing so far. It is unknown whether it is the cost or other factors that tend to be prohibitive.

This is contrary to the situation in some Asian and European countries where probiotics have been successfully introduced and are used extensively now.

Summary

The flora of the chicken's digestive tract can play a major role in resistance to ingested pathogens. In the past, prophylactic levels of antibiotics have been used to prevent or alleviate infection by poultry and food-borne pathogens. Antibiotic resistance of the pathogens now calls for antibiotic alternatives.

Probiotics, and possibly prebiotics would seem to be a solution to this challenge. Some have been shown to have major benefits on host health. However, the identification and enumeration of all organisms of the flora to determine its adequacy remains a challenge. To optimise probiotic benefits, more information is required to determine which probiotics will be effective and which dietary changes or prebiotics may be required to allow the beneficial organisms to colonize the GI tract of the chicken. Full characterization of the GI flora and probiotic effects is required to meet health regulations required for approval at a higher level than GRAS (generally regarded as safe) status.

Modern broiler production practices are sub-optimal for early development of the broiler chick GI flora. More broiler producers are trying probiotics as alternatives to antibiotics. Increased benefits from probiotics would result from further research into: identification, enumeration and culture procedures for organisms of the flora; feasible methods of measuring flora composition; interactions among the organisms of the flora; and nutrients or prebiotics to optimize beneficial organisms of the flora. Information obtained in these areas would assist and expedite their approved use by Health Canada.

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