The Living Gut

2nd Edition

By W.N. Ewing

with contributions by L.A. Tucker

An introduction to the identity, location and activity of the gastro-intestinal microflora and their benefits on health and performance of animals
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WN Ewing

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Dr Wesley Ewing has worked on bacterial supplements to improve animal performance and health since the early days of commercial probiotics in the 1980’s. Since then he has travelled within Europe, North America and Asia studying the sector and searching for the best solutions to maximise the performance of The Living Gut.

Dr Ewing is a postgraduate of Nottingham University, has worked for Cargill and Provimi in management positions and is now a partner in Context Products Ltd.

The author acknowledges the contribution of Dr Lucy Tucker in updating and compiling the 2nd Edition.

Dr Tucker received her first degree in Biological Sciences from the University of Lancaster, and went on to complete a Ph.D. in monogastric nutrition and digestion at Harper Adams University College. Since 1996 she has worked in commercial animal nutrition, specialising in research, development and marketing of technical feed ingredients to improve nutrient delivery and digestion, and promote gastro-intestinal health. Based in New Zealand since 2005, she now works as a consultant nutritionist, reflecting her wide-ranging interest in topics allied to nutrition.

Special thanks are also due to Dr Juha Apajalahti of Alimetrics, Finland for his contributions to the gut microbial section.
The major function of the gastro-intestinal tract is the absorption of nutrients from the diet to support health and growth. This results from a series of complicated processes in the gastro-intestinal tract. In their simplest form these processes can be considered as hydrolysis and fermentation, with the end products being absorbed through the gut wall. The extent to which these processes take place is influenced by the nature of the food, with the consequence that feeds and foods have different digestibility values. Furthermore, the digestibility of the material, and ultimately the health of the animal, will also be influenced by the micro-flora that inhabits the digestive tract. The modern nutritionist frequently seeks to modify these various processes in order to improve health and performance status of the animals to yield better quality food for humans.

There are a multitude of hydrolytic enzymes produced endogenously by the gut or present in the feed. These are specific to certain dietary components – broadly classified as carbohydrate, fat and protein. The development of the digestive tract and its associated secretions is dependent on the early gastric experiences of the animal. For avian species, it is well known that the longer the onset of first meal, following hatching, the more retarded the growth of the integral structures of the gut become, and the slower the endogenous secretions (Sklan, 2004). This delay can restrict growth and the affected animal may lag behind its flock mates even through to slaughter age. It also delays the establishment of a suitable and stable gut micro-flora, potentially leaving the younger animal vulnerable to pathogenic challenge and disease. Work with newborn mammals, including pigs, calves and horses, has illustrated the importance of rapidly establishing gut function and bacterial flora in the context of growth and health.

In some young animals a particular enzyme, for example amylase, can have low activity at birth but will increase readily with age. In the new born pig, sucrase activity is non-existent and takes approximately 7 or 8 months to reach its maximum. In contrast, the lactase content of the gut is very high at birth, in order to meet the needs for digesting the predominantly milk diet of the young animal. However, its activity falls rapidly and, in the pig for example, declines considerably by 5 weeks of age (Figure 1.1).
The gut micro-flora is also inevitably linked to digestion and plays a particularly important role. In most species the micro-flora of the gastro-intestinal tract is substantial and the number of cells in the gastro-intestinal tract vastly exceeds the number of cells in the body. Most of these organisms are anaerobic, many being strictly obligate anaerobes. The micro-organisms colonise the mucosal epithelia either by attachment to the epithelial cells or by their presence in the mucus layers at the base of the villi. By the nature of their position in the tract they are closely associated with the digesta, and involved in digestion. In human and porcine species, gut micro-organisms are most numerous in the caecum and the large intestine, where they play an important role in the fermentation processes. However, the site of fermentation varies depending on species. Birds confine their bacteria to their caeca (hind gut), as do the semi-ruminants, such as rabbits and horses. Control of the niche inhabited by the individual microbial species can be important in maintaining the micro-floral balance, and limit the opportunities for pathogen proliferation.

It is not surprising that there have been many attempts to modify the health of the gut and therefore its efficiency in both man and his domestic animals. This is now a major focus of nutrition and the related sciences. The extent of success depends on many things, but it is important to recognise that, while the site of breakdown is important, the site of absorption is more so. For example, while the horse has considerable ability to absorb the products of fermentation in the hind gut, the pig, although having considerable capacity for fermentation, is much less able to absorb from the hind gut.

Companies and researchers developing gut-active products for the human market have played a particular role, since the last twenty years has seen considerable
challenges to the eating patterns of populations in many parts of the world. While cost of food and feed is still a major consideration, safety and health within the context of perceived healthy eating have assumed major importance.

‘Natural’ and ‘organic’ foods play an increasing part in the advertising and marketing of food today. A growing number of consumers have indicated their willingness to pay a premium for guaranteed ‘antibiotic-free’ meat produced in a ‘natural’ way. These changing attitudes and purchasing decisions have had a significant effect on the agricultural industry, especially in the areas of added value food and niche market sectors. This was seen in Great Britain during the 1989 salmonella scare, and the 1990 BSE (Bovine Spongiform Encephalopathy) crisis, when sales of eggs and beef respectively dropped drastically. The modern consumer now has an influence upon the systems of animal production. This covers topics such as health, welfare and nutrition.

Intensification of the livestock industry inevitably increases the risk of clinical and sub-clinical enteric diseases. This is mainly due to the higher stocking rates, allowing greater risk of horizontal transmission of diseases, and the fact the animals were bred to perform and grow as cost-efficiently as possible, putting extra strain on their physiology and metabolic processes. The increased incidence of new emerging diseases should also be considered. Thus, animals have become more vulnerable to harmful bacteria, such as *Escherichia coli*, *Salmonella* spp., *Clostridium perfringens* and *Campylobacter jejuni*. As a response to the problems of intensive animal production, there was a considerable reliance on antibiotics as growth promoters for use in feeds.

This has engendered other problems however such as bacterial resistance to antibiotics which was recognised quite some time ago. Smith and Halls (1967), showed that infective resistance is probably the most common form of drug resistance in *E. coli* inhabiting the alimentary tract of humans, calves, pigs and poultry. In a survey of 400 pork and beef carcasses, it was found that the majority yielded drug resistant strains of *E. coli*., (Walton, 1971). However recent data from Scandinavia has shown that levels of resistant bacteria have fallen as a result of the ban on antibiotic growth promoters (AGPs) in animal feed, although increasing reliance on therapeutic drugs may negate these benefits.

Concern regarding food safety has affected the eating habits of people in developed countries. A survey, conducted by the Food Policy Research Unit at the University of Bradford, examined the reasons (Figure 1.2) for consumers eating less meat. When offered eight different reasons which might have caused them to eat less meat, feed antibiotics, animal welfare issues, and the use of growth hormones were mentioned by approximately 10 per cent of consumers while the major reasons were cost and health (Woodward, 1988).
The administration of antibiotics to animals or humans at either therapeutic levels for a short time, or sub-therapeutic levels for a prolonged period, will increase the number of antibiotic resistant bacteria in the gastro-intestinal tract. There is always the risk that this could establish a population of virulent bacterial pathogens with antibiotic resistance in both animals and humans (Barton, 2000). To what extent, antibiotic resistant bacteria contribute detrimentally to human health has not been determined conclusively. Nevertheless concerns about bacterial resistance were a major factor in the total prohibition of antibiotic growth promoters in the EU in 2006.

Furthermore, more than 2000 types of *Salmonella* spp. have been identified, the strain associated with the egg scare in Britain was *Salmonella enteritidis* PT4. Equally important, however, is *Salmonella typhimurium* that is often found in animals’ drinking water. *Salmonella* spp., like many other bacteria, can grow extremely quickly and, under optimum conditions, double in numbers every 15 minutes. Therefore, one strain of pathogen may be controlled while at the same time another develops.

A secondary factor has been growing concern over possible antibiotic residues in meat and other animal products. This has led to consumer pressure to reduce further the use of antibiotics in feed. However economically viable animal production necessarily requires high levels of animal performance and good animal health. This has led to an increased interest in alternative ways of enhancing animal performance and helping the animal withstand disease. Effective management of the gastro-intestinal tract is extremely important here.

Bacteria resident in the gastro-intestinal tract are broadly classified into two types, pathogenic and non-pathogenic, although some species may cross from...
one group into the other under certain circumstances. There is a delicate balance between beneficial and pathogenic bacteria in the gastro-intestinal tract, and many symbiotic and competitive interactions occur between them. However many gastro-intestinal bacteria are beneficial and essential to a healthy living gut. They are normally and naturally present within the gut of all domestic animals and birds.

Pathogenic strains, such as *E. coli*, cause disease and can reach high numbers in the gastro-intestinal tract when the body’s natural defence mechanisms such as gastric acidity, other bacterial populations and antibody protection are deficient or when either stress or infection reduces their protective effect. A variety of different factors may be considered to be “stresses”, all of which result in a change in the balance of gut flora in favour of the pathogenic species. Gut-active compounds, by various means, typically stabilise this balance in favour of the beneficial bacteria, leading to maintained or improved animal health and performance.

*Lactobacilli* and other lactic acid bacteria are recognised as beneficial bacteria which are also clearly important to man. This association has involved the manufacture of various human foods and also various beneficial interactions in different parts of the body. Interest in the role of the intestinal micro-flora has focused on *Lactobacilli*, particularly *Lactobacillus acidophilus*, as lactic acid-producing bacteria assumed generally to be beneficial and non-pathogenic. These are very common organisms in nature, being found in milk, cheese and plant material. They are involved in silage and yoghurt fermentation, and can be found in human saliva.

Correct maintenance of the gut environment and flora by using the various compounds now available can provide a natural alternative to antibiotics or chemical growth promoters, without the associated problems of tissue residues and therefore the dependence on drugs can be reduced. Some of the major materials capable of enhancing gut function are:

- Viable (live) bacteria
- Non-viable (dead) bacteria
- Live yeast
- Yeast by-products
- Certain fibres
- Enzymes
- Other fermentation products, including lactic acid
- Mineral or organic acids
These have frequently been described collectively as additives although some more precise terms have been proposed for these materials which are in fact bioactive molecules yet are not direct nutrients. “Pronutrient” has been suggested as being a more appropriate term for materials previously considered simply as additives. (Rosen, 1996). Adams (1999) has introduced the term “nutricines” to describe these bio-active feed ingredients which are not direct nutrients. Therefore a pronutrient or a nutricine can be defined as a compound or material that contributes to the health and nutrition of an animal, without necessarily being a nutrient itself. A good example would be the many commercial feed enzymes and organic acids. There has been significant commercial development of these products in farm animals and birds where preparations are fed to establish a desirable intestinal microbial balance and, consequently, to improve health and productivity. Such effects are achieved partly through the control of pathogenic organisms.

Another major group of products with gut modifying abilities are undoubtedly the probiotics, which is the term for those products that contain single or combined cultures of beneficial bacteria that are used to ‘seed’ the gut, and maintain the presence of beneficial micro-organisms, especially where the individual has been exposed to strong antibiotic therapy that has stripped the gut of its micro-flora. Instead of killing microbial cells, a probiotic product is designed to promote the proliferation of the beneficial species of bacteria within the gut environment.

The concept of probiotics as applied to preventative medicine has been claimed to originate from Eli Metchinkoff in 1907 and the concept is therefore not new. He postulated that the long life of certain Balkan people was due to the regular consumption of fermented milk (yoghurt) containing *Lactobacillus bulgaricus*. Metchkoff (1908) stated that *horros autointoxicos* (the production of toxins by putrefying organisms) could be reduced by the continuous consumption of a fermented lactobacillus culture.

Metchnikoff’s ‘prolongation of life’ (1907, 1908) was based on the ability of certain lactic acid bacteria to reduce the numbers of pathogenic gastro-intestinal micro-organisms. He stated that some microbes in the gut produced substances that were harmful to the host. Eating food containing the beneficial organisms, which he believed were contained in yoghurt, would improve the intestinal conditions. Through the constant addition of ‘good’ microbes in the diet, colonisation of the gastro-intestinal tract by disease-causing (pathogenic), ‘bad’ organisms was prevented, and thus health and life expectancy improved. This was the beginning of the probiotic concept of microbial inoculation based on the principle of competitive exclusion. At the time his observations were treated with scepticism by some medical practitioners. However, his work was supported by other researchers and during the 1920’s *Lactobacillus acidophilus*
cultures were used therapeutically, especially when it became known that it was one of the most common inhabitants of the intestinal tract of many animals.

Since then there have been many references to the application of probiotics in animal and human health (Alm, 1983; Crawford, 1979; Geier et al., 2007; Gill and Guarner, 2004; Gotz et al., 1979).

Bohnhoff et al., (1954) removed the natural micro-flora by oral treatment of mice with a large dose of streptomycin and demonstrated an increase in susceptibility to Salmonella typhimurium in the order of 100,000 fold. This was confirmed by later reports of Miller and Bohnhoff (1963) and Bohnhoff et al., (1964) who demonstrated the role of the gut micro-flora in protection against a range of pathogenic enteric bacteria.

Certain aspects of Lactobacilli, when included in the diet, can have a beneficial effect for both humans and animals (Gilliland, 1979; Sandine, 1979) by helping to control the growth of undesirable micro-organisms in the intestinal tract. They have also been involved in the improvement of lactose utilisation in persons classified as lactose malabsorbers (Kim and Gilliland, 1983). Most commercial probiotics have some lactobacilli species within their composition. Lactobacilli spp. continue to form the basis of many human and animal probiotic products today. Research into the effects of probiotics is still an active topic (Scharek, 2007).

The definition of gut-active compounds based on micro-organisms as probiotics has received much attention because it includes those that are viable (live) cultures of bacteria, and those that are non-viable (dead) fermentation products (Sakai et al., 2006; Sashihara et al., 2006). Fuller (1989) proposed that the definition for probiotics should be a “live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance”. This definition excludes fermentation products such as lactic acid and obviously dead bacteria. Atherton and Robbins (1987) defined a probiotic as “any product which can help the normal flora to maintain their domination over pathogenic organisms” which fits well into the approach taken here. However, the term probiotic has been used by Lilley and Stillwell (1965) to describe substances produced by one protozoan that stimulated another, while Parker (1974) used it to describe organisms and substances that contribute to the intestinal microbial balance.

Chesson (1993a) stated that correct maintenance of the gut bacterial environment was “the property of the normal adult flora to resist the overgrowth of component strains and the establishment of foreign strains”, and that “probiotics are intended to reinforce this state or re-establish it where this has broken down because of environmental stress or as a result of extended treatment with antibiotics at therapeutic levels.”
There are clearly many products and molecules which can act as gut modifiers. Therefore perhaps gut modifiers should be defined as any material (live, dead or chemical) that has a beneficial effect on the gastro-intestinal micro-flora. This encompasses those other materials of that do not have probiotic effects, but supply beneficial molecules to the animal (pronutrients or nutricines).

An indirect approach to modifying the living gut is through the use of prebiotics which are typically fibre-based products. These can directly provide a substrate for the beneficial gut bacteria to ferment such as fructo-oligosaccharides, thereby encouraging certain desirable species to proliferate. Alternatively they may act as decoys for bacterial attachment, preventing the adherence and invasion by pathogenic strains (e.g. mannan-oligosaccharides).

Today’s agriculture already relies heavily upon biotechnology for the improvement of livestock feed and feeding systems (Figure 1.3). Its involvement in a wide range of applications has grown as technologies developed. Some current applications are listed below.

- Phytase - improves the digestibility and availability of phosphorus in poultry and pig diets (Beers and Jongbloed, 1992; Ketaren et al., 1993).
- Enzyme preparations containing predominantly β-glucanase, arabinoxylanase and protease activities for addition to cereal-based diets for broilers, to improve performance (Bedford, 2000, Bedford and Partridge, 2001).
- Mixed enzyme preparations in pig diets in enhancing growth rate and preventing enteric disease (Bedford and Schulz, 1998; Close, 1992).
- Modified Lactobacillus preparations in controlling silage fermentation (Sharp et al., 1992).
- Gene manipulation to allow the enhancement of wool production (Rogers, 1990).
- Gene manipulation to allow the monogastric animal to improve digestion.
- Oligosaccharides (prebiotics) to control bacterial populations (Chesson, 1993b).
- Bacterial additives as probiotics (Fuller, 1989).
- Fungal probiotics to enhance fibre breakdown and protein flow to the abomasums (Wallace and Newbold, 1992).
- Manipulated bacteria to degrade plant toxins (Jones and Megarry, 1986).
Figure 1.3 Biotechnology in the improvement of livestock food and feeding systems (after Robinson and McEvoy, 1993)

As scientific knowledge continues to improve the understanding of the dynamic interactions between feed components and bacteria within the gut, so we are more able to make the links between health, physiological status and nutrient requirements. The aim of this book is to bring together those factors influencing the gut environment and function, across species, that can improve the health, growth and welfare of humans and animals.
The complexities of the gastro-intestinal tract differ from species to species, and from site to site within the gut and also depend upon the health of the animal. Food must be initially digested by endogenous enzymes, in order to facilitate the absorption of nutrients through the gut wall, where they are taken into the hepatic portal vein for distribution and use. Digestive mechanisms can be thought of, in the simplest way, as hydrolysis and fermentation.

Man, pigs, dogs, cats and poultry are classified as monogastrics (non-ruminant, simple stomached animals (Figures 2.1 and 2.2), and do not rely on a symbiotic relationship with the microbial flora within their gastro-intestinal tract to facilitate nutrient release to the same degree as animals possessing a rumen. They do possess important gastro-intestinal bacterial populations, but these are contained further down the tract, in the hind gut or caecal regions. As a result, fermentation plays a smaller role in nutrient breakdown compared to semi- and full ruminants.

A more limited micro-flora population, in comparison with ruminants, means they have a higher nutritional demand (especially for amino acids and vitamins) from their diet. They also lack the ability to utilise poor quality feedstuffs, containing large quantities of fibrous material, including cellulose and lignin and structural carbohydrates, resistant starch or denatured protein caused by heat processing methods.

Figure 2.1 Gastro-intestinal tract of the pig (simple stomached).
Ruminant animals, however, have considerable microbial fermentation occurring within the gastro-intestinal tract (Figure 2.3). This fermentation takes place in the rumen before digestion in the small and large intestines. These animals have the ability to synthesise amino acids and most vitamins through microbial action on nitrogen and carbon sources. Also, unlike pigs and poultry, they are able to breakdown both starch and structural carbohydrates, such as cellulose (Figures 2.6 and 2.7) making the digestion of roughage possible.

The heavy microbial load in the rumen, just like the host, also requires nutrients to survive, and there is a cost associated with this increased digestive capacity. In addition, when high quality feeds are given, this microbial maintenance.
demand means the animal converts high quality feedstuffs into body tissue, or milk, less efficiently than would a non-ruminant. The microbial protein may also be of lower quality than the original high quality feed.

Horses, although herbivores, do not have a rumen but have an enlarged caecum (Figure 2.4), and are classified as semi-ruminant (as are rabbits), as much of their nutrient supply is derived from fermentation. The caeca of horses are very large, and require a constant supply of fibre to maintain activity, much like ruminants. Horses are very sensitive to changes in diet that impact on the bacterial populations resident in their gut. This is manifested as various ailments collectively described as ‘colic’. They are also sensitive to disturbances in the bacterial profile that results in the production of toxic metabolites, such as fructan fermentation, which is linked to the development of laminitis (inflammation of the feet), which is particularly common.

![Gastro-intestinal tract of the horse](image)

**Figure 2.4** Gastro-intestinal tract of the horse (simple-stomached herbivores)

The role of gut bacteria appears to be less important in humans compared to adult pigs, who demonstrate great fermentation capacity. However, the maintenance of correct gut bacteria is now recognised as important in humans. This has resulted in the commercial production of specialised drinks and supplements which purport to aid digestive health by promoting or contributing to an ideal bacterial profile.

Human gastric evolution and development, as an omnivore, means that the human gut is more closely related to that of the pig or dog (Figure 2.5).
**Figure 2.5** Gastro-intestinal tract of the human

**Figure 2.6** Illustration of action of cellulase with the final liberation of glucose molecules.
The major starch-degrading enzymes include $\alpha$ and $\beta$ amylases, gluco amylases, pullanases and isoamylases. Starch exists in two forms, amylose (linear) and amylopectin (branched). Amylase hydrolyses $\alpha$ 1,4 bonds, while examples of debranching enzymes (which break the $\alpha$ 1,6 linkages) are pullanase and isomylase.

A wide array of bacteria have been isolated from healthy animals, most of which are natural to the environment in which they live. Donaldson (1964) detailed the bacteria of the intestines and described them and their distribution in the environment (Table 2.1).

**Organs and digestion**

Different organs assume various degrees of importance in different species (Figures 2.1 - 2.5) and have different enzyme and digestive activities (see Table 2.2). Consequently a short description of the important organs is necessary.

**Monogastric animals**

*The mouth*

The action here is largely mechanical with the particle size being reduced by mastication, although in avian species this is bypassed, with feed being stored for a while in the crop before transfer to the stomach. Saliva, secreted by the
### Table 2.1 Bacteria isolated from the intestinal tracts of healthy animals.

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Distribution</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroides:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteroides funduliformis</td>
<td>Intestinal tract; mouth, respiratory tract</td>
<td>Gram-negative, strictly anaerobic, non-sporulating, frequently encapsulated slender rods, with many bizarre pleomorphic forms</td>
</tr>
<tr>
<td>B. fragilis</td>
<td></td>
<td></td>
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<tr>
<td>B. putidus</td>
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<td></td>
</tr>
<tr>
<td>B. pneumosintes</td>
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<td></td>
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<tr>
<td>B. serpens</td>
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<tr>
<td>Enterobacteria (coli-</td>
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<tr>
<td>Enterobacteria (coli-</td>
<td>Enterobacteria (coli-</td>
<td></td>
</tr>
<tr>
<td>enterogenes)</td>
<td>enterogenes)</td>
<td>Lower intestine; plants; soil</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td></td>
<td></td>
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<tr>
<td>E. freundii</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterbacteria (proteus-</td>
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<tr>
<td>Enterbacteria (proteus-</td>
<td>Enterbacteria (proteus-</td>
<td>Lower intestine; soil; sewage; plants</td>
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<tr>
<td>providence):</td>
<td>providence):</td>
<td></td>
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<tr>
<td>Proteus vulgaris</td>
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<tr>
<td>P. morganii</td>
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<tr>
<td>P. mirabilis</td>
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<td></td>
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<tr>
<td>P. rettgeri</td>
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<td></td>
</tr>
<tr>
<td>Klebsiella rhinoscleromatis</td>
<td>Aerobacteria aerogenes</td>
<td></td>
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<tr>
<td>K. oxytoca</td>
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<tr>
<td>Enterococci:</td>
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<tr>
<td>Streptococcus faecalis;</td>
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<tr>
<td>Var. zymogenes</td>
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<td>Var. liquifaciens</td>
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<tr>
<td>S. faecalis</td>
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<td>S. durans</td>
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<tr>
<td>S. bovis</td>
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<tr>
<td>S. lactis</td>
<td></td>
<td></td>
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<tr>
<td>Lactobacilli:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactobacillus acidophilus</td>
<td>Milk; intestinal tract (infants);</td>
<td>Gram-positive, micro-aerophilic or anaerobic long slender rods, which are often pleomorphic and occur singly or in chains</td>
</tr>
<tr>
<td>L. bifidus</td>
<td>mouth; stomach; vagina</td>
<td></td>
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<tr>
<td>L. brevis</td>
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<td>L. exilis</td>
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<tr>
<td>L. casei</td>
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<tr>
<td>Pseudomonads:</td>
<td></td>
<td></td>
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<tr>
<td>Pseudomonas aeruginosa</td>
<td></td>
<td></td>
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<tr>
<td>P. fluorescens</td>
<td></td>
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<tr>
<td>P. ovalis</td>
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<td></td>
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<tr>
<td>Alcaligenes faecalis</td>
<td></td>
<td></td>
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<tr>
<td>Clostridia:</td>
<td></td>
<td></td>
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<tr>
<td>Clostridium perfringens</td>
<td></td>
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<tr>
<td>C. tetani</td>
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<tr>
<td>C. botulinum</td>
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<tr>
<td>C. fibermentans</td>
<td></td>
<td></td>
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<tr>
<td>C. sporogenes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** For a complete visualization, consider using a chart or diagram to represent the information. This description provides a textual overview of the bacterial species and their characteristics as described in the table.
Table 2.2 Source and action of digestive enzymes.

<table>
<thead>
<tr>
<th>Source</th>
<th>Enzyme/digestive secretion</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salivary glands (mouth)</td>
<td>Amylase</td>
<td>Glycogen, dextrin and starch broken down to branched oligosaccharides and maltose</td>
</tr>
<tr>
<td>Gastric glands (stomach)</td>
<td>Pepsin</td>
<td>Causes hydrolysis of peptide bonds in polypeptides</td>
</tr>
<tr>
<td></td>
<td>Histamine</td>
<td>Causes protein to swell. Antibacterial effect</td>
</tr>
<tr>
<td>Pancreatic glands (pancreas)</td>
<td>Trypsinogen</td>
<td>Breaks protein to polypeptides</td>
</tr>
<tr>
<td></td>
<td>Chymotrypsinogen</td>
<td>Breaks protein to polypeptides</td>
</tr>
<tr>
<td></td>
<td>Carboxypeptidase</td>
<td>Breaks polypeptides to small peptides and amino acids</td>
</tr>
<tr>
<td></td>
<td>Collagenase</td>
<td>Hydrolyses collagen</td>
</tr>
<tr>
<td></td>
<td>Elastase</td>
<td>Hydrolyses fibrous proteins</td>
</tr>
<tr>
<td></td>
<td>Lipase</td>
<td>Attacks fats</td>
</tr>
<tr>
<td></td>
<td>Phospholipase A</td>
<td>Degrades phospholipids by removing a fatty acid</td>
</tr>
<tr>
<td></td>
<td>Cholesterol esterase</td>
<td>Attacks cholesterol</td>
</tr>
<tr>
<td>Glands in mucosal microvilli (small intestine)</td>
<td>Aminopeptidases</td>
<td>Breaks polypeptides to small peptides and amino acids</td>
</tr>
<tr>
<td></td>
<td>Dipeptidases</td>
<td>Breaks dipeptides to amino acids</td>
</tr>
<tr>
<td></td>
<td>Phosphatase</td>
<td>Attacks organic phosphates</td>
</tr>
<tr>
<td></td>
<td>Monoglyceride lipase</td>
<td>Hydrolyses monoglycerides to fatty acids and glycerol</td>
</tr>
<tr>
<td></td>
<td>Lecithinase</td>
<td>Hydrolyses lecithin to fatty acids and phosphoric acid</td>
</tr>
<tr>
<td></td>
<td>Sucrase</td>
<td>Converts sucrose to glucose and fructose</td>
</tr>
<tr>
<td>Gall bladder (liver)</td>
<td>Bile</td>
<td>Emulsifies fat, Stabilises emulsions, Neutralises acid chyme, Accelerates action of pancreatic lipase</td>
</tr>
</tbody>
</table>

The gastro-intestinal tract

Salivary glands, has a lubricating effect on the food. In mammals it contains mucin, water and, in certain herbivores and man, the amylase, ptyalin, which catalyses the breakdown of starch to maltose. The extent of amylase activity in the mouth is variable, being high in man and lacking in cats, dogs and horses. Little starch digestion is thought to occur in the mouth due to an unfavourable pH.

**Stomach**

The stomach is an organ for digestion and storage. In the adult pig, it has a capacity of about 8 litres. While it is a single compartment, there are four main
zones in the stomach, which is representative of other single stomached mammals, and each has a different structure, environment and therefore microbial loading and type. The organ has a very low pH due to hydrochloric acid secretions, which are necessary for initiating protein digestion by denaturation, and consequently microbial activity is very much reduced.

The four zones of the stomach of mammals are categorised as oesophageal, cardiac, gastric and pylorus (Figure 2.8) The hormone pylorin, produced in the pylorus, stimulates the production of gastric juice which contains, in addition to water, hydrochloric acid, pepsinogens, inorganic salts, mucus and the factor involved in the absorption of vitamin B12. The pH of the stomach of the pig is about 2. The pepsins involved have two pH optima of 2.0 and 3.5. Protease enzymes within the digestive juices break the polypeptides into shorter amino acid chains (see Figures 2.9-2.11). Eventually the protein is broken down to peptides or single amino acids.

![Figure 2.8 The regions of a pig's stomach](image)

The oesophageal region which joins directly from the oesophagus has no glandular secretions, unlike other parts of the stomach. Its surface is very similar to that of the crop of birds, with the associated micro-flora comprising mainly Lactobacilli, which can be found here at all ages in the animal’s life (Tannock and Smith, 1970). The type and number of bacteria, specifically Lactobacilli and Streptococci, have been shown to depend on their ability to adhere to and colonise the surface. Common bacterial types include L. fermentum, L. salvaricus,
The gastro-intestinal tract

Figure 2.9 The break-down of protein in the food by the action of protease enzymes.

Figure 2.10 Illustration of the structure of a tri-peptide chain of amino acids. 
$R = \text{functional group.}$

Figure 2.11 Illustration showing partial hydrolysis of a tri-peptide to a di-peptide plus a single amino acid.
and *S. salivarus* in younger pigs. As the pig gets older, pepsin and HCl are produced in larger quantities. Milk-fed piglets have a stomach pH in excess of 3.4, while at 2-3 weeks of age there is a much lower pH. This will reduce the level of *L. acidophilus*.

The stomach of the animal is affected by the conditions in which it lives. When a high microbial load is present, the level of luminal fermentation increases while gastric digestion is delayed in pigs. The pH of the stomach is inversely related to the microbial load in the animal’s environment. The resultant microbial fermentation leads to the production of organic acids which act as a buffer, demanding further HCl to be secreted to lower the pH for pepsin activity. When dietary protein is high, its buffering capacity can prevent the pig’s stomach achieving a low pH.

Digestion at the upper portion of the stomach (oesophageal area) is unaffected by gastric juice produced lower in the organ. This allows favourable pH, moisture, temperature and substrates for microbial development and fermentation. The extent of fermentation also depends on the composition of the feed (*e.g.* lactose and sucrose, will produce higher levels of fermentation than starch). Other factors include particle size of the feed and the extent of starch gelatinisation after pelleting.

As the food moves down to the gastric region, the pH is lower, microbial activity decreases, and pepsin activity increases.

*The small intestine*

This long, convoluted organ starts at the pylorus and ends at the ileal-caecal junction. It can be up to 20 metres in length in the pig and account for one third of the volume of the gastro-intestinal tract. It is made up of three areas: the duodenum, jejunum and ileum; but the jejunum accounts for about 85% of its length. Food is partly digested by the time it reaches the small intestine. The digesta becomes mixed with the secretions of the duodenum, liver and pancreas.

The small intestine is an important site of digestion, largely by the host enzymes but, to a certain extent, by fermentation. It is populated with a micro-flora which increases in population as the large intestine is approached. It is also a major site of absorption of the end products of the digestive processes. Absorption is made easier by the great increase in surface area which is provided by the villi in the small intestine (Figure 2.12). Plates 2.13 and 2.14 show the villi of pigs pre- and post-weaning. The villi of pigs post weaning can be seen to be slightly stressed and reduced in length.
Figure 2.12 Illustration of villi on intestinal surface

Plate 2.13 Healthy villi in pigs pre-weaning (by courtesy of John Bourne)

Plate 2.14 Shortened stressed villi in pigs post-weaning (by courtesy of John Bourne)
The large intestine comprises the colon and caecum. Most of the nutrients capable of being hydrolysed will have been absorbed by the time the digesta reaches the large intestine. The largely cellulose and various hemicellulose materials are subjected to a certain degree of breakdown by the large microbial population, which includes *Lactobacilli*, *Streptococci*, coliforms, *Bacteroides*, *Clostridia* and yeast. The end products of fermentation include acetic, propionic and butyric acids, together with indole, skatole, phenol, hydrogen sulphide, amines and ammonia, plus certain vitamins.

The extent to which the end products of digestion are absorbed is open to question. While there is a certain amount of absorption of volatile fatty acids, the primary function of the caecum and colon is to maintain the water and electrolyte balance. Table 2.3 illustrates the relative volumes and lengths of the main regions of the pig’s digestive tract.

<table>
<thead>
<tr>
<th>Region</th>
<th>Volume (l)</th>
<th>Length (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td>Small intestine</td>
<td>11.5</td>
<td>18.3</td>
</tr>
<tr>
<td>Large intestine</td>
<td>9</td>
<td>4.9</td>
</tr>
</tbody>
</table>

The large intestine allows animals to make full use of their food by the salvaging of energy from dietary carbohydrates such as resistant starches, non-starch polysaccharides (dietary fibre), sugars and oligosaccharides which have been neither digested nor absorbed in the small intestine (Gibson, Macfarlane and Cummings, 1993). This process relies on anaerobic bacteria to breakdown the carbohydrates to short chain fatty acids by fermentation. Fermentation can also help utilise dietary proteins and host derived substances, such as pancreatic enzymes, mucus and sloughed off epithelial cells (Cummings and Macfarlane, 1991). The entire process relies on the natural production of hydrolytic enzymes by anaerobic bacteria.

**pH**

The pH of the gut varies dramatically from the mouth and stomach to the colon and caecum. The stomach is kept highly acidic due to the release of hydrochloric acid, proteases and mucus bile and can be as low as pH 1.5. However, the pH is usually around 2.5-4.5 for adult pigs and 4.5-7 for young, unweaned pigs. The stomach is highly oxidised, thus further limiting microbial growth.
The caecum and colon are more neutral, at pH 6-8, and are consequently the site of a lot of microbial activity. Table 2.4 indicates the approximate pH of the different regions of the pig’s digestive tract. A higher pH will give gut pathogens such as *E. coli* the opportunity to multiply at a quicker rate. Table 2.5 shows the typical pH range for the growth of common micro-organisms. Some micro-organisms are acid tolerant (acidophilic), while others are alkaline tolerant (alkalinophilic).

**Table 2.4 pH of contents of various regions of pig’s digestive tract.**

<table>
<thead>
<tr>
<th>Region</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>1.5-6.0</td>
</tr>
<tr>
<td>Duodenum</td>
<td>6.0-8.5</td>
</tr>
<tr>
<td>Caecum</td>
<td>6.0-9.0</td>
</tr>
<tr>
<td>Rest of large intestine</td>
<td>8.0-9.0</td>
</tr>
</tbody>
</table>

**Table 2.5 The pH ranges for growth of common micro-organisms**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Minimum</th>
<th>Optimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>3.0-4.0</td>
<td>6.0-7.5</td>
<td>9.0-10.0</td>
</tr>
<tr>
<td>Yeasts</td>
<td>2.0-3.0</td>
<td>4.5-5.5</td>
<td>7.0-8.0</td>
</tr>
<tr>
<td>Moulds</td>
<td>1.0-2.0</td>
<td>4.5-5.5</td>
<td>7.0-8.0</td>
</tr>
</tbody>
</table>

**Poultry**

The digestive system of poultry differs considerably from other monogastric animals, (Figure 2.2). For example, they do not possess teeth for mastication of food.

A further major difference is that the chicken has a crop (diverticulum) which is a pear-shaped reservoir in the oesophagus for holding food. There is some microbial activity in this organ with the production of lactic and acetic acids. The crop is linked by the proventriculus to the gizzard which is similar in function to the pyloric part of the pig stomach.

Starch enters the crop, which is a storage organ, and is hydrolysed by microbial activity, but sucrose does not undergo any microbial action. The production of volatile fatty acids in the crop indicates that some of this bacterial activity is from anaerobes. However, their level is not always high. *Lactobacilli* are the major bacterial inhabitants and are involved in the correct maintenance of microbial balance. Antibiotics can affect both the *Lactobacilli* level and the total microbial biomass, with a reduction in the total level being related to improvement in performance.
The small intestine of poultry is much shorter than that of pigs but still has a significant micro-flora. Poultry have large caeca and a small colon. The caeca assume the role of mixing the digesta together with fermentation and absorption. Figure 2.15 illustrates the major microbes in the main areas of the digestive tract of pigs and poultry.

**Figure 2.15** Micro-organisms in the main areas of microbial activity of the digestive tract. Gram-positive (+) and Gram-negative (-)

### Ruminants

The major characteristic of this group of animals is the presence in the adult of a large organ of considerable fermentative capacity. It is divided into four compartments, the rumen, the reticulum, the omasum and the abomasum. Table 2.6 details the typical maximum volume and length of the regions of the cow’s digestive tract.

**Table 2.6 Typical volumes and length of main regions of the cow’s digestive tract.**

<table>
<thead>
<tr>
<th>Region</th>
<th>Volume (litres)</th>
<th>Length (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rumen</td>
<td>182</td>
<td>-</td>
</tr>
<tr>
<td>Reticulum</td>
<td>23</td>
<td>-</td>
</tr>
<tr>
<td>Omasum</td>
<td>68</td>
<td>-</td>
</tr>
<tr>
<td>Abomasum</td>
<td>32</td>
<td>-</td>
</tr>
<tr>
<td>Small intestine</td>
<td>91</td>
<td>45</td>
</tr>
<tr>
<td>Caecum</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Large intestine</td>
<td>32</td>
<td>10</td>
</tr>
</tbody>
</table>
The juvenile animal, while suckling, behaves like a monogastric animal. There is little development of the rumen and reticulum whilst suckling, with milk passing down the oesophageal groove into the omasum and abomasum. The groove is formed as a reflex action by the oesophagus closing when milk is drunk from a teat.

As the young ruminant eats solid food there is considerable development of the rumen and reticulum. Eventually, they occupy more than 80% of the capacity of the total stomach, with the oesophageal groove no longer in effect. Saliva plays an important part in the dilution of food. In the rumen, food is broken down physically and chemically. The rhythmic contractions of the rumen walls mix the food, which is regurgitated to be chewed and swallowed again.

The chemical breakdown which occurs in the reticulo-rumen results from the enzymes produced by anaerobic bacteria, protozoa and fungi (Table 2.7). The resulting fermentation produces volatile fatty acids, methane and carbon dioxide with the rumen usually functioning at a pH of about 5.5-6.5. A summary of the action of the rumen on food is given in Figure 2.16. Many species of bacteria have been identified in the rumen (Table 2.8) and are found at about $10^9-10^{11}$/ml of rumen content. Protozoa; *Istrotirichia*, *Dasytricha*, *Entodinium*, *Diplodinium*, *Epidinium* and *Ophyosolex*, are present in much smaller numbers.

**Table 2.7 Products of rumen microbial fermentation**

<table>
<thead>
<tr>
<th>Type of organism</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose fermenting</td>
<td>Acetic, succinic and lactic acid</td>
</tr>
<tr>
<td>Starch and sugar fermenting</td>
<td>Lactic, butyric, formic, succinic, propionic and acetic acids</td>
</tr>
<tr>
<td>Lactic acid and succinic fermenting microbes</td>
<td>Propionic and acetic acid</td>
</tr>
<tr>
<td>Vitamin producing microbes</td>
<td>Vitamin B and others</td>
</tr>
</tbody>
</table>

![Figure 2.16 The breakdown of food in the rumen](image-url)
The epithelial tissue of the rumen is a very special environment, because large amounts of urea diffuse through the rumen wall and must be transformed to ammonia to avoid toxic effects. The taxonomically distinct population of approximately 23 bacterial species that colonise the rumen wall includes several species that produce large amounts of urease, with this enzyme essential for normal physiological function.

The mature rumen is also host to a large population of yeast (fungi). Yeast grow by budding and dividing which involves the formation of a bud on the mother cell which grows to the size of the mother and then the cells separate. After division, the cells are distinguishable, as the mother cell retains a bud scar for each daughter cell produced. A population of yeast cells which is growing has a changing cell age distribution. Under some growth conditions the buds do not separate from the mother cell and long, branched chains of cells (pseudomycelia) will form. Under optimum conditions yeast may divide in as little as 45 minutes, but generally it takes at least twice as long.

**Sites of microbial fermentation**

The form of the gastro-intestinal tract will determine the nature of its digestion. While ruminants have considerable capacity for fermentation in the rumen, some simple stomached animals have well developed hindguts.

The main sites of microbial fermentation differ from species to species. Animals have been classified on this basis (Table 2.9) and can be considered in the simplest form to be pre-gastric, hindgut or colonic fermenters, depending on the major site of fermentation.
Herbivores are largely pre-gastric fermenters. Ruminants, such as cattle, sheep and goats are particularly well developed for microbial fermentation, with the multi-compartment stomach retaining food for a long time. Non-ruminant pre-gastric fermenters have large, complex stomachs, but they do not ruminate or regurgitate their food for secondary chewing.

Hind-gut fermenters rely primarily on the caecum and colon for microbial fermentation. Semi-ruminants have a very well developed caecum which accounts for more than 40% of the digestive tract. However, the rabbit is unusual in that coprophagy is an important aspect of its nutrition. Others, such as the horse and pig, rely to a greater extent on the colon. Animals vary considerably in their ability to absorb nutrients digested at this level of the digestive tract. The horse has considerable ability, while some species have little.

Microbial fermentation results in the liberation of different fermentation products, depending on the organism (see Table 2.10). For example *Lactobacillus* produces lactic acid from pyruvate, while *Saccharomyces* liberates carbon dioxide and ethanol.

**Table 2.9** Classification of animals based on gastro-intestinal anatomy

<table>
<thead>
<tr>
<th>Class</th>
<th>Species</th>
<th>Dietary habit</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pregastric fermenters</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ruminants</td>
<td>Cattle, sheep, deer</td>
<td>Grazing herbivores</td>
</tr>
<tr>
<td></td>
<td>Antelope, camel</td>
<td>Selective herbivores</td>
</tr>
<tr>
<td>Non-ruminants</td>
<td>Colobine monkey</td>
<td>Selective herbivore</td>
</tr>
<tr>
<td></td>
<td>Hamster, vole</td>
<td>Selective herbivore</td>
</tr>
<tr>
<td></td>
<td>Kangaroo, hippopotamus</td>
<td>Grazing and selective herbivores</td>
</tr>
<tr>
<td></td>
<td>Hoatzin</td>
<td>Folivore</td>
</tr>
<tr>
<td><em>Hindgut fermenters</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caecal</td>
<td>Capybara</td>
<td>Grazer</td>
</tr>
<tr>
<td></td>
<td>Rabbit</td>
<td>Selective herbivore</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>Omnivore</td>
</tr>
<tr>
<td><em>Colonic digesters</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sacculated</td>
<td>Horse, zebra</td>
<td>Grazer</td>
</tr>
<tr>
<td></td>
<td>New world monkey</td>
<td>Folivore</td>
</tr>
<tr>
<td></td>
<td>Pig, man</td>
<td>Omnivores</td>
</tr>
<tr>
<td>Unsacculated</td>
<td>Dog</td>
<td>Carnivore</td>
</tr>
<tr>
<td></td>
<td>Cat</td>
<td>Carnivore</td>
</tr>
</tbody>
</table>

Microbial fermentation results in the liberation of different fermentation products, depending on the organism (see Table 2.10). For example *Lactobacillus* produces lactic acid from pyruvate, while *Saccharomyces* liberates carbon dioxide and ethanol.

**Table 2.10** The variation in fermentation products produced by the metabolism of pyruvate by different micro-organisms

<table>
<thead>
<tr>
<th>Organism</th>
<th>Fermentation product</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus</em></td>
<td>Lactic acid</td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td>Lactic acid</td>
</tr>
<tr>
<td><em>Clostridium</em></td>
<td>Acetone, butyric acid, butanol, isopropanol</td>
</tr>
<tr>
<td><em>Saccharomyces</em> (yeast)</td>
<td>Carbon dioxide, ethanol</td>
</tr>
</tbody>
</table>
The relative fermentative capacities of different species vary considerably, as do the various parts of the digestive tract in their capacity for fermentation (Table 2.11).

**Table 2.11** Fermentative capacity expressed as a percentage of the total digestive tract

<table>
<thead>
<tr>
<th>Species</th>
<th>Reticulo-rumen</th>
<th>Caecum</th>
<th>Colon and rectum</th>
<th>Total fermentative capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>64</td>
<td>5</td>
<td>5-8</td>
<td>75</td>
</tr>
<tr>
<td>Sheep</td>
<td>71</td>
<td>8</td>
<td>4</td>
<td>83</td>
</tr>
<tr>
<td>Horse</td>
<td>-</td>
<td>15</td>
<td>54</td>
<td>69</td>
</tr>
<tr>
<td>Pig</td>
<td>-</td>
<td>15</td>
<td>54</td>
<td>69</td>
</tr>
<tr>
<td>Capybara</td>
<td>-</td>
<td>71</td>
<td>9</td>
<td>80</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>-</td>
<td>46</td>
<td>20</td>
<td>66</td>
</tr>
<tr>
<td>Rabbit</td>
<td>-</td>
<td>43</td>
<td>8</td>
<td>51</td>
</tr>
<tr>
<td>Man</td>
<td>-</td>
<td>32</td>
<td>29</td>
<td>61</td>
</tr>
<tr>
<td>Man</td>
<td>-</td>
<td>-</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Cat</td>
<td>-</td>
<td>-</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Dog</td>
<td>-</td>
<td>1</td>
<td>13</td>
<td>14</td>
</tr>
</tbody>
</table>

(Parra, 1978)

Furthermore, the strict division into pre-gastric and hindgut fermenters may be too simplistic. For example, it has been shown in the pig, which is classified as a hindgut fermenter, that considerable fermentation may have taken place before the digesta reaches the large intestine. In one example, 9.3% of the crude fibre and 17.9% of the non-starch polysaccharides had disappeared before the ileum, with a further 30.0% and 49.2% being absorbed from the large intestine (Table 2.12).

**Table 2.12** Examples of digestion and absorption in different parts of the digestive tract of the pig

<table>
<thead>
<tr>
<th>Starch (%)</th>
<th>Crude fibre (%)</th>
<th>Non-starch polysaccharides (%)</th>
<th>Fatty acids (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Disappearance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>By ileum</td>
<td>97.2</td>
<td>9.3</td>
<td>17.9</td>
</tr>
<tr>
<td>In large intestine</td>
<td>2.5</td>
<td>30.0</td>
<td>49.2</td>
</tr>
</tbody>
</table>

Taking pigs as a further example, some fermentation of carbohydrates appears to begin in the first few days of life with activity throughout the gut even at this early age. The potential substrates for this fermentation are any of the carbohydrates consumed by pigs and it is likely that both microbial and host enzymic activity continue in parallel in the whole of the stomach and small intestine. By the end of the small intestine, the amounts of endogenous amylase...
present are very low and microbial enzyme activities, including proteases, high. However, the concentrations and activities of enzymes from microbial and endogenous sources show that endogenous activity predominates in the stomach and much of the small intestine of the pig. The rapid flow of digesta in this region does not favour high levels of bacterial growth but the slower digesta movement in the ileum and large intestine permits bacterial concentrations of up to $10^{11}/g$ digesta. Many of the enzymes in or on bacteria operate slowly on complex, and often branched, polysaccharides, with this occurring in the distal part of the gut.

The specific bacterial carbohydrates found in animals have not been studied in detail, but many reports provide indirect evidence that they have a very wide range of activity because of the great variety of substrates that can be fermented, for example, by pigs. Most of the fermentation leads to production of lactic, acetic, propionic and butyric acids, with the evolution of carbon dioxide, water, hydrogen and methane. It has been widely assumed that the stochiometry of this activity in pigs follows the same principles as those which apply in the rumen, but this is not entirely so because of the low levels of methane which have been found (Zhu et al., 1988).

The bacteria of the gut produce a wide range of enzymes and currently there is interest in the use of exogenous enzymes, produced by microbial fermentation. The genus *Bacillus*, for example, is well known for producing a wide variety of enzymes, including polysaccharidases, proteases and nucleic acid-hydrolysing enzymes (Table 2.13). Amylase from *B. subtilis* was considered an industrial enzyme even by 1917.

<table>
<thead>
<tr>
<th>Table 2.13 Enzymes produced by <em>Bacillus</em> spp. of bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organism</strong></td>
</tr>
<tr>
<td><em>B. amyloliquefaciens</em></td>
</tr>
<tr>
<td><em>B. licheniformis</em></td>
</tr>
<tr>
<td><em>B. coagulans</em></td>
</tr>
<tr>
<td><em>B. amyloliquefaciens</em></td>
</tr>
<tr>
<td><em>B. circulans</em></td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
</tr>
<tr>
<td><em>B. stearothermophilus</em></td>
</tr>
<tr>
<td><em>B. licheniforms</em></td>
</tr>
<tr>
<td><em>B. amyloliquefaciens</em></td>
</tr>
</tbody>
</table>
Bacterial numbers in the gastro-intestinal tract

The total number of organisms within the tract per gram of contents also varies between different regions. The stomach generally contains the least $10^6$-$10^7$/gram of contents while the colon contains the most $10^9$-$10^{10}$/gram of contents.

Digestion is dictated by the arrangement of the digestive tract, age and physiological status of the animal (e.g. exposure to disease and production demands) along with the nutrients supplied. These factors dictate the fermentation conditions in the rumen or hind-gut.
Bacteria that are resident in the gut attach themselves to the gut wall as a necessity, as otherwise they are unable to multiply and colonise the niches available within the gut environment. Adherence of bacteria to the intestinal mucosa is of research interest because attachment is an important initial event for setting up infectious disease, and is also related to the immune response mounted by the host.

For example, lactic acid bacteria are thought to stimulate the production of antibodies and phagocytic activity against pathogens in the intestine and other tissues of the body (Fuller, 1989). It has been claimed that bacteria contain some common antigens (Figure 3.1) that can cross react with some pathogenic micro-organisms and thus immunise against an invasion of pathogenic micro-organisms (Perdigon et al., 1986a, 1986b).

**Figure 3.1** Antigens from non-pathogenic bacteria potentiate the host’s immune response to pathogens. Pathogens are repelled from enterocyte receptors.
Supplementary bacteria may also activate the immune system involved in resistance by their antigens, (Morland and Midvedt, 1984; Perdigon et al., 1986a. *Lactobacillus* antigens could include lipoteichoic acids (Setoyama et al., 1985; Op Den Camp et al., 1985) and cell wall proteins (Conway and Kjelleferg, 1989). Increased leukocytic activity in gnotobiotic animals inoculated with *Lactobacilli* has been observed, which suggests that *Lactobacilli* may be involved in the immune response of conventional animals (Pollman et al., 1980c).

The gut of all animals represents an interface between the external environment (food and other ingested items) and the internal body tissues. As a result, certain gastric secretions, such as mucin and acid, have evolved to ensure physical barriers to any potential harmful micro-organisms ingested. The gut-associated lymphatic tissue (GALT), structures that maintain immune cells and related systems that detect and respond to any potential invasive organisms that could cause disease, have evolved in order to facilitate the required response to invading pathogens as efficiently as possible.

Early exposure of any animal’s gastro-intestinal tract to a variety of bacteria, via feed or other ingested substances, is important in the early and correct establishment of immunity. The immune system must learn to differentiate between benign and pathogenic micro-organisms, and develop a memory of response, via circulating immune cells and signalling entities such as immunoglobulins, in order to ensure efficient removal of pathogens.

Gut microbial populations, being a mixture of bacteria, yeasts, protozoa and viruses, require time to establish themselves in a stable and sustainable state. Research conducted with young chickens has shown that ready access to suitable feed from birth has a major bearing on the correct establishment of the microbial flora, which influences nutrient availability and efficient conversion of feed into useable nutrients (Sklan, 2004).

In addition to feed and water intake, young animals become exposed to microbes present within their environment, from bedding, as well as from their parents, in the case of suckling mammals. The consequent development of a suitable microflora is essential for the correct function of the gut, as well as the development and interaction with the GALT, and, hence, it will influence immunity.

In order to understand elements of immunology, it is essential to become familiar with some of the terms used in this field. Using chickens as an example, the avian GALT includes several tissues and organs; the bursa of Fabricius, caecal tonsils (CT) and Meckel’s diverticulum (MD) as well as Peyer’s patches (PP), intraepithelial lymphocytes (IEL) and variously distributed immune cells residing in the intestinal wall.
The major histocompatibility complex (MHC) is a key part of immunity, and its genetics are expressed in all avian and mammalian species, where its main function is to differentiate between ‘self’ and ‘foreign’ material. As a result, the MHC is able to regulate immune function, and prevents inappropriate antibody responses to the animal’s own body tissues.

Immunity is classified broadly into innate and acquired systems. The innate system includes barrier mechanisms such as acids and mucin, as discussed above, plus specialized cells including macrophages and natural killer cells (NK) that can destroy virus-infected cells, and those which can engulf and destroy pathogens. The innate immune system is stimulated by the production of ‘complement’ proteins that circulate in the blood and may also be found in the gut wall. Cells, such as NK cells, are stimulated by the presence of certain markers on infected cell surfaces.

Adaptive immunity involves thymus-derived T-cells and humoral Bursal-derived B-cells. The protein markers on immune cells determine their function, for example on the surface of a T-cell, a CD4 marker shows it is associated with T-helper cells, which initiate the immune response.

Immunity response is regulated by a complex network of communication chemicals, e.g. cytokines and interferons, which stimulate the multiplication of the types of cells required to defeat an invading pathogen. Protein markers on the cell’s surface identify its role within the whole cascade of the immune response.

Humoral immunity refers to immune components circulating in the body, such as immunoglobulin antibodies (Ig). Shane and Tucker (2006) defines their uses as follows:

- Binding antibodies to a pathogen (usually a virus), to prevent attachment to target host cells
- Opsonisation (where antibodies cover bacteria, to improve phagocytosis)
- Activate protein complement to initiate phagocytosis.

**Bacterial adherence and immune stimulation**

Very little information exists describing the development, function and activation of cells of the innate system. Innate immune cells are pre-programmed and are always available as the first line of defence, making their function independent of prior exposure to bacterial antigens. They provide the first line of defence and are extremely important in early life when the ‘adaptive’ (acquired) immune
system is undeveloped. Epithelial cells are now known to play a major role in innate immunity, forming a highly specialised physical and functional barrier to dietary and microbial antigens.

The tight junctions between cells provide a strong seal, forming a physical barrier to prevent pathogens crossing over from digesta into tissue. In mammals that rely on immunoglobulin transfer via maternal colostrum, these junctions remain unsealed for a certain amount of time following birth, allowing the uptake of immunoglobulins from initial sucklings. Gut wall enterocytes also secrete mucin and anti-microbial peptides that inhibit bacterial attachment and invasion of the epithelium, and secrete cytokines and chemokines into both the gut lumen and sub-epithelial lymph that alter both gut epithelial and lymphoid cell function. These capacities are crucial for host defence, but their development in animal gut epithelial cells and their influence on microbial colonisation, environment, weaning age and diet are currently undefined.

The cells lining the gut can recognise specific cell-surface patterns via receptors that detect and respond to the presence of bacteria or bacterial fragments. A number of receptor systems are expressed on epithelial cell surfaces that recognise bacteria and communicate to underlying lymphoid cell populations, preparing the immune system for a bacterial challenge.

**Detecting bacteria for launching an immune response**

Membrane receptors called Toll-like receptors (TLRs) are now known to be important in bacterial recognition (Takeda et al., 2003). They have the ability to recognise typical molecular patterns such as the ‘pathogen-associated molecular patterns’ (PAMPs) and ‘commensal-associated molecular patterns’ (CAMPs) shared by large groups of bacteria and other gut micro-organisms (Kelly, 2004).

TLRs are found on both epithelial and lymphoid cells and, in addition to their important function in bacterial attachment ligand recognition, they also send signals that result in altered epithelial and lymphoid cell function. Currently, in humans there are ten known TLRs, some of which appear to respond to only one PAMP, whereas others respond to several.

The development and regulation of TLRs due to bacterial colonisation of the gut has only been investigated in rodents to date. As TLRs are receptors that can signal to other cells and systems, the impact of TLR binding and gene expression on the function of epithelial and underlying lymphoid cells is very important.
**Linking immunity and gut digesta**

The gut wall has many types of cell making up its surface, the most common being epithelial and M cells, which are linked to the immune system. Epithelial cells and M cells provide the first point of contact with intestinal bacteria, as they are in direct contact with digesta moving through the gut lumen. The interactions between bacteria and these cells are interesting as there is good evidence to support dynamic interactions which profoundly influence the function of the epithelial barrier and the underlying lymphoid tissues.

Many studies have revealed highly evolved systems of communication involving modulation of cellular changes and functions from the surface receptor through to the nucleus of the cell. Previously much of this work has been devoted to the study of pathogens, however research is increasingly being directed towards the role of beneficial commensal bacteria. Irrespective of the precise mechanisms, it is now clear that bacteria amplify certain genes in epithelial cells and so influence the expression of epithelial secretions, altering the intestinal barrier. As our understanding of interactions between commensal bacteria and gut cells improves, the potential for beneficial manipulation and modulation of the gut response increases.

Activation of adaptive immune responses begins with processing and presentation of antigen by certain cells (APCs). Dendritic cells are important APCs for both innate and adaptive immunity, although research is continuing to elucidate their full role and activities.

Genetic studies strongly suggest that the mucosal immune system remains relatively immature throughout commercial weaning periods. Taken together with the decline in maternal antibodies, derived from colostrum of via placental transfer, this data highlights the vulnerability of the recently weaned animal or young chick (Stokes et al., 2004). The factors involved in promoting immune cell function and the development of specific cells such as regulatory T-cells remain poorly defined.

**Microbial diversity and development of immunity**

Colonisation of the intestine by microbes is essential for the normal development of humoral and cellular responses (Hooper & Gordon 2001). Studies in germ-free animals have highlighted the importance of an intestinal microflora on the phased development of the mucosal immune system (Rothkotter et al., 1991; Pabst et al., 1988: Barman et al., 1996, Barnes et al., 1972; Willing and Van Kessel, 2007).
The gut is sterile at birth and is then colonised by microbes from the mother (in the case of mammals), feed (in poultry) and the environment. The microflora participates in gut health maintenance by forming a barrier along the gut lining and hence ‘outcompeting’ other invading organisms, preventing colonisation by pathogenic bacteria. Although much emphasis has been placed on the role of bifidobacteria and lactobacilli (which form less than 1% the normal colonic flora) in maintaining gut health, barrier function and for stimulating a healthy immune function, other species are emerging as potentially important candidates (e.g. gram-negative Cytophaga-Flavobacteria-Bacteroides and gram-positive Clostridia spp). Interestingly, some of these bacterial groups have previously been associated with disease, although modern DNA analysis is revealing the increasing complexity of gut microflora. Combining microbial DNA profiling with functional studies defining specific responses to colonising bacteria allows better identification of microbes with immune-potentiating/modulating properties (see later chapter on analysis of microorganisms).

**Monitoring bacterial populations and immunity**

New molecular techniques have facilitated investigations into bacterial diversity in the gut. Based on DNA sequences, research has shown that the majority of bacteria associated with the gut wall are not known organisms. Valuable information on immune development can be derived from molecular profiling studies investigating the influence of important environmental, microbial and dietary variables.

Currently, our understanding of the mechanisms by which microbial colonisation affects immune development and modulates the adaptive immune response is poor. Unravelling the specific responses targeted at bacterial antigens against a complex background of colonising flora which are involved in modulating the host response, represents a significant challenge. It is likely that even within the complex ecosystem of the gut, dominant bacterial antigens determine the immune response. Certain bacterial components are recognised to be highly immunogenic and potent immune-modulators.

Investigations into signalling pathways induced in epithelial cells following exposure to beneficial commensal and disease-causing pathogenic bacteria, have shown that both bacterial groups possess molecular patterns that recognise and activate epithelial TLR receptors. Importantly, it has been found that some bacteria can regulate the host response, by modulation of signalling pathways and DNA expression (Figure 3.2) (Kelly and Tucker, 2004a, b). Products generated from bacteria, following contact with epithelial cells, appear to be
able to infiltrate beyond the epithelium. They interact with and regulate cells of the adaptive immune system which has important consequences for adaptive immunity.

Immuno-modulators and stimulants present on the surfaces of colonising bacteria are now known to exist, and identifying them and their mode of action may provide strategies for promoting natural immune defence mechanisms during early gut development. The application of genomics provides an opportunity to unveil important biological effects involving gut microbes on the early development of the innate and adaptive immune systems (Corthésy-Theulaz et al., 2005; Eliot and Ong, 2002).

A correctly functioning immune system requires exposure to a comprehensive and representative population of bacteria, if the host animal is to successfully develop the ability to identify benign materials from potentially dangerous ones. The role of commensal bacteria in the activation of immunity and the preservation of a suitable microbial flora in the gut is now well established. As a result, issues regarding feed materials and ingredients to promote correct microflora development that, in turn, improve immuno-competence and favourably affect the animals natural response to pathogens, can be more clearly resolved.
Various forms of enteritis and diarrhoea are common in animals and man. For example, after weaning, a number of farm livestock exhibit diarrhoea which is associated with production of enterotoxins by *E. coli* and *Clostridium* spp. The move to a dry food gives a ‘carbohydrate’ overload where undigested carbohydrates provide a medium for these potentially harmful bacteria. At the same time, the lactic acid producing bacteria are adversely affected by the removal of the milk diet.

In this chapter two particular organisms, *E. coli* and *Salmonella*, have been chosen for detailed consideration because of their widespread involvement in problems in the digestive tract. Not only do they cause problems to humans but they present major problems in the livestock industry.

**E. coli** and diarrhoea

*E. coli* is a non spore-forming, medium-size bacillus. It is a cylindrical bacterium about 2-3 µm (1/5000th of an inch) long and 0.5 µm wide with rounded ends. Some species are capsulate and the majority are motile. The very thin projections of *E. coli* (called pili or fimbriae) act as anchors to the gut surface receptors (Figures 4.1 and 4.2). They comprise lectins on the surface of the bacteria which recognise carbohydrate residues. These fimbriae form the basis of the antigenic structure of *E. coli*. The various serotypes may be identified very precisely depending on the O, K or H antigens. The O antigen corresponds to terminal sugars on the cell surface, the K antigens reside in the capsule and the H antigens are found in the flagella. There are more than 170 O antigens, over 100 K antigens and over 50 H antigens. The adherence property of the K antigens makes them very virulent and they include K88 and K99 which are associated with diarrhoea in young animals.
Diarrhoea in young animals is often caused by *E. coli* overgrowth, but it is only when pathogens attach to the gut wall that they produce the toxins associated with diarrhoea. The toxins result in an increased rate of peristalsis.

*E. coli* are found everywhere and even attach to dust particles in the air. Relative to particle size, greater numbers of *E. coli* attach to the smaller-sized particles than larger ones. These are the most numerous in the air, stay suspended the longest, and are most likely to penetrate the deeper parts of the respiratory tract.
Infections of the digestive tract

(lungs and airsacs) when inhaled by the birds and animals (Barnes, 1987). In poultry, the main site for E. coli and Salmonella colonisation are the crop and caecum (Weinack et al., 1981). The normal gastro-intestinal tract of domestic animals maintains a stable flora (Donaldson, 1964) and it is only when the gut is disturbed or a very pathogenic E. coli strain is present that the balance is tipped in favour of disease.

Bacterial translocation

The body possesses natural defence mechanisms to prevent pathogenic bacteria from crossing the gut wall into the body. The mechanisms are essential as pathogenic organisms are more likely to have lethal effects when they have crossed the gut wall. These defence mechanisms have been reported as:

- A complete intact mucosal layer to act as a barrier to entry
- A healthy population of non-pathogenic gastro-intestinal micro-flora to prevent overgrowth by pathogenic bacteria (Steffen and Berg, 1983)
- A natural host immune defence system.

Pigs

Colibacillosis (‘scouring’ or diarrhoea caused by E. coli in piglets) is a major source of economic loss to the livestock industry. Losses result from both increased mortality and reduced growth rates. It has been found that acutely ill pigs have low E. coli levels in the stomach but high levels in the anterior portion of the small intestine. Enterotoxigenic E. coli strains cause diarrhoea by attaching to the intestinal mucosa and by producing enterotoxins that cause an influx of sodium ions and water into the intestine.

Post-weaning diarrhoea in pigs has been associated with increased metabolic activity of E. coli in converting proteins to amines. Since amines are irritating and toxic they increase intestinal peristalsis, causing diarrhoea (Porter and Kenworthy, 1969) (Figure 4.2). It should, however, be noted that the pathogenicity of E. coli is restricted to certain strains, with the vast majority of strains causing no ill effects. Colibacillosis is caused by the invasion of the anterior small intestine of the piglet by pathogenic strains of E. coli that possess at least two virulence factors. Organisms possess colonisation antigens of fimbriae (adhesions or pili) that enable the bacterial cells to recognise and attach to specific mannose receptor sites on the mucosal brush border (Figure 4.1). In addition, bacteria must produce enterotoxins which act upon the secretory cells located in the
crypts of the villi. *S. typhimurium* and *E. coli* have been suggested to share common receptor sites and a common mechanism for attachment (Soerjadi *et al.*, 1981).

The attachment of the pathogenic *E. coli* to the mucosal cells enables them to overcome the mechanical clearance of the intestine caused by peristalsis, and facilitates colonisation of the gastro-intestinal tract by these harmful organisms. Once the bacteria have colonised the gut, they start to produce the enterotoxins, which can be divided into two main classes:

- Heat labile toxin characterised by a high molecular weight
- Heat stable toxin characterised by a lower molecular weight

The toxins inhibit the absorptive processes within the intestine, thereby increasing liquid levels in the lumen. The villi become much shorter and consequently have a smaller surface area for absorption. The volumes of fluid are too great to be re-absorbed from the large intestine and this consequently causes ionic imbalances and the development of diarrhoea (Figure 4.3).

**Figure 4.3** The villi of healthy pigs and those which have become shortened during colibacillosis.

The piglet is born free from circulating antibodies and obtains them with the first feeding of colostrum (Underdahl, 1983a). Sow vaccination with either live *E. coli* or a typical antigen *e.g.* K88 has been found to successfully reduce neonatal piglet mortality (Chidlow, 1979). Pigs pre-treated with a K88+ non-pathogenic strain of *E. coli* were more resistant to infection with a pathogenic strain of *E. coli* which shared the same K88 adhesion. The treated group suffered less diarrhoea and fewer animals died.
Villi in the intestine of the piglet increase in length up to 10 days of age, but appear to decrease after this. This reduction may be due to the nature of the feed being more abrasive and concentrated. These, generally solid, feeds are taken in large quantities after weaning. However, a whole series of events is associated with the change of nutrition at this stage. The importance of dietary lactose has been stressed by Powles and Cole (1993) who also illustrated its role as a substrate for Lactobacilli. *E. coli* are normal gut inhabitants whose numbers increase immediately after weaning, with the risk of a pathogenic strain developing (McAllister, Kurtz and Short, 1979). When a pathogenic strain establishes, toxin production starts and fluid secretion from the intestinal wall increases. This is followed by depletion of body electrolytes, dehydration, acidosis and finally death.

Pathogenic bacteria however, directly reduce cell effectiveness and lead the animal to increase the villi number to compensate for this loss in villi length. In the small intestine they cause damage either by producing toxins or by entering the enterocyte.

The piglet, for example, is more susceptible to colibacillosis than the fowl, due to a lower internal body temperature and reduced gut mobility which encourages luminal adherence (Arp and Jensen, 1980). Antibiotics can be used to treat colibacillosis but they may accentuate the problem by facilitating the absorption of endotoxin due to cell disintegration

**Poultry**

*E. coli* infection is an example of a “production disease” which has become increasingly important as production of poultry has intensified. A considerable amount of money is lost annually as a result of *E. coli*. For example, a 1985 survey of turkey health in Minnesota showed that over 40% of the loss from disease could be directly or indirectly attributed to *E. coli* infection (Barnes, 1987). In the USA alone, 4% of all broiler chickens and 6% of all turkeys die from *E. coli* related disease every year (Barnes, 1987).

In poultry, the crop and caecum are the main sites of colonisation for *E. coli* and *Salmonella* spp. The presence of non-pathogenic bacteria has been associated with a lengthening of the villi and increased turnover of their epithelial layers (Cook and Bird, 1973). Deeper crypts appear, and there is an increase in the rate of cell migration. This change in the villi of chickens was found to be more prominent in the upper than lower parts of the small intestine. The native gut micro-flora can reduce colonisation by *E. coli* at these sites by competitive exclusion and this can be enhanced by probiosis (Weinack *et al.*, 1981; 1982).
Two other important effects of *E. coli* have been reported in turkeys in two different ways. Firstly, they may infect the respiratory system. A cheese-like residue forms over the air sacs and induces death due to lack of oxygen. Birds are susceptible from 3 weeks of age until slaughter. Secondly, *E. coli* septicaemia, usually considered a secondary disease, may be precipitated by another disease or stress. The systemic nature of this disease includes symptoms such as an enlarged spleen and liver damage. Birds die very quickly with *E. coli* septicaemia, while with *E. coli* infection of the respiratory tract, the effects last for several days. *E. coli* septicaemia usually occurs between 42 and 84 days of age.

Although levels of *Salmonella* contamination in poultry rations may be low, young chicks can become infected from feed containing only one salmonella per gram, so every attempt must be made to eliminate these bacteria. The day-old chick is readily infected by *Salmonella* but develops a substantial resistance within the first few weeks of life, so that considerably greater numbers of *Salmonella* spp. are necessary to establish infection after this time. Modern poultry husbandry often precludes the development of proper intestinal micro-flora which may play a role in protecting birds against other enteric infections (Lloyd *et al.*, 1977; Snoeyenbos *et al.*, 1978). The recent introduction of stricter bio-security measures on farms to curtail the spread of new infectious diseases has allowed much greater control of bacterial infections.

Resistance of young chicks and poult’s to *Salmonella* infection can be substantially increased by early oral administration of intestinal contents of faeces from selected adult chickens (Rantala and Nurmi, 1973; Lloyd *et al.*, 1977; Snoeyenbos *et al.*, 1979; Weinack *et al.*, 1981; Soerjadi *et al.*, 1981). These authors reported that the protective mechanism appeared to be a consequence of competitive exclusion of *Salmonella* by “normal” micro-flora of the gastro-intestinal tract. The higher susceptibility of young chicks, compared with older birds, to *E. coli* infections, was thought to be due to an immature protective micro-flora. ‘Normal’ intestinal micro-flora create a more stable ecosystem helping to inhibit the establishment of opportunistic pathogens such as *Salmonella*. There can be no doubt that the proper intestinal micro-flora of the chicken and turkey plays a significant role in protection against infection by at least some *Salmonellae*.

Contamination of birds from the environment has been reported to be the biggest source of infection by *Salmonella enteritidis* in poultry (Watson, 1989). Environmental sources including drinking water and even the slaughter house have been implicated (Patrick, Collins and Goodwin, 1973). A further source of contamination has been reportedly due to vertical transmission of *Salmonella enteritidis* where infected breeding stock have produced contaminated eggs which spreads the contamination to the grower flocks (Borland, 1975).
It is important therefore that the control of salmonella in laying hens starts at the breeder and multiplier flock level. Elevated caecal salmonella levels of up to four times normal values have been found in problem breeder flocks. Various management programmes are now implemented as standard in meat-rearing and egg farms, and include:

- Proper control and storage of floor shavings prior to use.
- The use of feeds that are pelleted at higher temperatures
- Dedicated feed storage and haulage vehicles.
- Organic acids in the feed.
- Chlorination of drinking water
- Disinfection and cleanliness of staff and visitors
- Limited access to animals
- Use of foot baths and vehicle washing facilities

Coccidiosis is another common disease of poultry and young mammals, caused by parasites of the genus *Eimeria*, however it is in the rearing of poultry where most research has been focussed. Although it does not transfer between meat and consumer, it represents an important economic gastric pathogen for producers. Seven species – *E. acervulina*, *tenella*, *maxima*, *mitis*, *necatrix*, *praecox* and *brunetti* - are known to infect chickens (Conway and McKenzie, 2007). Other poultry species of commercial importance, such as turkeys, pheasants, quail, and geese, are infected by other species of *Eimeria*. As the worldwide poultry industry has developed and intensified, so has the economic impact of the disease.

The *Eimeria* parasite has a complicated life cycle, with some stages taking place inside the bird (where they invade the lining of the intestine or caeca) and some outside. Each life cycle is associated with a huge increase in the number of infective parasites. Coccidia are spread by the faecal-oral route. The different species of coccidia favour different regions of the intestine. In chickens, mixed infections are common in the field, with the species mix varying with the age of bird. No gross lesions are seen with some species (*e.g.* *E. praecox*), which are thought to have little or no impact on the host. Other species (*e.g.* *E. necatrix*) are associated with severe haemorrhagic lesions and bloody enteritis.

Where clinical signs, such as blood in the droppings and intestinal lesions, are present, the disease is known as coccidiosis. Sub-clinical disease (also known as coccidiasis) is the consequence of a mild infection although the economic
impact can be severe, with reduced growth and poorer feed conversion rate (Williams, 2005). In broiler chickens, coccidial infection is also one of several known risk factors for necrotic enteritis. This disease is an enterotoxaemia cased by types A and C of *Clostridium perfringens* which occurs when high numbers of bacteria coincide with damage to the intestinal mucosa. Mortality rates can be high, with sudden onset of disease. Characteristic lesions occur in the gut and the mucosa may take on a “Turkish towel” appearance.

Coccidiosis in poultry is controlled by in feed anti-coccidials (chemical coccidiostats and/or ionophores) or vaccines. Ionophores have the additional benefit of helping to control Gram-positive bacteria including *Clostridia*. The number of authorised anti-coccidials has declined in recent years, especially in the EU, and remaining products must be managed properly, to avoid problems with residues in meat or eggs. Another problem is the inevitable development of resistance by the parasites, which can impact on bird performance. Vaccines offer an alternative solution with none of the problems of resistance or residues. Live (administered by spraying on chicks, giving in feed or water, or by injection into embryonated eggs) attenuated or non-attenuated, and inactivated vaccines (given by injection to breeding birds) are available for chickens in different regions of the world.

**Calves**

Diarrhoea is a common occurrence in calves. Probably much of it is not infectious in origin but is the result of the artificial manner in which many calves are reared. This type of diarrhoea features prominently among sick animals. High *E. coli* levels are often found in the abomasums of animals suffering from diarrhoea (Ingraham, 1962).

For a strain to be able to produce diarrhoea in a particular species of animal, it must possess at least two properties; the ability to produce an enterotoxin active in the small intestine, and an ability to proliferate in the small intestine of that species.

Neonatal diarrhoea in calves has been reported to be due to colonisation of the intestine by *E. coli* strains producing K99 and/or F41 fimbriae (Gaastra and DeGraaf, 1982). However, other fimbriae have been reported in Belgium (Contrepois and Girardeau, 1985; Morris *et al.*, 1985; Pohl *et al.*, 1982, 1984). Epidemiological studies have revealed that *E. coli* F17+ strains are associated with other outbreaks of coligenic diarrhoea such as enterocolitis. In summary therefore, pathogenic cells have adapted different fimbriae to colonise to different receptors on the gut epithelium (see Figure 4.4).
**Horses**

Young foals are as susceptible to scours as calves, and require the same diligent monitoring and treatment for successful control. Mature horses can experience digestive upsets due to changes in diet, stress levels or poor feed quality. Most of these fluctuations are more likely to be caused by changes in the microfloral population, due to insufficient fibre intake, rather than by the invasion of a pathogenic microbe. However, maintaining the balance and correct population is very important in all aspects of equine nutrition and gut health.

Imbalances in the gastric microflora can lead to the production of toxins from certain bacterial species. Horses, and especially ponies, can be very sensitive to these toxins, exhibiting inflammation or allergic reactions. This can lead to laminitis (an inflammation of the sensitive laminae in the hooves caused by bacterial endotoxins circulating in the blood), colic (general gut pain), dermatitis or lumpy rashes.

Typically digestive problems were treated with antibiotics, but are now more likely to be dealt with by changing the diet, or adding a supplement to alter and maintain the microfloral profile.

**Companion animals**

Dogs are arguably the more likely to become exposed to gastric pathogens due to behavioural characteristics related to scavenging potentially contaminated
food. Diarrhoea in dogs is not uncommon, and these have been associated with the presence of *E. coli*, *Klebsiella pneumonia* (Olson *et al.*, 1985), spirochetes resembling *Helicobacter* spp. combined with *Campylobacter* spp. and *Anaerobiospirillum* spp. (Misawa *et al.*, 2002). A condition frequently referred to as ‘bacterial overgrowth’ in canines is often related to gastric disturbances.

In cats, as in dogs, the occurrence of spirochete-like organisms akin to *Helicobacter* spp. have been a major isolate from individuals experiencing digestive problems, including diarrhoea (Foley *et al.*, 1999; Kipar *et al.*, 2001).

The main treatment used in pet animals with symptoms of digestive disorders is antibiotics. This is primarily because the most likely treatment sought by the owner is veterinary. Recurrent problems may result if underlying dietary or contamination aspects are not addressed, or if bacterial balance is not reinstated following treatment.

**Contamination risks and controls**

Levels of contamination in feed supplies can vary greatly according to mill hygiene and cleanliness and the types of raw material used in the formulation, which may introduce contamination.

*Salmonella enteritidis* accounted for more than 36% of the reported *Salmonella* infections in 1993. The organism is rarely found in ruminant animals with most strains occurring in poultry (phage types 4,6,8). *Salmonella enteritidis* can be found in high numbers in the caeca (Brownell *et al.*, 1970, even when the animal may show no clinical signs of infection, nor suffer any performance defect.

Although animal feedstuffs are a main source of infection, it has been shown that the level of contamination is very low (Watson, 1989), (Table 4.1). Agricultural feedstuffs, and eggs, in particular, have been highlighted as the main source of the contamination in humans with little evidence to support it. However, other materials have from time to time been affected on a large scale, for example, tomatoes in the USA. It is just as likely that the infections may arise from poor storage, processing and cooking of foodstuffs.

The levels shown in Table 4.1 have particular significance in relation to two orders introduced in the United Kingdom to control contamination of animal feed. They are “The Disease of Animals (Protein Processing) Order, 1981” and the “Importation of Processed Animal Protein Order, 1981”. Contamination is commonly controlled by the use of organic acids.
Table 4.1 The level of *Salmonella* contamination in common feedstuffs as a % of screened samples.

<table>
<thead>
<tr>
<th>Source</th>
<th>Salmonella contamination (% of samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal</td>
<td>2.9</td>
</tr>
<tr>
<td>Meat and bone</td>
<td>2.7</td>
</tr>
<tr>
<td>Extruded soya</td>
<td>2.3</td>
</tr>
<tr>
<td>Broiler feed</td>
<td>0</td>
</tr>
<tr>
<td>Layer feed</td>
<td>0</td>
</tr>
</tbody>
</table>

The control of such disease continues to be problematic due to the media focus on human health, and the ‘scares’ that seem to occur so often. However infections of the digestive tract are probably at their most tightly controlled following several such incidents. The introduction of schemes such as HACCP in both farms and slaughterhouses has done much to remove any potential sources of infection from human populations. Any carcass contaminated with gut contents are normally condemned under these hygiene schemes, limiting the risk of potential contamination with organisms such as *Salmonella* spp. or *E. coli*.

For non-food animals, the risk posed by gastro-intestinal infections is one of welfare rather than food safety. Companion animal and equine species are normally treated by a veterinarian and kept under strict monitoring, should they encounter gastric disease. However, in horses, digestive infections can be difficult to treat, as they may induce colic symptoms or laminitis which may not be initially linked back to gastric problems. Where diets contain high levels of poorly digested protein or carbohydrate, imbalances in the microflora of the caeca can cause these symptoms, including gas production, colic and laminitis. Many of the treatments include direct action to prevent the major symptoms and make the animal comfortable. In many cases, full recovery of the digestive tract and replenishment of an optimised microflora is not made a priority, and so recurring cases in individuals is common.
The analysis of microbial populations has undergone major changes in recent years, primarily due to the increased application of genetic methods in its evaluations. Microbes were first viewed within their own environments, *e.g.* in water, via microscopes. However, the need to identify certain bacteria, especially pathogens associated with disease, lead to the development of culturing methods. Samples known to contain bacteria were isolated, and then grown in low population numbers, to allow identification and study of their characteristics and behaviour.

**Conventional microbiology**

The most commonly used method of identifying bacteria and other microbes is cell culture where samples containing the microbe are diluted and grown on culture medium, which may be a set gel or a liquid. The media contains nutrients (energy and nitrogen source plus macro-minerals and trace elements) that allow the target organism to grow. The most common form is basic agar, which is derived from seaweed. However, each bacterial species has an ideal environment in which they will grow and proliferate.

Further refinement of the media can improve the growth and isolation of the desired organism. This is termed ‘enrichment culture’, and is tailored to certain requirements. Examples of compounds used in enrichment culture include antibiotics, acid, sulphur or other specialised compounds that are required for certain bacterial proliferation or to prevent the growth of highly competitive organisms that are not required. Some microbes have very specific requirements, *e.g.* *Clostridia* spp, which are grown on high protein cultures, based on lean meat.
The sample (normally diluted into a liquid form) is spread on the surface of solid media or within a liquid culture by means of a sterilised small metal loop. In plating, the diluent is streaked across the surface to one side, and then spread out at approximately 90º to the original streak. The objective being to spread the sample thinly enough to produce single colonies (derived from one cell) on the final plate. For liquid or other cultures, it may be dipped into the centre of the media.

Once sown onto the medium, the plates are incubated under specific environmental conditions geared to the growth of the target organism. After a set period of incubation, the plates are removed and the growth and appearance of colonies compared, using standard stock cultures of known bacterial species. They can be counted (each colony being grown from a single micro-organism), and back-calculated to estimate the levels of this organisms that were present in the original sample.

For testing gut-active components that may impact microbial growth, the test material may be included in the agar and compared against a non-enriched control agar to compare growth retardation or promotion. Alternatively, using an actively growing culture, small wells of a standard size can be cut out of the gel, the test compounds poured into the wells, and then incubated again, and any changes in the colonies noted. Normally, in the case of an anti-bacterial compound, a zone of inhibition or promotion in microbial growth appears as a halo of clear agar around the well, or more actively growing colonies respectively. This can be measured and used as a comparison for different strengths of the compound. Anti-bacterial compounds can be included directly into the poured agar. The resulting colony growth and presence/absence of certain species can be used to determine minimum inhibitory concentration (MIC) of the compound, as well as its potential effects on population dynamics and the inter-relationships between microflora species.

In the case of identifying and quantifying gut microflora populations, culturing is a useful way to provide an indication of the main organisms present – although these will be confined mostly to those that can tolerate oxygen. Only organisms that remain viable following sampling can be used to generate new colonies. Anaerobic organisms are particularly difficult to culture, as it is hard to prevent their exposure to air during preparation and plating. True anaerobes can only survive in non-oxygen atmospheres, and must be collected, prepared and plated under strict conditions to ensure successful culturing. This is a major problem for the investigation of gastric populations of microbes, as many are anaerobic. The limitations of culturing lead to the belief that *E. coli* formed the majority of the populations found in the gut. We now know that this species, in reality, only
comprises a minor component of the microflora (Simon et al., 2004). There are many other types of micro-organism that are deemed ‘unculturable’ by standard plating methods, whether due to their sensitivity during sampling, or by their highly specific requirements that cannot be easily met within the limits of current culturing techniques.

**Genetic analysis**

The advent of analytical methods that negate the need for obtaining viable cells which can be grown and isolated has led to a major improvement in the understanding of gut microbial populations and their dynamics (Simon et al., 2004). Mullis won a Nobel Prize in 1993 for the development of the PCR (polymerase chain reaction) method which he developed in 1983. This allowed the amplification of DNA fragments, facilitating many more opportunities for genetic manipulation and study. Following this breakthrough, investigations into various DNA material resulted in new methods for analysing and characterising microbial populations. Initially, Lane et al. (1985) demonstrated that DNA extracted from bacterial ribosomes (rDNA) could be used as an identifier of bacterial group and phylogenetic (classification) origin. As genetic material can be extracted from live or dead bacteria, such analytical methods make it possible to identify all microbes, including sensitive anaerobic and unculturable species.

The chicken has become a useful research animal in modelling digestive bacteria using genetic methods, due to its relative small size and rapid development as well as the large amount of information regarding its gastric activity and microbiology.

**Primer-based genetic systems**

‘Primer’ analysis requires that the short sections of isolated DNA that are used to identify a bacterial species to be already characterised in a library, which is available for fragments comparison. The ‘primer’ refers to the initial identifying genetic sequence at the start of each gene, which is important in gene replication.

In the last twenty years genetic analytical methods have progressed rapidly, and libraries of generic marker material for both well-known and uncharacterised bacteria are now available. The reliability of identifying a complete population of bacteria resident in the gut is entirely dependent on the scale and accuracy of the gene library available.
Analytical methods using primers include denaturing gradient gel electrophoresis (DGGE), which examines the speed of movement of DNA through a gel (Holben et al., 2004). This is achieved by pulling the DNA through the gel via electrophoresis for a known period of time. The final gel will contain bands of DNA fragments of different densities. The degree of travel and density within each band dictates the length of the fragment, and, when run in lanes next to genetic material from a known species, can be used to identify the bacteria present in the sample.

Restriction fragment length polymorphism (RFLP) is a technique used for genetic fingerprinting. Amplified DNA (using PCR) is cut into shorter pieces (‘restriction fragments’) and then separated on an electrophoretic gel, in a similar manner to DGGE. Different species, and even individuals within a species, show their own characteristics in genetic fragment length, when using this preparation method, and so the banding is unique to the bacteria present.

Some methods rely on genetic fragments derived from the 16S rDNA genes. These fragments are compared against those held within the library, as done in the other methodologies, and, as they are unique to each bacterium, allow identification.

Primer methods account for less than 80% of the total bacteria within the digestive tract. However, 16S sequencing, which has been used to characterise bacterial microflora in chickens, is ‘primer dependent’, i.e. it needs to have already identified genetic fragments that are maintained in a gene library and used for direct comparison. This is true for all primer-based systems.

Although these methods have their limitations, they have still provided useful data regarding the bacterial populations within animals. Using genetic methods, researchers have found that the ileum of the chicken contains predominantly lactobacilli, followed by clostridia type XIVa – a benign group that produce butyrate (Table 5.1).

Table 5.1 Major bacterial groups present in chicken ileum using 16S sequencing methods. Adapted from Lu et al. (2003) and Apajalahti et al. (2004)

<table>
<thead>
<tr>
<th>Data source</th>
<th>Lu et al., 2003</th>
<th>Apajalahti et al., 2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of birds</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>&lt; 5 %</td>
<td>&lt; 5 %</td>
</tr>
<tr>
<td>Clostridial cluster XIVa</td>
<td>6 %</td>
<td>10 %</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>&lt; 5 %</td>
<td>&lt; 5 %</td>
</tr>
<tr>
<td>Escherichia</td>
<td>&lt; 5 %</td>
<td>&lt; 5 %</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>86 %</td>
<td>80 %</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>&lt; 5 %</td>
<td>&lt; 5 %</td>
</tr>
<tr>
<td>Unknown genera</td>
<td>&lt; 5 %</td>
<td>&lt; 5 %</td>
</tr>
</tbody>
</table>
Analysing gut microbial populations

Caecal samples show that Clostridia (type XIVa) have the largest population, followed by type IV, Lactobacilli and Bifidobacteria (Table 5.2).

Table 5.2 Major bacterial groups present in chicken caeca using 16S sequencing methods. Adapted from Apajalahti et al. (2004), Holben et al. (2004), Lu et al. (2003) and Zhu et al. (2002)

<table>
<thead>
<tr>
<th>Data source</th>
<th>Geographic region</th>
<th>Number of birds</th>
<th>Finland 20</th>
<th>Global 500</th>
<th>Georgia, USA 5</th>
<th>Delaware, USA 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bacteroides</td>
<td>29 %</td>
<td>9 %</td>
<td>5 %</td>
<td>&lt;3 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bifidobacterium</td>
<td>&lt;3 %</td>
<td>8 %</td>
<td>&lt;3 %</td>
<td>&lt;3 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clostridial cluster IV</td>
<td>&lt;3 %</td>
<td>13 %</td>
<td>35 %</td>
<td>5 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clostridial cluster XIVa</td>
<td>42 %</td>
<td>42 %</td>
<td>45 %</td>
<td>3 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eubacterium desmolans</td>
<td>&lt;3 %</td>
<td>&lt;3 %</td>
<td>9 %</td>
<td>&lt;3 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Escherichia coli</td>
<td>&lt;3 %</td>
<td>&lt;3 %</td>
<td>&lt;3 %</td>
<td>&lt;3 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lactobacillus</td>
<td>12 %</td>
<td>11 %</td>
<td>&lt;3 %</td>
<td>&lt;3 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Others</td>
<td>13 %</td>
<td>14 %</td>
<td>5 %</td>
<td>90 %</td>
</tr>
</tbody>
</table>

Independent genetic methods

Several methods of genetic evaluation of microflora exist that do not rely on the availability of primer libraries. These include flow cytometry, which simultaneously measures and analyses multiple characteristics of cells as they flow through a beam of light. These characteristics can be analysed and used to identify DNA, along with a whole host of other attributes. Graphical representation of the characteristics can be used to determine which types of bacteria are present within the sample. Research by Apajalahti and his team in Finland (Apajalahti and Kettunen, 2006) have shown that using flow cytometry can identify both unculturable and genetically uncharacterised bacteria in the assessment of the total microflora within a chicken.

The %G+C provides a non-primer method of genetic profiling, which quantifies the amounts of guanine and cytosine (nucleic acids) present within chromosomal DNA (Holben et al., 2004). This is characteristic of bacteria, and, via detailed analysis of the resulting spectrum, allows the calculation of relative levels of certain species within the population (Figure 5.1).

Practical applications of genetic microfloral analysis

Apart from the investigative aspect, analysis of the microflora can be very useful in understanding what constitutes a ‘correct’ profile (i.e. the balance of
populations resident in the gut) versus one that may be susceptible to pathogens or already be diseased. Even through all the bacteria may not have formally been identified, microflora samples from animals with diagnosed disorders, or one which is not developing correctly, can be compared by genetic methods (e.g. %C+G) against a healthy individual. ‘Shifts’ in the trace between samples from different feeds or diseases states can then be used to investigate methods of stabilising the microflora.

Understanding the complete microbial profile of the gut has also enabled the development of more accurate in vitro models of digestive and fermentation processes (Apajalahti, 2005). This allows the modes of action and benefits or shortcomings of feed raw materials and specialised ingredients to be studied under more controlled conditions.

**Consequences of increased bacterial identification**

One of the major problems currently in microbiology is the archaic manner in which bacteria are named. Their groupings have previously depended principally on characteristics in common, such as cell wall structure or utilization of certain substrates. It is now realized that, in order to be exact, it is important to look at the genetic relationships between bacteria. This reflects the true evolutionary aspects of the individual species.
Apajalahti (2004) reported over 640 species of bacteria in the chicken gut, representing 140 different genus level groups (i.e. ‘families’ with common characteristics). As these names and groupings are not related to actual genetic character, identifying pathogens and commensal bacteria is problematic. For example, it may be surprising to learn that most species of Clostridia are completely harmless. The most abundant type in the gut, the cluster XIVA, is a benign group which produces beneficial butyrate, promoting villi growth. An interesting example of confusing bacterial classification is that the Lactobacilli species *L. crispatus* and *L. rhamnosus* are more distantly related than *L. crispatus* and *Enterococcus faecium*.

Even with these major advances, microbial analyses are still not completely free of bias. The accuracy of new genetic methods are entirely dependant on the extensiveness of the libraries and quality of methodology employed. Great care is needed when discussing ‘good’ and ‘bad’ bacteria, as much confusion surrounds the complexities regarding naming gut bacteria. Full species names, rather than genus alone, are important to determine whether a bacterium is a true potential pathogen or not.

The continued development of more accurate and detailed assessment of bacteria that reside in the gut is key to increasing understanding of what a ‘correct’ microfloral profile may be, and how feed and gut-active compounds can destabilise or maintain microbial populations.
The last twenty years or so has seen a major increase in interest, both academically and commercially, in the use of supplemental, or bioactive, feed ingredients (Adams, 1999; Rosen, 2001, 2002). Many of these affect the dynamics of both the microflora and more general gastric function, however the manner in which they operate may differ greatly. Certainly there has been a substantial amount of data generated from trials using various species, to show efficacy in terms of animal performance (Rosen, 2002, 2003, 2006a,b, 2007a,b). In some cases, there is also microbial data and mode of action has been determined. From the point of view of using such ingredients to alter or stabilise the gut directly, the following chapter will deal with some of the key products and compounds that have been best characterised. However, it should be remembered that this is a fast growing area, and that this should not be viewed as a definitive list.

The main factors driving the development and use of bio-active feed ingredients (nutricines or pronutrients) are two fold. Primarily, the need for more efficiencies in agricultural production, whether for meat, eggs or milk, in order to meet the increasing demands from retailers and consumers has promoted the investigation into further means to extract nutrients for growth and development, with less excreted losses. Many of the ingredients discussed in this section are viewed as ‘natural’ or ‘safer’ alternatives to other means of improving animal performance, which leads to the second, more recent driver, which is the limitations with respect to using antibiotic-based growth promoters. Since the mid 1990’s this has provided a market for the commercial application of such feed ingredients, initially in niche sectors, but increasingly, following the recent ban in the EU, into mainstream production.

The real or perceived advantages of natural alternatives for promoting gut function and health have also spilled over into the human markets, with many supplements now available and marketed for use in people, pet animals and horses. Although supporting scientific data for these species is often less available, there are several examples of work that demonstrate efficacy of these ingredients.
The following chapter concentrates on the following broad classifications of bioactive feed ingredients:

- Live yeasts
- Enzymes
- Pro-biotics: live bacterial supplements
- Pre-biotics: gut-active carbohydrates
- Acids
- Botanicals: phytogenics

**Antibiotics**

In order to fully understand the importance of pronutrients, the use and relevance of antibiotics in animal feeds should first be considered, as many nutricines have been developed to replace these compounds. Antibiotics are chemical compounds, which in small quantities are harmful to other organisms. They occur widely in nature but the term is generally restricted to compounds which are microbial in origin, particularly those used to control other harmful micro-organisms, generally bacteria.

The first antibiotic to be discovered was penicillin, by Sir Alexander Fleming. It is a classic example of a major scientific discovery by serendipity rather than as the result of objective research. In 1928, a culture plate of *Staphylococcus* was contaminated with spores of *Penicillium notatum*. Around the mould which was contaminating the plate, the *Staphylococcus* was destroyed. The pure culture was isolated and found to produce a substance which had a powerful antibacterial effect, and was named penicillin. Later, Sir Howard Florey’s team, at Oxford University, made particular studies of antibiotics in terms of their curative properties, isolation and production.

The last forty years has seen a considerable intensification of the livestock industry, with faster growth rates, greater stocking density, larger production units, etc. The increased pressure on production resulted in increased use of low level feed additives to improve performance and/or health. Also, in intensive systems, the health of animals is a problem, with high levels of stress and microbial challenge. Antibiotics became commonly used as feed growth promoters, and are still used at a great deal around the world as prophylactics, as well as being used at higher levels for therapeutic purposes. Some of the more common antibiotics that have been used in animal feeds are given in Table 6.1.
Table 6.1 Commonly administered oral antibiotics

<table>
<thead>
<tr>
<th>Type</th>
<th>Examples</th>
<th>Source</th>
<th>Mostly active against</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillins and</td>
<td>Penicillin G</td>
<td>P. chrysogen</td>
<td>G+ bacteria</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>Ampicillin</td>
<td>Semisynthetic</td>
<td>G+ and some G- bacteria</td>
</tr>
<tr>
<td></td>
<td>Cephalexin</td>
<td>Semisynthetic</td>
<td>G+ and some G- bacteria</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>Chlortetracycline</td>
<td>Streptomyces aureofaciens</td>
<td>G+ and G- bacteria</td>
</tr>
<tr>
<td></td>
<td>Oxytetracycline</td>
<td>Streptomyces rimosus</td>
<td>rickettsiae</td>
</tr>
<tr>
<td></td>
<td>Tetracycline</td>
<td>Streptomyces spp</td>
<td>G+ and G- bacteria</td>
</tr>
<tr>
<td>Macrolides</td>
<td>Erythromycin</td>
<td>Streptomyces erythreus</td>
<td>G+ bacteria</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>Streptomycin</td>
<td>Streptomyces griseus</td>
<td>G+ and G- bacteria</td>
</tr>
<tr>
<td></td>
<td>Neomycin B</td>
<td>Streptomyces fradiae</td>
<td>G+ and G- bacteria</td>
</tr>
<tr>
<td>Polypeptides</td>
<td>Bacitracin</td>
<td>Bacillus licheniformis</td>
<td>G+ bacteria</td>
</tr>
<tr>
<td>Others</td>
<td>Chloramphenicol</td>
<td>Streptomyces venezuelae</td>
<td>G+ and G- bacteria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Or synthetic</td>
<td>Including some</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Salmonella rickettsiae</td>
</tr>
<tr>
<td>Lincomycin</td>
<td></td>
<td>Streptomyces lincolnensis</td>
<td>G+ bacteria</td>
</tr>
</tbody>
</table>

However, in many countries there has been critical examination of their use. Their indiscriminate use is believed to have exacerbated some of the conditions that their development was intended to prevent, with the result that resistance to antibiotics has built up in important human pathogens.

The Swann Report (1969) recommended tighter governmental control on the use of antibiotics in feed in Britain and stated that antibiotics which produced cross-resistance should not be used in animal feeds. This led, in 1971, to the banning of the tetracyclines and other ‘therapeutic’ antibiotics, in feed, as growth promoters.

Bacitracin, virginiamycin, nitrovin and flavomycin, amongst others, were therefore promoted as safe antibiotics. However, their use has caused concern as it was proposed that they affected the natural ecological balance in favour of pathogens such as Salmonellae (Smith, 1971). It may be that that the increase in the incidence of antibiotic resistant bacteria is a result of their over-use. Due to such concerns, Sweden initially prohibited the use of antibiotics as growth promoters. Monitoring of resistance bacterial species as the ban swept across Scandinavia showed reductions in the problem, although in many cases the actual use of therapeutic drugs increased dramatically. The problems relating to using antibiotic prophylaxis continued across Europe, culminating in an EU-wide ban from 1st January 2006. In the couple of years leading up to the ban, many alternatives exerting non-resistance modes of action became available on the market. A further ban on coccidiostats will be introduced in the near future.
Antibiotics are still permitted for use in many countries around the world, and of course remain as a course of action for serious infections, which require veterinary or medical intervention.

The mode of action of antibiotics

The theory of improved efficiency from the reduction in microbial loading by the use of anti-microbial agents centres on the fact that the gut is the most demanding of the body’s organs both in terms of its energy and protein (Murumatsu et al., 1987). Any improvements in its nutritional efficiency will, therefore, have large effects on the animal’s ability to improve its performance.

There has been much discussion about the growth promoting mode of action of antibiotics which may have their effect through a variety of mechanisms, differing from one product to another. They have been shown to inhibit microbial growth, sugar metabolism, metabolite production, cell wall formation, and nucleic acid and protein synthesis.

One possible mode of action is that antibiotics suppress some types of bacteria. This would give rise to the direct absorption of some materials which would be a more efficient use than their fermentation by microbes. The micro-flora normally metabolises some of the ingested food, with carbohydrates, for example, being converted to lactic acid and volatile fatty acids under anaerobic conditions. These end products may be useful to the host after absorption, but this would depend on the site of fermentation. They are less useful and have less metabolisable energy than the original carbohydrates and, in the pig, for example, the major site of fermentation, the large intestine, is not regarded as an efficient site of absorption. Thus, antibiotics, if they act on the gut flora, may save energy by sparing carbohydrates from bacterial metabolism, reducing the production of volatile fatty acids, improving nitrogen availability by sparing essential amino-acids and reducing the levels of toxic amines. However, it is claimed that their growth promoting activity cannot be adequately explained solely by the suppression of some bacterial groups alone. Possible modes of action are summarised in Table 6.2, with a primary action possibly followed by a secondary anatomical or biochemical change.

A classification of antibiotics may be based on whether their effect is bactericidal or bacteriostatic (Table 6.3) but such divisions are not always clear (Manten and Meyerman-Wisse, 1962).
Table 6.2 Examples of modes of action of antibiotics on target bacteria

<table>
<thead>
<tr>
<th>Mode of action (affecting)</th>
<th>Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial cell wall</td>
<td>Penicillins, bacitracin</td>
</tr>
<tr>
<td>Bacterial cell membrane</td>
<td>Streptomycin group</td>
</tr>
<tr>
<td>Protein synthesis</td>
<td>Chloramphenicol, tetracyclines, lincomycin, neomycin, streptomycins</td>
</tr>
<tr>
<td>Nucleic acid metabolism</td>
<td>Griseofulvin</td>
</tr>
<tr>
<td>Intermediary metabolism</td>
<td>Sulphonamides</td>
</tr>
</tbody>
</table>

Table 6.3 Classification of antibiotics

<table>
<thead>
<tr>
<th>Mode of action</th>
<th>Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteriostatic</td>
<td>Tetracyclines, chloramphenicolns, macrolides, sulphonamides</td>
</tr>
<tr>
<td>Bactericidal</td>
<td>Penicillins, novobiocin, bacitracin, polymyxins (including colistin), nitrofurans</td>
</tr>
</tbody>
</table>

Effects of antibiotics in the host animal

Numerous influences of antibiotics have been reported, many of which are interrelated. First, harmful bacteria are inhibited. For example, *S. faecalis* has been shown to reduce growth rate in chickens (Eyssen and DeSommer, 1967), possibly by inducing malabsorption of fat with the influence of antibiotics improving weight gain and restoring fat absorption to normal. This may be due to nutrient sparing effects by inhibiting fermentation losses or by enhancing fat digestibility. (Fuller, 1984; Lev and Forbes, 1959; Smith, 1972; Stutz and Lawson, 1984).

A protein sparing effect is apparent through the reduction of faecal nitrogen loss which has resulted from antibiotic use (March et al., 1978; Eyssen and DeSommer, 1967; Visek, 1978). Furthermore, a sparing of amino-acids has been shown by Hedde (1984) who demonstrated that lysine was destroyed less in the gut of pigs when virginiamycin was included in the diet. This sparing effect has been observed as increased nitrogen retention in pigs (Lindsey, Hedde and Sokolek, 1985). Such improvements are associated with a decreased passage rate of digesta and mucosal cell turnover. A slower rate of passage allows a greater time for digestion so improving nutrient availability. Consequently, there is improved
nutrient absorption. This may also result from gut thinning (Gordon, 1952), which may partially account for improved absorption and increases in apparent digestibilities of protein and amino-acids.

Feed antibiotics help to reverse the effects of the normal flora on the gut wall, which after treatment will resemble that of a germ free animal (Coates, Davies and Kon, 1955). As many species derive essential nutrients from the activity of their resident microflora, and the gut needs to be maintained in a stable microbial state, reintroducing correct bacterial levels and ensuring they establish correctly is important following a course of therapeutic antibiotics.

Other likely effects of the use of anti-microbials include:

- Lower crypt cell proliferation leading to a reduction in protein turnover.
- Lower use of absorbed nutrients by microbial cells leaving increased quantities available to the animal’s tissues.
- A reduction in epithelial turnover and mucus secretion conserving nitrogen.
- An increase in digestive and absorptive ability.

The use of antibiotics on the farm

The main production effects of using antibiotics in poultry and other animals are improved health performance, growth and feed utilisation. However, the Swann Report in 1969 first attempted to separate the use of anti-microbials into “in feed” and “therapeutic” types. The report also stated that the routine use of low levels of antibiotics “in feed” might endanger human health because of the development of bacterial resistance. Therapeutic antibiotics (which are used at much higher levels) for animal use are now only available on prescription in the United Kingdom. Despite a bad press, linked with perceptions of them being ‘unnatural’, there are numerous reports to support the beneficial effects of antibiotics, for example in young calves and poultry (Bush et al., 1959; Eyssen et al., 1962; Radisson et al., 1956).

A typical on-farm antibiotic that was widely available was Avoparcin. However, under current EU legislation it is no longer authorised for use as an antibiotic growth promoter in feed. It has been claimed to have activity in the gut but not to be absorbed into the body. Avoparcin is claimed to:

- Increase the villi surface area.
- Enhance nutrient absorption.
Bio-active feed ingredients

• Alter fermentation patterns in the rumen.
• Reduce Gram-positive organisms which damage villi and reduce nutrient supply.
• Maintain a thinner gut wall.
• Reduce epithelial cell turnover leading to increase in enzyme secretion (especially dipeptidase).
• Have a protein sparing effect.

Because of the better intestinal activity and nutrient absorption, it is claimed feed utilisation is improved in cattle, pigs and chickens, with milk yield improvement in dairy cattle.

Avoparcin is produced by a natural fermentation on a nutrient medium by a bacterium isolated from soil. It was included at low levels of 10 to 40 mg/kg of feed.

In the ruminant animal improvements in performance, from in-feed antibiotics, e.g. monensin sodium, are claimed to be due to alteration of rumen fermentation by inhibition of specific rumen micro-organisms (Table 6.4). Fermentation in the rumen can therefore be restricted to more efficient and beneficial organisms.

Table 6.4 Rumen bacteria sensitive to monensin sodium and their fermentation products

<table>
<thead>
<tr>
<th>Monensin sodium sensitive</th>
<th>Products of fermentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruminococcus</td>
<td>Acetate</td>
</tr>
<tr>
<td>Methanobacterium</td>
<td>Acetate, Methane</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>Lactate</td>
</tr>
<tr>
<td>Butyrivibrio</td>
<td>Acetate, Butyrate</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>Lactate</td>
</tr>
<tr>
<td>Methanosarcina</td>
<td>Methane</td>
</tr>
<tr>
<td>Selenomonas</td>
<td>Propioniate</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>Acetate, Propionate</td>
</tr>
<tr>
<td>Veillonella</td>
<td>Propioniate</td>
</tr>
</tbody>
</table>

Antibiotic resistance

One of the main concerns over the use of antibiotics for farm animals is the development of resistance. This will make them less effective and has implications for human health (Barton, 2000). Most large bacterial populations contain mutants which are naturally less susceptible to a given drug than the remainder of the population.
Microbial resistance to anti-microbial agents may be due to physiological adaptation (phenotypic change) or to a mutation and selection (genotypic changes). Once a drug-resistant mutant has emerged in a bacterial population, the resistance can be transferred to other cells by the mechanisms of transformation (incorporation of DNA from resistant organisms into sensitive organisms), transduction (transfer of drug resistance by bacteriophage), or conjugation (sexual reproduction and direct transfer).

Other suggestions for drug resistance include:

- Decreased permeability of the organism to the drug.
- Increased destruction of the drug.
- Conversion to an inactive form.
- Increased formation of the metabolites which compete with the drug.
- Production of inhibitory (extra cellular) enzymes.
- Development of alternate metabolic pathways.
- Altered enzymes which can function in the presence of the drug.
- Change in ribosomal protein structure.
- Duplication of target site.
- Reduction in physiological importance of site.

Transferable antibiotic resistance has been shown to be possible via plasmids called R-factors. The feeding of antibiotics is thought to favour the selection of R-plasmid bacteria in pigs and poultry, possibly leading to transfer of resistance to pathogenic bacteria such as Salmonella. The transferring of genes has been confirmed in Gram-positive bacteria, e.g. Streptococcus, Bacillus, Staphylococcus and Clostridia. R-plasmids are spread widely and found in the intestinal microflora of animals which have not undergone the pressure of antibiotic treatment. Cells can adapt, mutate or gain natural resistance to an antibiotic.

**Contamination of animal products with antibiotics**

Antibiotics have been claimed to leave residues in tissues (Wu, 1987), cause disturbance of other microbes, kill desirable bacteria and cause pathogenic resistant strains (Wu, 1987). The level of antibiotic actually entering the animal’s tissues will depend on the degree to which it is absorbed from the intestinal tract. Tetracyclines are generally well absorbed and so are more likely to enter the tissues than streptomycin and neomycin, which are less well absorbed.
On the basis of long term microbiological studies, it has been suggested that the use of antibiotic growth promoters does not increase the prevalence of antibiotic resistance in humans (Lacey, 1988). Contrary to this belief other researchers have shown close relationships between drug resistance patterns in human and animal populations, with some reporters linking this to the utilisation of antibiotics as feed additives (Jensen et al., 1999). Various suggestions to reduce the development of resistant strains have been made:

- The use of high strength antibiotics only until infection is overcome.
- The use of chemically unrelated drugs with different modes of action.
- Stronger control of anti-microbials in farming.
- The use of more ‘natural’ alternatives for therapeutic purposes and for animal growth and performance.

New developments are resulting in antibiotics which are more selective in their action and, in some cases; the new antibiotics have little effect on the populations of beneficial, non-pathogenic micro-organisms within the gut environment. It has been suggested that it is desirable to find more natural methods of regulating the number of pathogenic bacteria, other than by the routine use of antibiotics. Antibiotics are, however, undoubtedly essential in agricultural systems and for the welfare and health of all other species, and their importance should not be underestimated.
Yeasts have been long recognised for their contribution as a natural feed ingredient. Indeed, yeast-based products are commonly admitted for use in organic farming, as the governing authorities consider them as part of the animal’s natural intake. Yeasts and yeast-rich products can be obtained as by-products from the baking and brewing industry as well as being specifically designed for in-feed ingredients.

Live yeasts are complete cells that are viable within the digestive tract. They are mainly used commercially in true and semi ruminants, *e.g.* cattle, sheep and horses. This is because their key activity is related to optimising the fermentation within the rumen or hindgut of these animals, especially for the control of acidosis that can be a major problem for these species.

Yeasts retain their function within the gut environment, and have important modes of action that contribute to a stable fermentation. Yeasts are aerobes, *i.e.* they utilise oxygen. Within the caeca or rumen, it is important to maintain an anaerobic environment to ensure the efficient working and proliferation of the resident bacteria, which break down the feed material, releasing nutrients for the host. When forage is ingested, it is often coated with a layer of air bubbles on their surface. Yeasts can utilise this available oxygen for their own metabolism, which reduces its potential for promoting aerobic conditions. Live yeasts strip away the protective oxygen shield and secrete peptides, which signal that the forage is unprotected. This encourages rumen bacteria to engulf and digest the forage, uninhibited by oxygen challenges. Improving the contact between the feed substrate and the bacteria increases digestive efficiency. For any starch present in the ration, it is important to control the lactic acid produced during its digestion, as this can decrease the pH, destabilising the rumen or caeca.

The second effect of live yeasts is their preference for carbohydrates such as starch. The breakdown of starch in the rumen or caeca leads to the establishment of acidic conditions (through the generation of lactic acid), which causes inefficient feed breakdown, digestive disorders and diarrhoea. Yeast have been shown to buffer the pH in this manner more effectively than other, chemical
treatments

The mode of action was first proposed by Dawson (1990) and the relationships between rumen metabolism and animal performance are given in Figure 7.1.

![Diagram](image)

**Figure 7.1** Model for the effects of yeast culture on microbial activities in the rumen (Dawson 1990)

Fungal cultures increase the total bacterial count in the rumen generally, and particularly, the cellulolytic population. For example, Harrison et al., 1988 found a 60% increase in total bacteria and an 82% increase in cellulolytic bacteria in the rumen of lactating dairy cows given 57g/day of yeast. *In vitro* increases of up to 461% in total bacteria have been shown.

It appears that the rate of fibre digestion is one of the major factors to be altered by the addition of yeast. It has been suggested that changes in the time course of digestion may increase the availability of nutrients in the rumen and have a significant impact on feed intake (Williams and Newbold, 1990; Dawson, 1990). Such increases in degradation rate would be particularly important for forages.

In ruminant animals the supply of amino acids to the small intestine is of major significance. As the rumen micro-flora influences this supply, there has been considerable interest in the so-called ‘by-pass protein’, i.e. protein that can reach the small intestine without being influenced by the rumen. A further approach is to manipulate rumen fermentation to enhance and modify the supply to the small intestine. It is generally accepted that the amino acid profile of the rumen bacteria is fairly constant. However, it has been shown that
this can be modified by the addition of yeast culture and that the supply of the
normally limiting amino acids to the duodenum (e.g. methionine and lysine) can
be increased (Erasmus, Botha and Kistner, 1992).

Acidosis, due to incorrect rumen function, results in poor animal performance,
as many nutrients are excreted and not available for the host. The combined
activities of live yeasts promote a stable rumen, with optimal bacterial
fermentation, which increases digestion and promotes better feed intake (Tuck,
pers. comm.). The graph below (Figure 7.2) illustrates how rumen pH can be
stabilised using live yeasts.

![Graph showing rumen pH over time with different yeast treatments]

**Figure 7.2** Improvements in rumen stability in cattle supplemented with yeast
(Tuck, pers. comm.)

The source of yeast dictates its efficacy *in vivo*, so the selection of a commercial
yeast-based product requires some diligence to ensure the product used is giving
good value for money. Trials comparing different live yeast sources have shown
that brewers yeast strains are more effective than bakery-derived ones (Figure
7.3).

Several live yeast products are available commercially, and are primarily aimed
in the dairy and beef cattle sectors, where they form an important part of a
good quality feeding program. The stabilised rumen will also result in higher
performance, due to increased nutrient availability and decreased digestive
upsets in ruminants.

Potential benefits from feeding live yeast to ruminants can be seen in Table 7.1
below.
**Figure 7.3** Comparison of the efficacy of two yeast sources on rumen aerobic bacteria proliferation (Newbold et al., 1996)

<table>
<thead>
<tr>
<th>Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased ammonia concentration</td>
<td>Harrison <em>et al.</em> (1988)</td>
</tr>
<tr>
<td>Altered VFA production</td>
<td>Harrison <em>et al.</em> (1988)</td>
</tr>
<tr>
<td>Increased ethanol concentration</td>
<td>Williams and Innes (1989)</td>
</tr>
<tr>
<td>Moderated ruminal pH</td>
<td>Williams and Innes (1989)</td>
</tr>
<tr>
<td>Decreased lactic acid concentration</td>
<td>Williams and Innes (1989)</td>
</tr>
<tr>
<td>Decreased soluble sugar concentration</td>
<td>Williams and Innes (1989)</td>
</tr>
<tr>
<td>Reduced methane production</td>
<td>Williams and Innes (1989)</td>
</tr>
<tr>
<td>Altered digestive patterns</td>
<td>Wiedmeier <em>et al.</em> (1987)</td>
</tr>
<tr>
<td>Stabilised fermentation</td>
<td>Harrison <em>et al.</em> (1988)</td>
</tr>
<tr>
<td>Increased concentration of anaerobic bacteria</td>
<td>Wiedmeier <em>et al.</em> (1987)</td>
</tr>
<tr>
<td>Increased concentration of cellulolytic bacteria</td>
<td>Harrison <em>et al.</em> (1988)</td>
</tr>
<tr>
<td>Increased concentration of yeast in the populations</td>
<td>Dawson (1990)</td>
</tr>
</tbody>
</table>

For hind gut fermenters such as horses, many compound feeds contain live yeast cultures as digestive enhancers, as the same problems require addressing as for ruminants. Trials have shown that feeding commercial live yeasts to growing horses can improve nitrogen availability and uptake (Glade & Biesik, 1986) (Table 7.2).
Table 7.2 Effect of feeding commercial live yeast on nitrogen metabolism in growing yearling thoroughbred horses (Glade and Biesik, 1986)

<table>
<thead>
<tr>
<th>Parameter (g/d)</th>
<th>Control</th>
<th>Yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed intake</td>
<td>113</td>
<td>113</td>
</tr>
<tr>
<td>Faecal N excretion</td>
<td>52</td>
<td>49</td>
</tr>
<tr>
<td>Digested N</td>
<td>61</td>
<td>64</td>
</tr>
<tr>
<td>Urinary excretion</td>
<td>54a</td>
<td>50b</td>
</tr>
<tr>
<td>Retained</td>
<td>7a</td>
<td>14b</td>
</tr>
</tbody>
</table>

Retained as % digested N: 12.6a vs. 22.6b

Means not sharing a letter differ significantly (P<0.05)

Research using exercised horses has shown evidence for enhanced athletic fitness in supplemented horses, probably due to an improvement in nutrient digestibility and assimilation (Glade and Campbell-Taylor, 1990).

Feeding trials with commercial live yeasts have reported improvements in microbial profile in the gut (Dawson, 1990), increased nutrient digestibility and more efficient conversion of nutrients into useable energy and protein (Glade and Pagan, 1988). Maintaining a correct microbial profile helps prevent the growth of undesirable micro-organisms, limiting the potential for the generation of toxic products that can cause laminitis or colic, especially for horses fed high starch diets (Medina et al., 2002). Improving digestibility and conversion of nutrients is essential for efficient digestion, making sure the horse gets the most out of the feed it is given.

Trials conducted in Brazil, using growing two-year olds, found that supplementing their diet with 30 g of live yeast significantly improved the digestion of protein (required for muscle and tissue development) and phosphorous (for bone growth) (Table 7.3).

Table 7.3 Feeding yeast improves nutrient digestibility in growing horses (Ribeiro et al., pers comm)

<table>
<thead>
<tr>
<th>Digestibility (%)</th>
<th>Control</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>(50% concentrate + 50% Hay)</td>
<td></td>
<td>(+ 30 g yeast)</td>
</tr>
<tr>
<td>Protein digestibility (%)</td>
<td>71</td>
<td>74.5*</td>
</tr>
<tr>
<td>Phosphorous digestibility (%)</td>
<td>59.5</td>
<td>69*</td>
</tr>
</tbody>
</table>

* significantly different from control diet
Other trials have considered the benefits in terms of weight gain in growing horses, as a direct effect of increased nutrient digestibility from feeding live yeast. Research conducted in Hungary showed that yearlings, receiving a hay and oat-based diet, gained an extra 7 kg in weight over the 79 days they received live yeast in their feed compared to those not supplemented (Hausenblasz et al., 1993, pers. comm.). Again, improvements in protein, dry matter and sugar digestion were seen, and fibre digestion was 3% higher than the unsupplemented feed.

Trials run in Korea reported that feeding live yeast significantly improved dry matter, fibre, ash and mineral digestibility (Kim et al., 2006), and numerous other studies have supported such finding, including improvements in microbial profiles and the dynamics of how live yeast operates in the gut of equines.

Adult pigs derive an appreciable amount of energy from hind gut fermentation, and feeding experiments with sows given diet formulated with live yeast have reported increased litter weights and daily gains of progeny from primiparous and multiparous sows (Kim et al., 2006). Other animals studied include lambs that, like calves, are subject to digestive problems during the transition from milk to weaned feed. Cole, Purdy and Hutcheson (1992) showed that feeding live yeast culture could reduce incidence of illness and improve feed intake during this period of transition, as well as increasing nitrogen and mineral metabolism.

Other fungi

From the other commercially available fungal groups, Aspergillus spp, A. oryzae, has received the greatest attention. Quite substantial responses to supplementation with live A. oryzae plus the growth medium have been reported, particularly with beef cows and calves on poor pastures. Growth rates of 800g/day have been achieved, compared with 570g/day for untreated animals (Wiedmeier, 1989). It has been suggested that research in dairy cows has tended to dwell too much on milk production with too little consideration of the associated changes in live weight and body composition (Williams and Newbold, 1990). Aspergillus oryzae was reported to have increased the total bacteria by 12% and cellulolytic bacteria by 56% (Wiedmeier et al., 1987) and by 80 and 188%, respectively (Frumholz et al., 1989).

Non-viable cells

Work by Ewing and Cole (1988) with non-viable cells confirmed earlier work by Porter (1986 pers. comm.) that it is possible to obtain growth benefits by feeding
Live yeast

non-viable, non-pathogenic bacterial cells to animals. The growth enhancing effect of probiotics can, in some cases, be improved when the material is killed. Studies have been carried out in young pigs showing an improvement in live weight gain of 15% (Table 7.4) and in young calves, improvements of 10% in live weight gain and food conversion have been observed (Gribben and Hughes, 1989, pers. comm.). Similar effects have also been seen in poultry (Sissins, 1988).

Table 7.4 Effects of a processed bacterial culture in weaned pigs over a 4 week period

<table>
<thead>
<tr>
<th>Performance parameter</th>
<th>Control</th>
<th>Processed culture (2kg/tonne)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily live weight gain (kg)</td>
<td>0.26</td>
<td>0.30</td>
</tr>
<tr>
<td>Relative weight gain (% control)</td>
<td>100</td>
<td>115.4</td>
</tr>
<tr>
<td>Daily feed intake (kg)</td>
<td>0.39</td>
<td>0.40</td>
</tr>
<tr>
<td>Relative daily feed intake (% control)</td>
<td>100</td>
<td>102.6</td>
</tr>
<tr>
<td>Feed/gain ratio (kg feed/kg gain)</td>
<td>1.51</td>
<td>1.32</td>
</tr>
<tr>
<td>Relative feed/gain ratio (% control)</td>
<td>100</td>
<td>87.4</td>
</tr>
</tbody>
</table>

Chromium yeast

Yeasts can be modified for specific purposes, for example chromium yeast, i.e. yeast with high chromium content. This is achieved by ‘feeding’ inorganic mineral sources to the yeast during fermentation culturing. The yeast has the capacity to store minerals in their organic form, bound to protein fractions. It is well established that Cr is an essential nutrient and beneficial effects of its supplementation have been shown in both children and farm animals. Central to its essentiality is its role as a Glucose Tolerance Factor (GTF). The classic work of Schwartz and Mertz (1957) showed that the addition of brewer’s yeast to the diet improved the rate of glucose removal and they named it the Glucose Tolerance Factor. In 1959, the same workers identified the GTF as involving trivalent Cr. Although its structure is not fully understood, it is now generally accepted that in addition to trivalent Cr, nicotinic acid, glutamic acid, cysteine and glycine are involved. The GTF apparently enhances the binding of insulin to cell receptors, thereby stimulating uptake of glucose by the tissues.

There is evidence that a marginal Cr intake is linked to elevated serum lipids in both humans and animals. For example, rats fed a low Cr diet had increased serum cholesterol, aortic lipids and plaque formation. The addition of 1 to 5 mcg Cr/l of drinking water reduced serum cholesterol. Athletes use chromium to build muscle mass and chromium yeast tablets are found in health food stores.
Chromium occurs widely in nature and, as it exceeds $1/3000$th of the earth’s crust, there are only seven more abundant metals. It is a transition element that occurs in a number of oxidation states. The most stable form is the trivalent state which is the one involved in the GTF. Many forms of chromium are particularly inert and unavailable to the animal and in some cases toxic (hexavalent Cr). Natural sources of Cr (such as Cr yeast) are more bio-available than chromic salts, with improvements of up to eight fold. The initial identification of a factor in glucose tolerance was in brewer’s yeast. Yeasts are able to incorporate Cr and provision of the correct fermentation medium results in a high Cr yeast which has been used in human nutrition and is now being used in animal nutrition.

Experiments in Louisiana State University (Page et al., 1991) have shown improvements in carcass quality with the inclusion of 200ppb Cr in the diet - in this case as chromium picolinate, which is used in human nutrition and thought to be chemically similar to part of the GTF in Cr yeast. Not only was lean increased and fat decreased but serum cholesterol was also reduced. The benefits of the added Cr were substantial and the work has given impetus for further research to define more accurately the responses of pigs to dietary Cr.

**Selenium yeast**

The requirements for selenium (Se) are intimately linked with those for vitamin E. Lack of knowledge on Se requirements in all species is further complicated by the relationship between Se content of plant materials in the animal’s diet and Se content of the soil. Selenium yeast may be used in place of the commonly used sodium selenite, because of its claimed greater availability (Figure 7.4) and reduced toxicity (relative to inorganic sources). As this form of mineral is bound to small peptides within the yeast, it is selectively taken up via the same routes at amino acids across the epithelial barrier. Therefore it does not contribute to the interferences and poor uptake seen in inorganic minerals, which carry a charge and have to be transported across the gut epithelium by less preferential routes.

Glutathione peroxidase (GSH-Px) contains 4-Se atoms/mole and is an important antioxidant enzyme in its own right. There is a major review of the role of Se available (Surai, 2006) which details the importance of this mineral in relation to animal and human health. It is certainly also important in controlling the oxidative damage caused by pro-oxidant mycotoxins that, when ingested, may harm gastric tissues.
Figure 7.4 Glutathione peroxidase activity in erythrocytes (a measure of Se absorption) of heifers after dietary supplementation of a selenium deficient diet with different selenium compounds (the total daily amount of selenium fed is given in parentheses) (Pehrson et al., 1989)

In many countries there is a desire to increase human dietary intake through levels in animal products such as meat and milk. The animal itself requires adequate Se for many functions and recent interest has centred on stimulus of the immune system.
Enzymes are used widely in animal feeds to enhance digestion (Table 8.1), and it may be argued that the development of feed enzymes for practical applications was a major step forward in animal nutrition in the late 20th century. Since their development, they have become increasingly commoditised as a feed ingredient, although the range available for compounders and farmers remains wide (Table 8.2). It is now possible to buy single or multi-activity enzymes, depending on the specific need to be addressed.

**Table 8.1 Examples of enzymes used in feed and food**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Action</th>
<th>Typical use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-glucanase</td>
<td>Beta-glucans to oligosaccharides and glucose</td>
<td>Barley-based diets</td>
</tr>
<tr>
<td>Pentosanase</td>
<td>Pentosans to low molecular weight products and glucose</td>
<td>Wheat-based diets</td>
</tr>
<tr>
<td>Amylase</td>
<td>Starch to dextrins and sugars</td>
<td>High cereal diets</td>
</tr>
<tr>
<td>Lipase</td>
<td>Fats to fatty acids</td>
<td>High fat diets</td>
</tr>
<tr>
<td>α-galactosidases</td>
<td>Breakdown oligosaccharides</td>
<td>Soybean meal, grain legumes</td>
</tr>
<tr>
<td>Proteinases</td>
<td>Protein to peptides</td>
<td>Numerous protein materials, e.g. soya and soya products</td>
</tr>
<tr>
<td>Phytase</td>
<td>Increased utilisation of phytate phosphorus</td>
<td>Phosphorus supplied by plant sources</td>
</tr>
</tbody>
</table>

**Enzyme structure, function and applications**

Enzymes come in many forms and activities. In order to understand the best applications for releasing nutrients *in vivo*, a basic comprehension of the different types available is required. The following tables illustrate some of the activities that are available for use in feed and food, and the specific catalysis they facilitate.
Table 8.2 Commercial enzymes used in feed and food processing with their International Union of Biochemistry (IUB) numbers

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Product Name</th>
<th>Description</th>
<th>IUB Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-amylases</td>
<td>Fungal amylase</td>
<td>Fungal alpha-amylase (A.) oryzae var.) for dextrinising and saccharifying starch</td>
<td>3.2.1.1.</td>
</tr>
<tr>
<td></td>
<td>Bacterial amylase</td>
<td>Bacterial alpha-amylase (B.) subtilis var.) for starch liquefaction at temperatures up to 90°C</td>
<td>3.2.1.1.</td>
</tr>
<tr>
<td></td>
<td>High temperature</td>
<td>Thermostable bacterial alpha-amylase (B.) licheniformis var.) for starch liquefaction at temperatures above 90°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>bacterial amylases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-glucanase</td>
<td>Bacterial beta-</td>
<td>Thermostable bacterial beta-glucanase (B.) subtilis var.) for the hydrolysis of cereal beta-glucan polysaccharides</td>
<td>3.2.1.6</td>
</tr>
<tr>
<td></td>
<td>glucanase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catalase</td>
<td>Micro-catalase</td>
<td>A standardised liquid enzyme obtained by the controlled fermentation of Micrococcus lysodeikticus which catalyses the decomposition of hydrogen peroxide to water and molecular oxygen</td>
<td>1.11.1.6</td>
</tr>
<tr>
<td>Cellulases</td>
<td>Cellulase</td>
<td>Fungal cellulose system (A.) niger var.), which is primarily active on soluble forms of cellulose</td>
<td>3.2.1.4</td>
</tr>
<tr>
<td></td>
<td>Cellulase TR</td>
<td>Multi-enzyme system (T.) reesei var.) with endo- and exoglucanase activity</td>
<td>3.2.1.4</td>
</tr>
<tr>
<td>Galactomannase</td>
<td>Hemicellulase</td>
<td>Fungal hemicellulase (A.) niger var.) specific for the hydrolysis of galactomannan gums and soluble cellulose</td>
<td>3.1.1.78</td>
</tr>
<tr>
<td>Glucoamylase</td>
<td>Fungal glucoamylase</td>
<td>Fungal glucoamylase (A.) niger var.), which is capable of hydrolysing both the linear and branched glucosidic linkages of starch and oligosaccharides resulting in essentially quantitative, yields of glucose.</td>
<td>3.2.1.3</td>
</tr>
<tr>
<td>Lipases</td>
<td>Pancreatic lipase</td>
<td>Pancreatic derived lipase that hydrolyses insoluble fats and fatty acid esters to yield monoglycerides diglycerides, glycerol and liberation of free fatty acids</td>
<td>3.1.1.3</td>
</tr>
<tr>
<td>Enzyme</td>
<td>Product Name</td>
<td>Description</td>
<td>IUB Number</td>
</tr>
<tr>
<td>-----------------</td>
<td>------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Lactase</td>
<td>Fungal lactase</td>
<td>Fungal lactase (A. oryzae var.) which hydrolyses lactose, forming glucose and galactose</td>
<td>3.2.1.108</td>
</tr>
<tr>
<td>Pectinase</td>
<td>Pectinase XL</td>
<td>Concentrated fungal pectic enzyme system (A. niger var.) for efficient depolymerisation of naturally occurring pectins</td>
<td>3.2.1.15</td>
</tr>
<tr>
<td></td>
<td>Pectinase AT</td>
<td>Fungal pectic enzyme system (A. niger var.) especially effective in low pH processes such as cranberry juice production</td>
<td>3.2.1.15</td>
</tr>
<tr>
<td></td>
<td>Pectinase ML</td>
<td>Fungal pectic enzyme system (A. niger var. and T. reesei var.) used in fruit maceration of liquefaction processes to maximise fruit juice and solids extraction</td>
<td>3.2.1.15</td>
</tr>
<tr>
<td></td>
<td>Pectinase APXL</td>
<td>Fungal pectic enzyme system (A. niger var.) used for hydrolysis of both soluble and colloidal pectic substances. Also high in arabinase activity for elimination of araban haze.</td>
<td>3.2.1.15</td>
</tr>
<tr>
<td>Proteases</td>
<td>Bacterial protease</td>
<td>Bacterial proteases (B. subtilis var.), which are effectively, hydrolyse proteins over the neutral to alkaline pH range.</td>
<td>3.4.24.28</td>
</tr>
<tr>
<td></td>
<td>Acid fungal protease</td>
<td>Acid fungal protease (A. niger var.) characterised by its ability to hydrolyse proteins under acidic conditions. (pH 2.5-3.5)</td>
<td>3.4.23.26</td>
</tr>
<tr>
<td></td>
<td>Fungal protease (dedusted also available)</td>
<td>Fungal protease (A. oryzae var.) containing endo- and exo- peptides with a broad substrate specificity for catalysing the hydrolysis of proteins</td>
<td>3.4.23.18</td>
</tr>
<tr>
<td></td>
<td>Bromelain</td>
<td>A protein isolated from the pineapple plant which hydrolyses plant and animal proteins to peptides and amino acids</td>
<td>3.4.22.33</td>
</tr>
<tr>
<td></td>
<td>Papain</td>
<td>A protease isolated from the papaya latex. The enzyme extensively hydrolyses proteins and has excellent stability at elevated temperatures.</td>
<td>3.4.22.2</td>
</tr>
<tr>
<td></td>
<td>Papain 6,000</td>
<td>A liquid protease used for hydrolysing proteins</td>
<td>3.4.22.2</td>
</tr>
<tr>
<td></td>
<td>Alkaline protease</td>
<td>Bacterial alkaline proteases, (B. licheniformis var.) for hydrolysing proteins under highly alkaline conditions.</td>
<td>3.4.21.62</td>
</tr>
</tbody>
</table>

(Kindly provided by Valley Research Inc., South Bend, IN, USA)
The IUB (1991) number relates to the specificity of the enzyme. Each number from left to right defines the enzyme specifically. For example, alpha-amylase 3.2.1.1 is translated as follows:

1. The first number shows to which of the six main categories the enzyme belongs.
2. The second figure indicates the subclass.
3. The third figure gives the sub-subclass.
4. The fourth figure is the serial number of the enzyme in its sub-subclass.

It is important when trying to improve the digestibility of the diet that the correct enzymes are chosen for the correct substrates. Consequently enzyme mixtures should be chosen for each particular application and it is necessary to consider the response of the various substrates individually.

The primary use of feed enzymes remains in commercial poultry and pig diets, with the vast majority of poultry diets now supplemented in most countries. In the 1980’s, when feed enzymes first became a commercial reality, their use was confined to barley-based rations, which are particularly difficult to digest. Since that time, research has shown performance and economic benefits for wheat- and maize-soya-based diets, along with efficacy in feeds formulated with by-products.

Feed enzymes are known to influence the bacterial activity and nutrients available within the gut due to their ability to increase digestion, and therefore control the amount of undigested material available for hind gut fermentation and the proliferation of bacterial species. In this capacity, research has shown that the application of feed enzymes can help stabilise and maintain the correct microfloral balance, and potentially rectify problems associated with poor microbial profiles and digestive disorders. This is covered in detail in the final section of this chapter.

The efficient output of quality end products is no longer the sole target of animal production. In recent years there has been considerable emphasis on the reduction of pollution and care of the environment. Drastic remedies have been sought in some countries, for example Singapore, where pigs are banned. In others, for example the Netherlands, legislation places limits on the quantities of materials such as nitrogen and phosphorus that can be applied to the land (Table 8.3). In the latter case, great efforts are being made to control output by livestock. In the first instance this involves the use of much greater precision in the construction of diets, so that high quality diets offering optimum nutrient intakes result in lowered outputs.
### Table 8.3 Permissible levels of phosphate (kg P2O5/ha) on farmland in the Netherlands

<table>
<thead>
<tr>
<th></th>
<th>1990</th>
<th>Up to 1994</th>
<th>After 1994</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arable</td>
<td>125</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>Grass</td>
<td>250</td>
<td>200</td>
<td>175</td>
</tr>
<tr>
<td>Maize</td>
<td>350</td>
<td>250</td>
<td>175</td>
</tr>
</tbody>
</table>

The following chapter details the basics of enzymes in feed, and how they impact on gut structure and microflora and the benefits derived by the host animal.

**Controlling pollution with enzymes**

The pollution problem of excessive phosphorus excretion is well established and serves as a good example of the nutritionists’ attention to the use of exogenous enzymes. Phosphorus has many roles in the body with the result that it is required in large quantities by the animal. It plays an important part in the formation of bones and is involved in muscle and nerve metabolism, energy transformation and acid-base balance of body fluids.

Two important factors are involved in minimising excretory phosphorus from animals, namely, its bio-availability in various feedstuffs and the level of supply. While phosphorus can be derived from plant, animal and inorganic sources, it is the plant ingredients that have the major problem of low availability. This results from phosphorus being in the form of phytate (or phytin), a salt of phytic acid (Figure 8.1). Phytases in plant material have generally been attributed little value. However, Jongbloed and Kemme (1990) demonstrated that wheat phytase, for example, could improve digestibility of phosphorus, by pigs, from 27 to 50%.

![Phytic acid](image)

*Figure 8.1 Phytic acid.*
Simons et al. (1990) has shown an increase in digestibility of phosphorus from 20 to 46% with the addition of microbial phytase to maize/soya diets for pigs. It has been reported that this greater breakdown of phytic acid took place mainly in the stomach (Jongbloed et al., 1992). It has been shown in young pigs (10-30kg live weight) that feed intake and growth rate were increased by the addition of microbial phytase, as well as improving digestibility of phosphorus by 20% (Beers and Koorn, 1990).

Predictions from the Netherlands suggest that large reductions in phosphorus excretion are possible through a better knowledge of requirements, a judicious selection of feed ingredients, and the use of microbial phytases. Diet quality is an important factor in minimising pollution. The needs of efficient production and care of the environment are entirely compatible (Table 8.4).

**Table 8.4 A prediction of the reduction in phosphorus excretion in pigs in the Netherlands**

<table>
<thead>
<tr>
<th>Improvement factor</th>
<th>Intake (kg/animal)</th>
<th>Excretion (kg/animal)</th>
<th>(% 1983 value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1983</td>
<td>1.61</td>
<td>1.18</td>
<td>100</td>
</tr>
<tr>
<td>1990</td>
<td>1.23</td>
<td>0.83</td>
<td>70</td>
</tr>
<tr>
<td>Using Microbial phytase</td>
<td>1.01</td>
<td>0.61</td>
<td>52</td>
</tr>
<tr>
<td>Using alternative feedstuffs</td>
<td>0.89</td>
<td>0.49</td>
<td>42</td>
</tr>
</tbody>
</table>

(As reported by Coppoolse et al., 1990)

Even higher responses have been achieved with poultry. Phytase is commonly used at about 400-1000 units of activity/kg feed, depending on the product. Trials conducted in New Zealand using broiler chickens, showed a linear increase \((P, 0.001)\) in P excretion over the range of 0.2-0.38% used in a complete diet. When a combined enzyme containing phytase was added (pers. comm. Alltech, USA) P excretion was reduced by around 35% in the diet with the lowest P levels.

Regarding other claimed benefits for phytase supplementation (such as amino acid and energy sparing), in a review of all published data at that point, Rosen (2003) reported that the benefits of supplemental phytase in broiler chicken diets depended on whether the basal diet was already deficient in phosphorous. The magnitude of performance response to phytase was found to be influenced by cereal type in the formulation, and the presence of anticoccidial prophylactics.

Excess nitrogenous compounds are also linked to pollution problems with drinking water and algal blooms. Increasing digestibility of protein by using
multi-enzymes has also been shown to reduce these problems (Figure 8.2) (Wu et al., 2001)

![Graph](image)

**Figure 8.2** Effect of enzyme addition on male broiler ileal nitrogen digestibility (Wu et al., 2001)

### Effect of feed enzymes on digestibility and nutrient release in the gut

Enzymatic feed ingredients work on several levels to influence digestion. Some, such as amylase and protease, work directly on specific target substrates to catabolise them into their component parts, which can then be taken up via the gut enterocytes through the gut wall, into the hepatic portal vein. Other, earlier, enzymes focused on the inhibition or breakdown of certain anti-nutritional factors that inhibited digestion. These included the viscous beta-glucans and arabinoxylans. In solution, these compounds form sticky gels that entrap nutrients within the gel matrix, effectively masking them from enzymes secreted in the gut. Dietary enzymes are used in situations when it is considered that the animal is unable to secrete sufficient enzymes from its own tissues, or the feedstuff supplied is poorly digested. They can be used in a variety of circumstances, for example, in pigs they are predominantly used in young animals that have immature digestive enzyme production. However, the fibrous cell walls of many plant feedstuffs are a particular problem for non-ruminant animals.

Anti-nutritional factors, such as non-starch polysaccharides (NSP), reduce the feeding value of many plant materials in the diet of monogastric animals. These animals have an inadequate production of enzymes, which affect hydrolysis and
absorption. They may, of course, subject them to fermentation in the later stages of the gastro-intestinal tract, but absorption is often poor at this point.

NSP vary from simple polysaccharides, such as β-glucans, to more complex arabinoxylans (pentosans). The important NSP in cereals are pentosans and β-glucans while in legumes they tend to be pectic substances (which are rich in uronic acid) and flatulence producing oligosaccharides (Table 8.5).

**Table 8.5 Levels of non-starch polysaccharides present in cereals (Dierick and Decuypere, 1994)**

<table>
<thead>
<tr>
<th>Feedstuff</th>
<th>*β-glucan</th>
<th>#Arabinoxylan</th>
<th>Raffinose</th>
<th>Stachyose</th>
<th>Verbascose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>4.3</td>
<td>6.0</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oats</td>
<td>3.4</td>
<td>6.6</td>
<td>0.5</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Rye</td>
<td>1.9</td>
<td>9.3</td>
<td>0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheat</td>
<td>0.7</td>
<td>6.6</td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triticale</td>
<td>0.7</td>
<td>7.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorghum</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beans (Vicia faba)</td>
<td></td>
<td></td>
<td>0.2</td>
<td>0.7</td>
<td>2.0</td>
</tr>
<tr>
<td>Beans (Phaseolus vulgaris)</td>
<td></td>
<td></td>
<td>0.5</td>
<td>2.6</td>
<td>0.1</td>
</tr>
<tr>
<td>Beans (Glycine max.)</td>
<td></td>
<td></td>
<td>2.3</td>
<td>1.9</td>
<td>0.1</td>
</tr>
<tr>
<td>Peas</td>
<td>0.7</td>
<td></td>
<td>0.7</td>
<td>2.3</td>
<td>2.1</td>
</tr>
<tr>
<td>Sunflower meal</td>
<td></td>
<td></td>
<td>1.9</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Cottonseed meal</td>
<td>3.6</td>
<td>0.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soyabean meal</td>
<td>0.7</td>
<td></td>
<td>0.7</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>Rape seed meal</td>
<td>0.5</td>
<td></td>
<td>0.5</td>
<td>1.4</td>
<td></td>
</tr>
</tbody>
</table>

*(1-3), (1-4) β-glucan (30:70) mainly soluble. # 60 xylose: 40 arabinose

Flatulence-producing oligosaccharides (raffinose, stachyose, verbascose and ajucose) are resistant to digestion and pass to the hindgut, where they are fermented and can produce large amounts of gas. The increase in gas emissions from intensive livestock systems is a concern regarding environmental pollution.

In farm livestock, particular emphasis has been given to improving the feeding value of cereals, which form the majority of the diet and can vary substantially in price. β-glucans are a problem in barley and oats, with pentosans being of particular significance in wheat, triticale and rye (Table 8.6).
Table 8.6 Fibre components of different grains (Cowan et al., 1996)

<table>
<thead>
<tr>
<th>Fibre type (g/kg)</th>
<th>Wheat</th>
<th>Barley</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble beta-glucan</td>
<td>5.2</td>
<td>33</td>
</tr>
<tr>
<td>Insoluble beta-glucan</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Soluble arabinoxylan</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>Insoluble arabinoxylan</td>
<td>54.5</td>
<td>52.1</td>
</tr>
<tr>
<td>Total soluble NSP</td>
<td>23.2</td>
<td>49</td>
</tr>
<tr>
<td>Total Insoluble NSP</td>
<td>65.5</td>
<td>66.1</td>
</tr>
<tr>
<td>Total NSP</td>
<td>88.7</td>
<td>115.1</td>
</tr>
</tbody>
</table>

Perhaps the two major characteristics of ß-glucan in barley are reduced feeding value and increased viscosity of the excreta which can be a big problem with poultry (e.g. sticky droppings).

Benefits of feed enzyme supplementation are derived through an increase in nutrient and fibre digestibility. The actual performance response to enzyme use may be mediated indirectly through subsequent changes in nutrient availability for the resident microbial populations. Recently, the understanding of how enzymes function has been advanced by the realisation that there may be two components to its activity – an ileal phase and a caecal phase. The ileal phase being governed by the effects on nutrient digestibility and the caecal phase focused on the response to the end products of feed enzyme activity e.g. volatile fatty acid production from undigested fibre material fermented by microorganisms.

Research has shown that problems with digestion and utilisation of nutrients occur not only between different species of grain, but are also influenced by harvest year (i.e. environmental and management conditions) and the cultivar or specific strain of that crop. These are all directly linked to performance of the animal (Table 8.7).

Table 8.7 Effect of wheat variety and harvest year on 7-21 d broiler performance (Waldron et al., 1993)

<table>
<thead>
<tr>
<th>Variety</th>
<th>Year</th>
<th>Weight Gain (kg)</th>
<th>FCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0.382b</td>
<td>1.708ab</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>0.405ab</td>
<td>1.693ab</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>0.349c</td>
<td>1.729a</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0.437a</td>
<td>1.577b</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0.427a</td>
<td>1.672b</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>0.404b</td>
<td>1.694ab</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>0.0136</td>
<td>0.0203</td>
</tr>
</tbody>
</table>
Soluble fibre derived from non-starch polysaccharides (NSP) have been shown to have a direct impact on digestibility and performance of broilers (Annison and Choc, 1994, Figure 8.3).

![Figure 8.3 Starch, protein and fat digestibility in broiler chickens fed increasing levels of isolated cereal arabinoxylans (Annison and Choc, 1994)](image)

There is a good relationship between intestinal viscosity and reduced feeding value in both pigs and poultry. The use of β-glucanase to improve the feeding value of barley is well established. In general it is used to bring the feeding value of barley up to that of other cereals (Pugh, 1972) (Table 8.8).

**Table 8.8 The use of β-glucanase to improve the feeding value of barley for broiler chickens (Pugh, 1972)**

<table>
<thead>
<tr>
<th>Performance parameter</th>
<th>Wheat</th>
<th>25% Barley + β-glucanase</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of birds</td>
<td>45000</td>
<td>45000</td>
</tr>
<tr>
<td>Age (days)</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td>Live weight (kg)</td>
<td>2.073</td>
<td>2.082</td>
</tr>
<tr>
<td>Food conversion ratio</td>
<td>2.10</td>
<td>2.09</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>7.7</td>
<td>7.3</td>
</tr>
</tbody>
</table>

The addition of pentosanase to improve the feeding value of rye for pigs has been well demonstrated. Improvements of 7% and 8% have been reported for growth rate and feed utilisation respectively (Thacker, 1988). Such differences have been associated with improvements in ileal digestibility of dry matter, nitrogen and NDF of 3.1, 4.2 and 7.2%, respectively (Buraczewska, 1988).
From a commercial point of view, wheat is a much more important feedstuff. There is an extensive published data resource detailing the benefits of using feed enzymes on diets formulated with all major cereals and legumes available to feed formulators. In a review of the productive performances achieved by using enzymes in monogastric diets, Rosen (2001, 2002, 2003) found that positive responses in trials were observed around 70% of the time, a similar level to other nutricines and antibiotics. These publications also describe the relationships between trial factors and the outcomes in terms of animal responses.

**Influence of enzyme supplementation on gut microflora**

When digestion is compromised due to poor digestion, more starch and protein is able to reach the lower gut, allowing the microflora to proliferate. Moreover, if there is a change in the type of substrate available such as higher starch and protein relative to fermentable fibre levels, not only will population density increase, but species balance and dominance can change (Wagner and Thomas, 1987; Vahjen et al., 1998). Pathogens, such as *Clostridia perfringens*, respond particularly to higher levels of nitrogen. High viscosity diets or those that are poorly digested have been reported to elicit such reactions (Vahjen et al., 1998). The animal will respond to such changes in gut environment via several mechanisms, including increasing digestive enzyme output (Angkanaporn et al., 1994) and pancreatic weight (Brenes et al., 1993). The intestines may enlarge (Brenes et al., 1993) in response to increased competition with micro-organisms for nutrients, which occurs due to recognition of bacterial polyamines that stimulate mucosal growth and gut enterocyte turnover (Deloyer et al., 1996; Noack et al., 1996; Seidel et al., 1985). This is an attempt to compensate for the reduction in the rate of nutrient absorption by increasing its digestive capacity. Villi enterocytes of the intestine grow and move up the villus more rapidly. Paradoxically these enterocytes are more immature and are less able to absorb nutrients efficiently due to a limited range and concentration of digestive and absorptive enzymes. In addition the surface glycoproteins in immature cells vary markedly from more mature cells. As a result, a totally new environment is presented to the intestinal bacteria, and a rapid change in species distribution takes place, which can lead to intestinal disorders.

Formulating with poorly digested protein meals have been shown to reduce feed efficiency (Table 8.9).

Trials have shown that enzymes can successfully improve the digestibility of nutrients from cereal sources. Table 8.10 shows the typical improvement in energy, protein and amino acid apparent digestibility in pig and poultry formulations.
Different commercial enzymes can vary in their ability to improve digestion, although data should be readily available for registered products.

**Table 8.9** Effect on performance when feeding increasing levels of sunflower meal (without enzyme supplementation) to 1-42 d broiler chickens (Musharaf, 1991)

<table>
<thead>
<tr>
<th>Sunflower (g/kg)</th>
<th>Weight gain (kg)</th>
<th>FCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.043b</td>
<td>1.80a</td>
</tr>
<tr>
<td>50</td>
<td>2.043b</td>
<td>1.84ab</td>
</tr>
<tr>
<td>100</td>
<td>2.070ab</td>
<td>1.86b</td>
</tr>
<tr>
<td>150</td>
<td>2.086ab</td>
<td>1.83ab</td>
</tr>
<tr>
<td>200</td>
<td>2.108a</td>
<td>1.83ab</td>
</tr>
<tr>
<td>250</td>
<td>2.102ab</td>
<td>1.87b</td>
</tr>
<tr>
<td>SEM</td>
<td>11.0</td>
<td>0.005</td>
</tr>
</tbody>
</table>

**Table 8.10** Effect of feed enzymes on digestibility of nutrients in poultry diets (Alltech, pers. comm.) and pig feeds (Partridge, pers. comm.)

<table>
<thead>
<tr>
<th>Poultry</th>
<th>ME (kcal/kg)</th>
<th>Protein (%)</th>
<th>Lysine (%)</th>
<th>Met+Cys (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2900</td>
<td>14.1</td>
<td>0.37</td>
<td>0.51</td>
</tr>
<tr>
<td>Enzyme</td>
<td>3075</td>
<td>14.9</td>
<td>0.39</td>
<td>0.54</td>
</tr>
<tr>
<td>Change %</td>
<td>6%</td>
<td>6%</td>
<td>5%</td>
<td>6%</td>
</tr>
<tr>
<td>Pigs</td>
<td>Ileal DE (%)</td>
<td>Protein (%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>69.8</td>
<td>72.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Enzyme</td>
<td>73.6</td>
<td>76.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Change %</td>
<td>5%</td>
<td>5.5%</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Improved nutrient and energy availability has an impact on growth and feed utilisation efficiency, especially in the ileum. Coon et al. (1998 pers. comm.) reported that feeding enzymes in a corn-based diet significantly increased ileal starch and energy digestibility, whereas no differences were found at the faecal level. These findings suggested that the bacteria probably fermented undigested starch, resulting in a higher ME level (although this energy may have been utilised by the bacteria rather than the host bird).

How much of the response to enzyme utilisation is due to improved nutrient utilisation and how much to reductions in microflora population is difficult to determine. It is impossible to separate the two effects because the
Table 8.11 Effect of addition of maize-targeted enzyme complex on ileal and faecal starch digestibility in broilers fed diets of varying protein content (Coon et al., 1998, pers. comm.)

<table>
<thead>
<tr>
<th>Digestibility</th>
<th>Control</th>
<th>Enzyme*</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ileal digestion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch (%)</td>
<td>75.4a</td>
<td>85.6b</td>
<td>+13.5</td>
</tr>
<tr>
<td>ME (kcal/kg)</td>
<td>2725a</td>
<td>2890b</td>
<td>+6</td>
</tr>
<tr>
<td><strong>Faecal digestion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch (%)</td>
<td>93.2a</td>
<td>88.5b</td>
<td>-5</td>
</tr>
<tr>
<td>ME (kcal/kg)</td>
<td>2810</td>
<td>2830</td>
<td>+0.7</td>
</tr>
</tbody>
</table>

Means not sharing a letter differ significantly (p<0.05)

Consequence of supplying more substrate to ileal flora and encouraging population growth often results in a subsequent reduction in digestive efficiency due to bacterial interaction with bile salts and digestive enzymes. Recent evidence suggests that the consequences of reduced rate of digestion are much more radical in the presence of an intestinal microflora than in the absence. For example, poor digestion of a maize-soya diet, due to the inclusion of a viscous pectin or β-glucan analogue in the ration of a bird with a developed microflora, depressed broiler performance (Schutte and Langhout, 1999; Smits and Annison, 1996). When a similar ration was fed to gnotobiotic (microflora-free) chickens, no such depression in digestibility or performance was recorded. These observations suggest that the negative effects of reduced diet digestibility were only significant if there is a resident microfloral population.

The negative potential of gut microflora on bird performance has been reported in work by Muramatsu et al. (1994) (Figure 8.4). Gnotobiotic birds offered the same diet grew substantially quicker, but captured less energy from the diet than their conventional counterparts with normally populated hindguts. The discrepancy in energy captured was ascribed to the fact that hindgut microflora remove a significant amount of energy from the diet, making it unavailable to the bird. These data suggested that the “energy cost” of the microflora was at least 10% of the total AME in this experiment, in which a semi-synthetic ration was used. The use of a more fermentable diet with higher fibre levels from cereals could be assumed to result in greater microbial activity, and hence higher microbial energy requirement.

In order for a particular microbial species to populate the hindgut, it needs a supply of nutrients, physical caecal space and an environment where there is a favourable balance between its substrate requirements and waste products.
Figure 8.4 Influence of microbial status of the intestinal tract on AME and body weight of leghorn chicks fed semi-synthetic diets (Muramatsu et al., 1994)

from other species in its proximity. The nutrients present in the intestines are derived from the dietary intake. Therefore diet, especially early feeding exposure, affects microflora development and bacterial species mix in the hindgut of monogastrics. Diets based on barley and/or rye (high viscosity cereal grains) are known to result in much larger anaerobic populations in the ileum (Hofshagen and Kaldhusdal, 1992; Wagner and Thomas, 1987) compared with corn- or rice-based (low viscous cereals) diets. As a result, such diets high in soluble non-starch polysaccharides are commonly seen to respond particularly well to inclusion of feed additives such as enzymes (Hofshagen and Kaldhusdal, 1992; Vranjes and Wenk, 1997).

Unlike ruminant nutrition, monogastric nutrition often neglects the fact that the diet is a source of nutrients for the microflora as much as it is for the target animal. Poultry, in particular, have the ability to process feed more rapidly than other farm animals. Due to the very low pH of the gizzard/proventriculus, feed entering the duodenum has been significantly depopulated of bacteria. The concentration of digestive enzymes, high oxygen tension and the presence of antimicrobial compounds, such as bile salts in the duodenum, further limits bacterial growth in this region of the gut. Further along the small intestine (ileum), the environment changes and becomes more favourable for bacterial growth due to a reduction of these factors (partly due to re-absorption and partly due to microbial de-conjugation of bile salts). Under conditions of optimal digestion, the high rate of nutrient digestion and absorption from the intestine ensures that there are few nutrients that can pass to the gut microflora for fermentation. Microbial populations in the ileum are kept to a minimum through limitations in nutrient availability in this region. However it is still important
to support the growth of commensal microflora populations in the gut, and this can be encouraged by increasing the flow of fermentable fibre, a nutrient source only gut bacteria can utilise.

Research from Australia (Pluske et al., 1996; Hampson et al., 1997) has illustrated the provocative nature of enzyme-sensitive dietary fibre (measured as both soluble and insoluble non-starch polysaccharides) on swine dysentery, a specific disease condition affecting the gut and associated with the bacterium *Serpulina hyodysenteriae* (Figure 8.5).

![Figure 8.5](image)

**Figure 8.5** The effect of soluble and total fibre level in different grains on the incidence of swine dysentery in pigs challenged with *Serpulina hyodysenteriae* (Pluske et al., 1996)

Hampson and Pluske’s work demonstrated that, as seen in chickens, different feed grains exacerbated the situation in *Serpulina*-challenged pigs, with soluble fibre in wheat, barley and oats forming the main provocative factor in these ‘viscous’ cereals. Maize and sorghum were also provocative, but this appeared to be more related to their ‘resistant’ starch content, which escapes ileal digestion and is available for microflora fermentation. Where diets were based on grains containing rapidly digestible starch and low levels of soluble non-starch polysaccharides, the incidence of the disease in challenged animals was significantly reduced or virtually non-existent (e.g. cooked white rice, steam flaked sorghum, steam flaked corn). These results demonstrated that increased digestibility of fibre (both soluble and insoluble) and starch can have a beneficial influence on the microbial population of the gut in challenged animals.

Effective feed enzymes have been shown to improve not only nutrient digestibility at the terminal small intestine but also increase digesta flow. A faster flow rate combined with more effective absorption of nutrients at the gut surface, due to enzymatic reduction in digesta viscosity and water-holding capacity indirectly
improves feed intake and reduces microbial proliferation in the gut. Current pig and poultry research suggests that certain types of fibre in the diet can be beneficial when it is fermented in the lower intestine. Data has shown that fermenting NSP-type carbohydrates in the large intestine produces volatile fatty acids, especially butyrate. Butyrate has been shown to be an essential energy source for the growth of cells forming the epithelial layer of the colon and promotes the development of the finger-like villi on the gut wall, improving absorptive capacity. Research has also shown that volatile fatty acids can act against diarrhoea by altering water absorption from the intestine and may inhibit the growth of intestinal bacteria by end product inhibition of fermentation pathways. Interestingly, feed enzymes appear to be compatible with increased VFA production in the hindgut in both pigs and poultry. Similar findings have been reported in studies by Apajalahti and Bedford (1998), who reported rises in total VFA production (mainly propionic acid) in the caeca of broilers offered xylanase supplemented wheat-based diets.

In the absence of an intestinal microflora, the adaptive mechanisms of the bird (increased pancreatic enzyme output, gut length and intestinal retention time) help to correct for the digestive inefficiencies imposed by the addition, for example, of viscous pectin. This is often at a nutrient or energy cost for the bird, so applying a suitable feed enzyme can be valuable in these cases. In a trial using layer pullets fed flaxseed in a maize-based diet, significant reduction in villus length and increased distances between villi at the top were observed, compared to the control. This change suggested that the pullet adapted its gut physiology to counteract the high viscosity levels (Scheideler et al., 1998). Feeding an enzyme product significantly increased faecal fat digestion and ME level in pullets at 9 weeks of age compared to the flaxseed fed control group. By 16 weeks of age the control group appeared to have adapted to the viscous environment because no differences were found between treatments (Scheideler et al., 1998). The adjustment to the viscous diet by the pullet might have included changes in the microflora of the caecal area, although this was not studied.

Data regarding actual effects on microbial populations due to feed enzyme supplementation is increasingly available. Much of this data, for enzymes and other nutricines, has been generated in response to the increasing legal limitations on the use of antibiotic growth promoters (AGP) to control digestive disorders and pathogenic bacteria in the gut. AGPs remove the bulk of the flora by interrupting their ability to replicate or destroying them directly by disruption of the outer cell wall. Both the direct antibiotic mechanism and the indirect enzyme mechanism are effective in reducing total ileal floral populations as shown in Figure 8.6 (Bedford, pers. comm.).
Figure 8.6 Influence of antibiotic growth promoter compared to feed enzymes on ileal populations of coliforms, lactic acid bacteria, and enterococci in 3-week-old broilers fed wheat-based diets

Whilst the AGP was more effective than the enzyme, as would be expected, since it targets the bacteria directly, the enzyme also shows a major reduction in bacterial numbers, relative to the control. Such changes are more pronounced when the quality of the base cereal used in the diet is low (Classen et al., 1995). The degree to which feeding an enzyme will result in altered microbial populations is related to the relative improvement in digestibility (Choc't et al., 1996; Hillman, 1999; Morita et al., 1998; Schutte and Langhout, 1999; Smits et al., 1998). This has been observed in barley, wheat and maize-based diets. Trials elucidating the different modes of action of enzymes versus AGPs have shown that the use of a single commercial enzyme preparation alone led to an average 5.9% improvement in FCR while growth promoters gave a 3.3% advantage (Tucker, pers. comm.). The combination of the two gave the greatest response, indicating that the two products do not substitute for one another, but work in tandem.

In the process of breaking down viscous β-glucans and arabinoxylans, and partially degrading cell wall arabinoxylans in cereal grain, enzymes produce small oligomers and free sugars as end products (Figure 8.7). (Apajalahti and Bedford, 1998). Many of these products are poorly absorbed, if at all. These sugars instead provide a fermentation source for certain bacterial species and, on entering into the caeca, stimulate their growth to varying degrees (Hartemink et al., 1996; Imaizumi et al., 1991; Jaskari et al., 1998).
Choc et al. (1996), (Figure 8.8) have reported this effect in greater detail. They fed birds either a sorghum-based control diet, the control diet supplemented with 3% viscous wheat arabinoxylan or the latter diet supplemented with a xylanase. Feeding the viscous arabinoxylan diet markedly depressed growth and feed efficiency and increased volatile fatty acid production in the ileum (indicating increased ileal microbial activity). No effect was noted at the caecal level. Addition of a xylanase restored performance of the birds and reduced ileal VFA concentrations to control levels (indicating reductions in ileal microflora populations). Most interesting was a significant three-fold increase in caecal volatile fatty acid concentration on xylanase addition.

Figure 8.7 Concentration of short-chain xylo-oligomers (chain length 500 and below) in the ileum of broilers fed wheat-based diets +/- xylanase (Apajalahti and Bedford, 1998)

Figure 8.8 Influence of diet on jejunal and caeca contents of volatile fatty acids in broiler chicks fed NSP and xylanase-based enzyme (Choc et al., 1996)
When examined in detail, the individual VFA’s were all increased in the presence of the xylanase-based feed enzyme (Figure 8.9).

![Figure 8.9](image)

**Figure 8.9** The effects of xylanase enzymes on individual and total VFA production in the caeca of 21 d broiler chickens (reported in Partridge and Tucker, 2000)

The most likely source of the volatile fatty acids was the fermentation of xylose and xylo-oligomers by caecal bacteria. Detailed analysis of the profiles of VFAs resulting from the fermentation of xylose has revealed that the molar ratios were altered in favour of increased propionic acid which is thought to reduce caecal carriage of *Salmonella* spp (Hume *et al.*, 1996; Kwon *et al.*, 1998). Furthermore, published work has indicated that adding xylanases to wheat based diets in broilers challenged with *Salmonella* spp. and *Campylobacter* spp. at 10 days of age results in a lower number of these bacteria found at 14 days of age (Fernandez *et al.*, 2000). It was suggested that the enzyme was providing sugars for fermentation by bacterial species that naturally compete with *Salmonella* spp and *Campylobacter* spp., in a similar mode of action as competitive exclusion products.

It is evident that feed enzymes impact the gut on several levels. First of all, they provide a means to increase nutrient availability through improved digestion. This increases gastric development and limits the over-production of endogenous enzymes and other secretions, which diverts nutrients and energy away from growth and maintenance. This reduces the amount of undigested nutrients flowing to the lower gut, reducing the proliferation and potential imbalances in resident microflora. The breakdown products of certain soluble fibres are associated with increased VFA production, which can improve gut development and also provide an extra energy source for the host animal and its resident microbes. Enzymatic processing of certain feed compounds has been shown to reduce the chances of developing gastric disorders, especially in immature animals with poorly developed digestive tracts.
Man has known about the ability of microbes such as lactic acid bacteria to ferment substrates for over one hundred years. They were known to cause fermentation and coagulation of milk. Weigmann (1899) produced the first definition of the lactic acid bacteria as those which produce milk acid (lactic acid) from milk sugar (lactose).

**Lactobacilli**

Lactobacilli are one of the most dominant groups of bacteria found in parts of the gastrointestinal tract. Classically, the cells are large, and appear as pairs or short chains. They are non-motile, non-sporulating and non-capsulate, grow over a wide temperature range (15-45°C), but have an optimum of 37°C. They prefer acidic conditions (pH 5.8). They have been shown to increase in number from birth, whereas other bacteria decline (Fuller, 1989). Common *Lactobacilli* in the intestinal flora include *L. acidophilus*, *L. bifidus*, *L. leichmannii*, *L. plantarum*, *L. casei* and *L. fermentum*.

Lactobacilli are known to produce bacteriocins (antibiotic-like compounds) that can attack other bacteria. Shahani and Ayebo (1980) isolated two of these: acidophilin from *Lactobacillus acidophilus* and bulgarican from *Lactobacillus bulgaricus*.

Lactobacilli have an ability that most micro-organisms lack, *i.e.* to utilise lactose. This ability is shared with a number of other intestinal bacteria such as *E. coli*. Lactose is produced only by the mammary gland of mammals and is secreted in milk which is ingested with the utilisation of lactose by micro-organisms. Lactose is a disaccharide that must be hydrolysed before it can enter the catabolic pathway for hexoses.

\[
\text{β-galactosidase} \\
\text{Lactose} \xrightarrow{\text{H}_2\text{O}} \text{D- glucose + D- galactose}
\]

The galactose is then phosphorylated and converted to glucose phosphate.
The original subdivision of the genuine lactic acid bacteria was into six genera; Betacoccus, Streptococcus, Tetracoccus, Betabacterium, Streptobacterium and Thermobacterium (Davis, 1960). The genus names Betacoccus and Tetracoccus have been replaced by Leuconostoc and Pediococcus. These same classifications are recognised today (Bergey, 1986). A further classification has resulted in two subdivisions depending on whether they are able to ferment glucose solely to lactate or to other products as well, i.e. homo-fermentative or hetero-fermentative. (Table 9.1).

**Table 9.1 Lactic acid bacteria classification depending on type of fermentation**

<table>
<thead>
<tr>
<th>HOMO-FERMENTATIVE $C_6H_{12}O_6$ $\rightarrow$ 2$CH_3-CHOH-COOH$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus cremoris</td>
</tr>
<tr>
<td>Streptococcus diacetilactis</td>
</tr>
<tr>
<td>Streptococcus faecalis</td>
</tr>
<tr>
<td>Streptococcus lactis</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
</tr>
<tr>
<td>Streptococcus salivarius</td>
</tr>
<tr>
<td>Streptococcus thermophilus</td>
</tr>
<tr>
<td>Pediococcus cerevisiae</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HETERO-FERMENTATIVE: $C_6H_{12}O_6$ $\rightarrow$ $CH_3-CHOH-COOH + CH_3CH_2OH + CH_3COOH$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leuconostoc mesenteroides</td>
</tr>
<tr>
<td>Leuconostoc cremoris</td>
</tr>
<tr>
<td>Lactobacillus brevis</td>
</tr>
</tbody>
</table>

**Homo-fermentative**

Homo-fermentative lactic acid bacteria metabolise glucose via the fructose-bisphosphate pathway (Figure 9.1), having all the necessary enzymes, including aldolase, and being able to use the hydrogen obtained from the dehydrogenation of glyceraldehydes-3-phosphate (to 1, 3-bisphosphoglycerate) to reduce pyruvate to lactate.

In this case, lactate, which is the product of glucose fermentation, can account for up to 90% of the end products. The extent to which other products occur, depends on oxygen supply.
**Figure 9.1** The Embden-Meyerhoff Parnas or Glycolysis (homo-fermentative) pathway which yields 2 moles ATP per mole of hexose fermented.

**Hetero-fermentative**

In the case of these bacteria, fifty per cent of the end products of glucose metabolism is lactic acid. In addition, large amounts of CO₂ (20-25%), acetic acid and ethanol are also produced. Mannitol is obtained from fructose in this sub-group and other determinative points include:
The Living Gut

- Gas produced during fermentation of glucose and gluconate.
- Fermentation of ribose to lactic acid without gas production.
- Thiamine is required for growth.
- Glucose-6-phosphate dehydrogenase activity is shown.

Examples of lactic acid bacteria in this group are:

i. *L. fermentum*. Growth at 45°C but none at 15°C.
ii. *L. cellobiosus*. Variable growth at 45°C and at 15°C, but no growth at 48°C.
iii. *L. viridescens*. Growth at 15°C, but none at 45°C.

The species classification can be confirmed by other complex methods, apart from temperature of growth, which include the chemical nature of the cell wall, cell wall membrane, antigenic determination, serological grouping (Sharp, 1970), vitamin requirement (Rogosa et al., 1961), amino acid sequences of the peptidoglycan and DNA composition (Gasser and Mandel, 1968) and DNA homology (Dellaglio et al., 1973, 1975; Simonds et al., 1971).

The hetero-fermentative lactic acid bacteria lack the important enzymes of the fructose-bisphosphate pathway; aldolase and triose-phosphate isomerase and details of the pathway are given in Figure 9.2.

Hetero-fermentative bacteria can convert the acetylphosphate, either partially or completely, to acetate gaining utilisable energy as ATP. They ferment fructose with the formation of lactate, acetate, carbon dioxide and mannitol:

\[
3 \text{fructose} \rightarrow \text{lactate} + \text{acetate} + \text{CO}_2 + 2 \text{mannitol}
\]

The fructose can also accept excess reducing equivalents e.g.

\[
\text{fructose} + \text{NADH} + \text{H}^+ \rightarrow \text{mannitol} + \text{NAD}^+
\]

**Bifidobacteria**

The hetero-fermentative lactic acid bacterium called *Bifidobacterium bifidum* takes its name from the Y shape of its cells (Latin *bifidus* means ‘divided into two’). It is dominant in the intestinal tract of babies, especially those that are breast fed (Hall et al., 1990; Benno and Mitsuoka, 1986). This specificity of their distribution to breastfed babies can be traced to the high requirement of this bacterium for sugars that contain N-acetylglucosamine, which is found only in human milk and not in cows milk. The members of the *Bifidobacterium* are strict anaerobes.
Figure 9.2 The pentose phosphoketolase (hetero-fermentative) pathway which yields 1 mole ATP per mole of hexose fermented.

**Lactic acid bacteria in agricultural and food production**

If non-sterile solutions containing sugars, complex nitrogen sources and accessory factors are left under anaerobic conditions they will soon become overgrown with lactic acid bacteria. These lower the pH to below 5 and suppress the growth of other anaerobic bacteria, which are less acid tolerant. The actual types of lactic acid bacteria that become dominant in these cultures depends on specific conditions. This sterilising and preserving effect of the lactic acid bacteria, due to their acid production, has led to their use for silage-making in agriculture and in food processing to manufacture yoghurt and other milk-utilising products.

**Silage production**

Silage is an important cattle feed, generally made from grass or other forage crops which are high in moisture. However, other materials, such as fish, can also
be preserved in this way. The objective in all silage manufacture is to produce sufficient acid to prevent the activities of spoilage organisms such as *Clostridia* spp. If, however, the activity of *Clostridia* is not prevented then butyric acid is produced, protein is broken down and feeding value is reduced. The pH required will depend on the conditions, but normally pH 4 is satisfactory. Consequently efforts are made to aid the processes of silage making. Various aids are used such as molasses, enzymes, acids and bacterial inoculants (see Table 9.2).

### Table 9.2 Current methods used to manipulate silage fermentation and preservation

<table>
<thead>
<tr>
<th>Application</th>
<th>Objective</th>
<th>Problems/concerns</th>
<th>Potential benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molasses</td>
<td>Supply simple sugars to feed lactic acid bacteria and thereby reduce pH due to lactic acid produced</td>
<td>Difficulty of application. Can stimulate less efficient hetero-fermenters</td>
<td>Cost effective. Provides residual energy and protein as well as improved fermentation</td>
</tr>
<tr>
<td>Acids</td>
<td>Lower pH of ensiled material to stabilise/ preserve silage</td>
<td>Can be corrosive and lock up natural minerals</td>
<td>No use of crop nutrients in unnecessary fermentation</td>
</tr>
<tr>
<td>Enzymes</td>
<td>Convert fibrous carbohydrates to sugars and allow naturally present lactic acid bacteria to lower pH</td>
<td>Activity needs careful controlling. Can be relatively expensive compared with other additives</td>
<td>Can work on higher dry matter and more fibrous crops. Natural crop sugars are not essential to feed lactic acid bacteria</td>
</tr>
<tr>
<td>Bacterial inoculants</td>
<td>Produce maximum amount of lactic acid from available soluble carbohydrates (sugars). Hence pH is reduced and conditions produce a stable silage</td>
<td>Viability and strain benefits need controlling. Adequate numbers must be supplied</td>
<td>Homolactic fermentation improves efficiency of lactic acid production and fermentation</td>
</tr>
</tbody>
</table>

The high moisture and physical compaction of forages exclude air and prevent spoilage by microbes which thrive in air. Acid fermentation occurs, in which the lactic acid bacteria present in the ensiled material ferment the sugars to lactic and other acids.

It has long been recognised that bacteria are involved in silage making, although it was originally thought that yeasts were of major significance. It is now known that yeasts play only a minor part in fermentation but are one of the main causes of loss during aerobic deterioration.

The main organisms playing a significant role in the ensilage process have been described as (Woolford, 1984):
- The lactic acid-producing bacteria *Lactobacilli, Streptococci, Leuconostoc* and *Pediococcus spp.*
- The endospore forming bacteria, *Clostridia* (generally associated with spoilage) and *Bacilli*.
- Fungi (yeasts and filamentous fungi) which are associated with spoilage
- Other groups such as propionic acid bacteria (which may increase with the greater production of lactic acid) and *Listeriae* are occasionally found.

**Microbial changes during ensilage**

In normal silage making there are two distinct stages of microbial development.

- Lactic acid bacterial development (always takes place)
- Clostridial development (may or may not take place)

The latter may or may not occur, depending on the conditions prevailing, e.g. dry matter and sugar content.

Providing anaerobic conditions have been established after harvesting, there will be a shift in the microbial population from the aerobes of the fresh crop to facultative and obligate anaerobes of the silage. This is usually associated with a change from Gram-negative to Gram-positive organisms (Woolford, 1984).

Coliform bacteria multiply until about the seventh day after harvesting (Beck, 1972) and then decrease. At the same time they are replaced by lactic acid cocci including *Streptococci, Leuconostoc* and *Pediococci spp.*, and finally by the higher yielding acid producing *Lactobacilli spp.* It is essential to control coliforms in the first seven days if quality is to be maintained.

Of the early appearing lactic acid bacteria, *Pediococci spp* and *Lactobacilli spp* dominate in the fermented silage. Total numbers of viable organisms following ensilage are usually in the order of $10^9$ to $10^{10}$ per gram fresh weight, with the majority being lactic acid bacteria. This dominance is due to their powers of survival (tolerance to acid) rather than their ability to grow more rapidly than other species (Gibson and Stirling, 1959). *Pediococci spp*, tend to be more acid tolerant and consequently maintain higher numbers than some other strains which are acid limited, such as *Streptococcus lactis*. Also, the lactic acid bacteria may produce substances which are inhibitory to other native grass microorganisms. As the type of fermentation exhibited by the lactic acid bacteria changes, (within 4 days of ensilage) there is a move from homo-fermentative to
hetero-fermentative fermentation again possibly due to the change in pH and increase in acetic acid.

Figure 9.3 shows how the pH of the silage drops progressively with time until a stable crop of pH4 is produced (about day 10). The figure also indicates the pH optima of different organisms showing which would dominate at a given pH. It also shows how, when *Clostridia* are present (from soil contamination), the pH can rise, making the silage more suitable for coliforms. This rise in pH can be associated with an increase in proteolytic enzymes, which break down protein to ammonia and free amides.

*Figure 9.3* Qualitative changes which take place in the silage micro-flora during fermentation. ---- preferred pH of silage organism (after Woolford, 1984)

Figure 9.4 illustrates the overall changes in nutrients from ensiling a fresh crop to stabilisation of a preserved crop. There are many different fermentation pathways involved within this general summary. For example, the fermentation of glucose is affected by whether the organisms are homolactic or heterolactic fermenters. Homolactic fermenters produce twice as much lactic acid and are therefore more efficient in producing conditions to preserve the crop (Figure 9.5).

*Clostridia* spp. although often associated with the later stages of ensilage may also multiply in the first few days of ensilage. After this, their viable count decreases (Gibson *et al.*, 1958). If, however, lactic acid bacteria ferment silage carbohydrates to butyric acid (causing spoilage), these conditions are conducive to the multiplication of proteolytic *Clostridia*. 
Figure 9.4 The effect on crop nutrients of ensiling of organic matter.
The process can, therefore, be effective in the preservation of the ensiled crop, or, under adverse conditions, produce poorly stabilised silage of low feed value, from which valuable nutrients have been lost due to poor fermentation. Lactic acid bacteria play a critical role in this process (especially homolactic fermenters) and are consequently widely used in many commercial silage additive products.

Silage inoculants

The theory of commercial inoculants centres around grass being a relatively poor source of lactic acid bacteria, especially those which are efficient converters of silage dry matter to fermentation acids (homo-fermentative). After ensilage, their numbers can be low allowing the coliforms or Enterobacteriaceae to dominate, producing acetic acid and ammonia.

In order to overcome this it is claimed that the correct bacteria should be present, i.e. homo-fermentative organisms which convert natural sugars to lactic acid. The beneficial organisms used include Streptococci, Pediococci, Lactobacilli spp. A range of organisms is often used to provide organisms with different pH tolerances (See Figure 9.3). The stabilisation sequence would start with
organisms such as *Streptococcus* being dominant. Finally *Lactobacilli* spp., which have a lower pH tolerance, take over. The addition of organisms leads to an increase in the total numbers of lactic acid bacteria in the crop (Figure 9.6) and allows a faster rate of lactic acid production (Figure 9.7). This enables the silage to reach peak lactic acid levels in fewer days, ensuring fewer nutrients are lost, and limiting the chances of challenge by spoilage organisms.

**Figure 9.6** Lactic acid bacteria levels in control and inoculant treated silage in the first seven days of ensilage. Thomas and Slater (1986).

**Figure 9.7** Lactic acid bacteria levels in control and inoculant treated silage in the first seven days of ensilage Thomas and Slater (1986).
Fermented milk products

Man has theorised for years about the many beneficial effects of yoghurt in the human diet. It has been claimed to increase life-span, and on a day-to-day basis maintain the balance of bacteria and “settle” the environment within the digestive tract. Yoghurt has its origins stretching back to before Egyptian times when it was known as “Benraib”. It was made by allowing the sugars in milk to ferment, using its natural bacteria. These bacteria were mainly lactic acid bacteria and they converted the lactose of milk to lactic acid. Late in the sixteenth century, Francis I of France was reported to have enjoyed the benefits of yoghurt. A persistent intestinal disorder could not be cured, but under advice from the Sultan of Ottoman Empire, yoghurt was prescribed and the problem disappeared.

Other fermentations of lactobacilli are equally important in the production of bread and beer.

In 1908, Metchnikoff won the Nobel prize for his work on yoghurt at the Pasteur Institute in Paris. He concluded that longevity was a result of maintaining a healthy intestine. This was accomplished by eating the correct diet. His research subjects were the Balkan mountain people and people of the middle eastern countries who relied on yoghurt containing live bacteria for a large part of their diet.

Lactose is a simple carbohydrate present in milk and provides the energy necessary for the fermentation of milk. Lactic acid is a product of its fermentation and may be responsible for some of the beneficial effects of yoghurt. Other beneficial effects of yoghurt appear to be related to the functions of the live bacteria in the digestive tract. It is claimed, unlike most other ingested bacteria, that those in yoghurt can survive digestion in the stomach and small intestine, where they produce their effects on the native intestinal micro-flora.

The bacteria used in yoghurt are not major inhabitants of the intestinal flora of humans, but help to maintain the balance of this endogenous flora. Work on babies has shown that those fed live yoghurt had a significantly lower susceptibility to intestinal infections than babies fed on cow’s milk (Larue, 1960; Mayer, 1962). Later work by Niv (1963) even found yoghurt to reduce diarrhoea in children more quickly than antibiotics, but antibiotics were still more effective at removing persistent long term problems. Bifidobacteria have been implicated as being important in the digestive process by maintaining a healthy balance of both good and bad bacteria (Lubis, 1983).

Manufacture of yoghurt

There are six main factors necessary for the correct fermentation of milk by bacteria:
Probiotics/live bacterial supplementation

- Specific and selected live bacteria must be added.
- The temperature of the milk must be correct for fermentation (45°C).
- The bacteria must replicate by growing on the lactose present in the milk for 2-3 hours.
- No foreign organisms must be present. The milk is therefore pasteurised to destroy any harmful pathogens.
- As in other commercial fermentations, light agitation must be applied frequently.
- When fermented the medium must be chilled to prevent further secondary fermentation (4°C).

There are normally two types of bacteria used in the manufacture of yoghurt.
- *Streptococcus thermophilus*.
- *Lactobacillus bulgaricus*.

Commercially, various forms of milk and cream are inoculated with cultures of lactic acid producing bacteria, which act as ‘starters’ for fermentation. Examples of such products are given in Table 9.3. Again these products are a way of storing materials through the production of lactic acid, which lowers pH and suppresses other anaerobic, but harmful bacteria. Sour milk products are also used in cases of milk allergies and lactose intolerance.

**Table 9.3 Examples of sour milk products**

<table>
<thead>
<tr>
<th>Product</th>
<th>Culture</th>
<th>Temperature and time of incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sour cream and buttermilk</td>
<td><em>Streptococcus lactis, S. cremoris, Leuconostoc cremoris</em> or <em>S. diacetilactis</em></td>
<td>22°C, 18h</td>
</tr>
<tr>
<td>Yoghurt</td>
<td><em>Streptococcus thermophilus, Lactobacillus bulgaricus</em></td>
<td>43-45°C, 2.5-3h</td>
</tr>
<tr>
<td>Cottage cheese curd</td>
<td><em>Streptococcus lactis, S. cremoris, Leuconostoc cremoris</em></td>
<td>22°C, 18h or 35°C, 5h</td>
</tr>
<tr>
<td>Kefir</td>
<td><em>Streptococcus, Lactobacillus</em> and yeast</td>
<td>15-22°C, 24-36h</td>
</tr>
<tr>
<td>Kumiss (made from donkey milk)</td>
<td><em>Lactobacillus bulgaricus</em> and Torula yeast</td>
<td>15-22°C, 24-36h</td>
</tr>
</tbody>
</table>
Yoghurt has become particularly important commercially with the development, not only of many flavours but also of many variations, e.g. ‘low fat’, ‘creamy’, and ‘set’. The pasteurised, homogenised whole milk is inoculated with *Streptococcus thermophilus* and *Lactobacillus bulgaricus* to make yoghurt. An interesting feature is that there is a synergistic effect between these two organisms (Figure 9.8). On the one hand *L. bulgaricus* produces free amino acids, particularly histidine, which encourages the growth of *S. thermophilus* and this, in turn, produces formic acid-like compounds and CO₂ which stimulate the growth of *L. bulgaricus* (Tamine and Deeth, 1980).

![Diagram](image)

**Figure 9.8** The lactic acid producing effects of *S. thermophilus* and *L. bulgaricus* when fed singly or in combination (based on Tamine and Robinson, 1985).

The beneficial effect of fermented milk products in the diet to treat gastro-intestinal disorders is brought about by modifying the gut flora to the detriment of the harmful bacteria.

It is known that *L. bulgaricus* is able to survive through the gastro-intestinal tract of infants and also to inhibit some *Enterobacteriaceae*. When added to human milk in the diet of infants aged between one and four months, it has been claimed to improve weight gains. In the adult, a large increase in the numbers of *Lactobacilli* spp. recovered in the faeces after yoghurt had been included in the diet for one or two weeks has been reported. Three weeks after yoghurt was removed from the diet the faecal flora had returned to its pre-treatment state. Initially there was a ten-fold reduction in coliform count, but after a further week, it returned to the pre-treatment level. An interesting feature of this work was that although there was a large increase in *Lactobacillus* spp. count, they were not the same strains as contained in the yoghurt (Waites and Tibble, pers. comm.). It was suggested that the reasons for such a phenomenon may include a failure of *L. bulgaricus* to colonise and survive the large intestine, and also that it was a poor competitor with the indigenous species. Even the feeding
Probiotics/live bacterial supplementation

of unfermented milk containing \( L. \text{ acidophilus} \) to humans has resulted in a significant increase in \( Lactobacilli \) in the faeces.

**Specific probiotic products for animal feed and human food**

The knowledge now surrounding the application of direct-fed microbial products for both man and animals has spawned a major industry for promoting gastric health and disease control in agricultural and companion animals, and for maintaining correct digestive function in humans. In order to derive any benefit from their addition it is essential that the viability of the preparation is maintained during storage and application to feeds or food, particularly if undergoing any form of processing. Another approach is to use bacterial spores as these are more resistant to high temperature and high moisture conditions associated with pelleting, than live cultures. However they must germinate in the small intestine if they are to be of benefit.

The characteristics which need to be fulfilled by an organism in order to be useful as a “live” probiotic/direct-fed microbial include:

- **Not harmful to the animal**  The organisms should not cause disease nor should they be toxic (Fuller 1989).

- **Acid and bile resistant** If a live micro-organism is to survive passage through the stomach and reach the intestine alive it must be acid and bile resistant. However, it is likely that they may be protected by the ingested food. Micro-encapsulation, for example, using \( \beta \)-glucans, has been used as a means of increasing the ability of the organism to survive.

- **Ability to colonise the gut** Only specific bacterial strains are able to effectively adhere to the intestinal epithelium, which is necessary if their mode of action is competitive exclusion. For example, it has been suggested in the pig, that only bacteria isolated from domestic pigs and the closely related wild boar are able to adhere to the squamous epithelial cells of the stomach (Barrow \textit{et al.}, 1980). Thus it is important to select bacteria common to the gastro-intestinal tract of the host animal (Shahani and Ayebo, 1980; Gilliland, 1987). They will need to possess the appropriate colonisation or attachment factors (Fuller 1989).

- **Ability to inhibit pathogen activity** The probiotic should be able to show its inhibitive powers towards pathogenic bacteria in the laboratory, if live microbial activity is required. The organisms chosen should produce acid or other materials so aiding in the inhibition of Gram-negative pathogenic micro-organisms, such as \( E.\text{coli} \) (Tramer, 1966; Sandine, 1979).
- **Stable and viable under manufacturing conditions** Organisms in “live” probiotic products; must be able to withstand freezing and the high temperatures of processing to be a viable product.

- **Stable and viable under storage conditions** If an anaerobic bacterium is chosen, it must be kept in anaerobic conditions until it is fed to the animal, if it is to survive (unless it is a spore former). Strictly speaking, anaerobic bacteria cannot be used in feed if viability is to be guaranteed and is of importance. The micro-organisms would also need to be kept at very low temperatures to avoid sporulating and/or death.

Bacteria that only remain viable for short periods of time are unsuitable for commercial application as live probiotics. Facultative organisms such as *L. acidophilus* are not harmed by the presence of oxygen and, thus, are often selected for this reason.

**Dosage**

The minimum effective dose of live bacteria is not easily identified (Fuller 1989). In trials about 1g/kg feed is commonly used. Commercial probiotic products come in various forms, such as pellets, powder, capsules, paste and granules. All of the product is not, therefore, active biomass, making it difficult to assess true dose level. One typical live probiotic was stated to consist of $1 \times 10^8$ million CFU’s/g *L. acidophilus* and $70 \times 10^7$ CFU’s/g of *S. faecium* compared with the populations of gut micro-flora supposedly in the region of $1 \times 10^{14}$ micro-organisms. Figure 9.9 illustrates the perceived benefits of feeding live non-pathogenic micro-organisms to the new-born animals.

![Figure 9.9](image)

**Figure 9.9** Theory of gut protection. When bacterial additives are fed to newborn animals with an incomplete natural flora, a stable population forms and the host animal is protected from the effects of pathogenic organisms.
Types of direct-fed microbial organisms

Many organisms have been used commercially to produce direct-fed microbials (Table 9.4), the most common include:

**Lactobacilli**

Lactobacilli are commonly used in probiotics as they are known to be non-pathogenic and are also natural inhabitants of the gastrointestinal tract with many beneficial effects. It is also generally assumed that they are responsible for the health benefits associated with yoghurt.

**Bacillus**

This is a spore-forming organism and therefore more stable than Lactobacilli and Streptococci; viability should, in theory, be less of a problem. It has been claimed that Bacillus subtilis could reduce the number of E. coli in faeces, the digestive tract and blood (Pollman, 1986). Fuller (1989) has stated that B. subtilis is not an intestinal organism, but is a strict aerobe and he questioned its use as a live probiotic. However, it is established that pools of oxygen may be present in the gut of monogastric animals, such as the young pig.

**Streptococci**

Streptococci were originally classified as part of the Lactobacillaceae family, due to their common physiological traits. However, it is now a member of the Streptococcaceae family (Deibel and Seeley, 1974) and they are often called enterococci. They are Gram positive, facultatively anaerobic, non-motile cocci, spherical or ovoid, occurring in pairs or chains. They have complex nutritional requirements and have a fermentative metabolism (homo-fermentative), producing D-lactic acid from glucose (Deibel and Seeley, 1974). They are located in the mouth, and in the entire intestinal tract. They are very tolerant to a range of temperatures, bile, NaCl and low pH (Frobisher et al., 1974). Streptococci are lactic acid bacteria which have been claimed to have a role in maintaining a balanced micro-flora and for this reason have been selected for their probiotic potential. However, less attention has been given to Streptococci spp. than Lactobacilli spp., probably because some species of Streptococcus can be pathogenic. Streptococci are found attached to the squamous epithelium but in lower numbers than Lactobacilli spp. This is probably due to their greater sensitivity to pH and pepsin (Barrow et al., 1980).
### Table 9.4 Some common organisms used as direct-fed microbials.

<table>
<thead>
<tr>
<th>Organism classification</th>
<th>Genus</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
<td></td>
<td></td>
<td>bifidus</td>
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<tr>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>bulgaricus</td>
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<td></td>
<td>casei</td>
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<td></td>
<td></td>
<td>cellobose</td>
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<tr>
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<td></td>
<td>curvatus</td>
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<td></td>
<td>delbruecki</td>
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<tr>
<td></td>
<td></td>
<td>fermentum</td>
</tr>
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<td></td>
<td></td>
<td>lactis</td>
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<td></td>
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<td>salvaricus</td>
</tr>
<tr>
<td></td>
<td>Bacillus</td>
<td>cereus</td>
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<td></td>
<td>licheniformis</td>
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<tr>
<td></td>
<td></td>
<td>pumilus</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>toyoi</td>
</tr>
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<td>Bacteroides</td>
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<td></td>
<td>capillosis</td>
</tr>
<tr>
<td></td>
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<td>rumicola</td>
</tr>
<tr>
<td></td>
<td></td>
<td>suis</td>
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<tr>
<td></td>
<td>Bifidobacterium</td>
<td>adolescentis</td>
</tr>
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<td></td>
<td>animalis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>bifidum</td>
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<td></td>
<td></td>
<td>infantus</td>
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<tr>
<td></td>
<td></td>
<td>longum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>thermophilum</td>
</tr>
<tr>
<td></td>
<td>Streptococcus</td>
<td>cremorius</td>
</tr>
<tr>
<td></td>
<td></td>
<td>diacetilactis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>faecium</td>
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<td></td>
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<td></td>
<td></td>
<td>lactis</td>
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<tr>
<td></td>
<td></td>
<td>thermophilis</td>
</tr>
<tr>
<td></td>
<td>Pediococcus</td>
<td>acidilacticii</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cerevisiae</td>
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<td></td>
<td></td>
<td>faecium</td>
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<tr>
<td></td>
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<td></td>
<td>pentosaceus</td>
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<td></td>
<td></td>
<td>thermophilus</td>
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<td></td>
<td></td>
<td>mesenteroides</td>
</tr>
<tr>
<td>FUNGI</td>
<td>Leuconostoc</td>
<td>cerevisiae</td>
</tr>
<tr>
<td></td>
<td>Saccharomyces</td>
<td>boulardii</td>
</tr>
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<td></td>
<td></td>
<td>candida</td>
</tr>
<tr>
<td></td>
<td>Torulopsis</td>
<td>niger</td>
</tr>
<tr>
<td></td>
<td>Aspergillus</td>
<td>oryzae</td>
</tr>
</tbody>
</table>
Fungi

Fungi have also been used to manipulate the gut environment. As far as farm livestock are concerned this has been largely, but not exclusively in ruminants. For this application *Saccharomyces cervisiae* and *Aspergillus* spp. have received most attention although there is current interest in the use of *Saccharomyces boulardii* in the diets of monogastric animals. The former have been used to alter microbial digestion in the rumen and so enhance productivity and are listed under ‘live yeasts’ in this book.

Beneficial effects and possible modes of action

A number of suggestions have been put forward to explain how beneficial microorganisms might help promote health and combat the proliferation of pathogenic species of bacteria, and at present it is unclear which of these might be the most important. It is possible that several factors together might be involved.

Competitive exclusion

This theory has received considerable attention. It proposes that some microorganisms could prevent the colonisation of the gastrointestinal tract by other micro-organisms including pathogens. In its simplest form it can be seen as the beneficial bacteria occupying adhesion sites in the gut that would otherwise be populated by harmful bacteria. The complex interaction of good and bad micro-organisms protecting the animal has been termed ‘bacterial antagonism’ (Freter, 1956), as well as the increasingly common term ‘competitive exclusion’ (Lloyd *et al*., 1977). The precise mechanism controlling the principle of gut colonisation is not well known but involves specific recognition of receptor sites (oligosaccharides) by bacterial fimbriae (lectins). *Lactobacilli* that colonise the stomach and associate with the non-secreting epithelium have been considered to be important in preventing populations of coliform bacteria (*e.g.* *E. coli*) and its pathogenic and non-pathogenic relatives (Adler and DaMassa, 1980) from rising to high numbers. They are normal inhabitants of the large intestine and it is when they become prolific in the small intestine that problems occur for example, in pigs (Barrow *et al*., 1977).

In poultry, significant protection against *Salmonella* spp. has also been found by administration of intestinal micro-flora from selected donor birds (Luckey 1963; Snoeyenbos *et al*., 1978; Vincent *et al*., 1955; Weinack *et al*., 1979). In competitive exclusion, *Lactobacilli* attach to the wall of the crop and compete with *E. coli* (Fuller and Brooker, 1974), *Salmonella* and other pathogens. These observations add support to the hypothesis suggested earlier by several investigators that
‘competitive exclusion’ helps prevent attachment of the pathogens to the intestinal mucosa. It has also been reported that newly hatched birds treated with the protective micro-flora, had partial protection within a few hours, and reached full potential in about 32 hours (Soerjadi et al., 1981).

Good responses have also been seen in pigs and rodents (Muralidhara et al., 1977; Savage, 1969; Shahani and Ayego, 1980).

Competitive exclusion (Figure 9.10) has been reported to stop gut colonisation by *Salmonella* spp. when the contamination level is very high. Salmonella excretion levels of chicks treated with probiotics have been shown to be at least two logs lower than untreated chicks (Weinack et al., 1979). Furthermore, chicks which came into contact with *Salmonella* following treatment with normal gut micro-flora had reduced colonization by pathogens and toxin production was not seen (Soerjadi et al., 1981). Meynell (1963) and Bohnhoff et al.,(1964) reported that volatile fatty acids were produced in the caeca of mice by bacteria and linked the VFA produced with the protection against *Salmonella*. A molecular mechanism for attachment and invasion of epithelial cells by *Salmonella* has been reported by Finlay et al. (1989). Other mechanisms include competition for nutrients, reducing the redox potential and non-specific activation of the immune system (Hill et al., 1986).

**Figure 9.10** A) represents a mixed population of bacteria with a substantial component of pathogenic bacteria. B) shows competitive exclusion of pathogens due to preferential attachment of non-pathogens.
Competitive exclusion can take place throughout the digestive tract as non-pathogenic organisms such as lactic acid-producing bacteria are always present in the food.

It should be noted that the recognition of receptor sites (oligosaccharides) by the bacterial fimbriae (lectins) is very specific for different types of organisms. The lactic acid-producing bacteria flow from the stomach into the small intestine where their lactic acid production may mediate, at least in part, the inhibition of the development of pathogenic Gram-negative bacteria. *L. acidophilus* has been shown to be effective in adhering to the intestinal epithelium of the pig (Conway et al., 1987), and it is agreed (Jones and Rutter 1972; Linton and Hinton, 1988) that *E. coli* colonisation is necessary for its pathogenic activity. This is further supported by observations of reduced numbers of *Lactobacilli* and an increase in *E. coli* colonising the intestine walls in scouring pigs compared with their healthy counterparts (Muralidhara et al., 1973). Intestinal material has been used to provide bacteria to block salmonella from attachment sites (Pivnick and Nurmi, 1982; Mead and Impey, 1984). Large scale field trials looking at competitive exclusion have also reportedly produced good results, (Mead and Impey, 1987).

It has also been reported that *Lactobacilli* can successfully dominate other bacteria in the competition for nutrients in the gut and therefore survive to colonise the intestine (Muralidhara et al., 1977; Roach et al., 1977) (Figure 9.11).

![Figure 9.11](image_url) Non-pathogenic bacteria and pathogenic bacteria often compete for nutrients, e.g. carbon, nitrogen and minerals. Non-pathogenic bacteria can compete successfully and so colonise the intestine to a greater extent.
Competitive exclusion could also occur as a result of aggregation of non-pathogens to pathogens, preventing binding to attachment sites and leading to their removal from the gut (Figure 9.12).

**Figure 9.12** Aggregation or grouping of non-pathogens to pathogens in areas of quick digesta flow may allow their removal from the gut.

It has been suggested that the protective flora may be host specific (Snoeyenbos *et al.*, 1979) but there is some degree of uncertainty about this process as some trials have shown that gut micro-flora from both chickens and turkeys were reciprocally protective. However, it has been claimed that the turkey was less well protected than the chicken by competitive exclusion (Weinack *et al.*, 1981). Differences in specificity between these species may be present, with Mead and Impey (1984) finding no protection of turkeys from a partially defined flora that protected chickens.

**Reduction in toxic amine production**

Metabolic activity of the intestinal micro-flora produces amines and ammonia which may have deleterious effects on the host animal. For example, amines produced after weaning have been associated with diarrhoea (Porter and Kenworthy, 1969). They are irritating and toxic, and increase intestinal
peristalsis, which may account for the diarrhoea (Muralidhara et al., 1977). It has been shown that the level of amines produced within the gut can be reduced by Lactobacilli such as L. acidophilus (Schaedler and Dubos, 1962; Porter and Kenworthy, 1969; Hill et al., 1970) and this may be important in maintaining a high health status. It is also claimed that they can detoxify pathogenic toxins, for example L. bulgaricus was shown to neutralise E. coli enterotoxin (Mitchell and Kenworthy, 1976).

Bile salts, produced within the liver, are surface-active chemicals which aid digestion by forming polymolecular aggregates with water-insoluble lipids and fat-soluble vitamins (Sandine, 1979). Bile is important in blocking the passage of many live organisms into the lower intestine and has been shown to inhibit the growth of enteric anaerobic bacteria, the mechanism of inhibition have been described by many authors, e.g. Floch et al. (1970). Specific strains of Lactobacilli can release free bile acids into the intestinal tract, and could, as a consequence of this, influence the balance of bacteria present within the gut (Sandine, 1979). Unconjugated (free) bile acids are much more inhibitory than conjugated forms (Floch et al., 1972).

Antibiotic production

There have been several reports of antibiotic production by lactic acid producing species of Streptococcus and Lactobacillus (Hirsch and Grinsted, 1951; Hirsch and Wheater, 1951; Mattick and Hirsch, 1944; Oxford, 1944; Su, 1948; Vincent et al., 1959; Whitehead, 1933; Wheater et al., 1952). In a few instances (Berridge, 1949; Oxford, 1944; Whitehead, 1933), it was suggested that these antibiotics may have been polypeptides. Numerous antibacterial substances have been identified as products of lactic acid bacteria (Table 9.5).

Table 9.5 Examples of antibacterial substances produced by lactic acid bacteria.

<table>
<thead>
<tr>
<th>Antibacterial substance</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nisin and diplococcin</td>
<td>Mattick and Hirsh (1944); Oxford (1944)</td>
</tr>
<tr>
<td>Acidophilin</td>
<td>Vakil and Shahani (1969)</td>
</tr>
<tr>
<td>Lactocidin</td>
<td>Vincent et al. (1959) Shahani et al. (1976, 1977)</td>
</tr>
<tr>
<td>Acidolin</td>
<td>Hamdan and Mikolajcik (1973)</td>
</tr>
<tr>
<td>Lactolin</td>
<td>Kodama (1952)</td>
</tr>
<tr>
<td>Bulgarican</td>
<td>Shahani et al. (1977)</td>
</tr>
</tbody>
</table>
The presence of anti-enterotoxic activity

Toxins produced by pathogens at times of disease can bind to epithelial receptors preventing colonisation of bacteria (Figure 9.13). The enterotoxin, produced by a number of *E. coli* strains, that causes fluids to be lost from the intestine, can be neutralised by live probiotic type bacteria. This is the case with lactic acid bacteria (Mitchell and Kenworthy, 1976; Fuller and Cole, 1988). There is also anti-enterotoxic activity in *L. bulgaricus* and *S. faecium*, although it is not common (Mitchell and Kenworthy, 1976).

![Figure 9.13](image)

**Figure 9.13** Endotoxins produced by pathogens at times of disease prevent colonisation by bacteria.

The role of bacteria in digestion of protein

In monogastric animals, microbial fermentation takes place mainly, but not exclusively, in the large intestine. The extent to which absorption takes place in the large intestine is open to some debate. Once ammonia has been absorbed, it cannot be used and is excreted (Just, 1983). Although a simple approach of assuming that digestion is by hydrolysis before the ileo-caecal junction and by fermentation in the large intestine is often used, this is not entirely the case. For example, up to 20% of fermentation in pigs can take place ahead of the ileum with the advantage of enhanced absorption.

Bacteria are involved in the digestion of endogenous compounds. Where no bacteria are present, in germ-free animals, it has been shown that endogenous protein can collect in the caecum causing expansion of this organ (Luckey, 1963).
Bacteria can also break down compounds produced by the gastro-intestinal tract but which are not hydrolysed by enzymes in the stomach and small intestine. These products can include urea (Delluva et al., 1968; Levensen et al., 1959; Okumura et al., 1976), bacterial mucoproteins, mucosal residues, mucus and uric acid (Mason, 1980). Bacteria inhabiting the caecum have also been shown to be capable of catabolising all L-amino acids in vitro (Fauconneau and Michel, 1970).

Breakdown of protein leads mainly to the production of ammonia but small quantities of amide, indole and phenolic compounds, keto acids and carbon dioxide have been shown to be produced in the pig (Just, 1983). A beneficial side-effect is that bacterial action helps circulation of urea which may in turn help the production of non-essential amino acids.

**Increased absorption and enzyme activity**

The gastro-intestinal micro-flora is well known to influence the levels of absorptive enzymes in the microvillus membranes (brush borders) of intestinal epithelial cells in laboratory rodents. Although the mechanism by which it achieves this is unknown, it has been shown that gastric *Lactobacilli* can alter the levels of enzymes in germ-free mice. This suggests that *Lactobacilli* may affect the absorption of nutrients by the animals, as the microvillus enzymes are known to mediate such absorption (Savage, 1983). Bacteria are more likely to be involved in nitrogen metabolism when an amino acid or protein is in short supply (March, 1979).

Additional dietary *Lactobacilli* have been claimed to affect the metabolism of the hosts, micro-flora (Fuller and Cole, 1988; Gilliland and Speck, 1977), due to the effect of certain *Lactobacilli* on enzyme activity. It has been shown that *Lactobacilli* can increase or decrease enzyme activity, providing beneficial effects to the host. Galactosidase is an enzyme of bacterial origin which is required by mammals to break down lactose (milk sugar). The feeding of yoghurt (containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus*), has been shown to increase the concentration of this enzyme in the small intestine (Fuller, 1989).

**Production of hydrogen peroxide**

Under certain circumstances, some lactic acid-producing bacteria form detectable amounts of hydrogen peroxide (Price and Lee, 1970; Sandine, 1972; Speck, Calloway and Hadley, 1970). This will inhibit the growth of many bacteria, especially pathogenic Gram-negative types. It can also be involved in the
activation of the lactoperoxidase-thiocyanate system in the gut. In this system, lactoperoxidase combines with hydrogen peroxide and then oxidises thiocyanate to an intermediary oxidation product. This substance can inhibit bacterial growth, and may be bacteriocidal at low pH.

**Other effects of Lactobacilli**

*Lactobacilli* and other non-pathogenic bacteria have also been claimed to be involved in:

- Vitamin production (Carlstedt-Duke *et al.*, 1987; Coates *et al.*, 1963; Shahani and Ayebo, 1980).
- Lowering gut pH (Torrey and Kahn, 1923) and improving gastric homeostasis.
- The production of D-leucine (amino acid with inhibitory properties) (Gilliland and Speck, 1968).
- Production of other beneficial unidentified by-products (Hamdan and Mikolajcik, 1974).
- Deoxy-D-glucose production which has inhibitory properties (Muralidhara, 1974).
- Production of natural flavours and aromas to enhance food palatability (Schindler and Schmid, 1982) and possibly appetite stimulation.
- Production of beneficial nucleotides (Eyssen *et al.*, 1965).
- Anti-microbial effects (McCormick and Savage, 1983).
- Anti-cholesteremic effects (Gilliland *et al.*, 1985).
- Anti-tumour effects (Friend and Shahani, 1984).

The responses to lactic acid producing bacteria sought in man are likely to relate to better health of the digestive tract while in domestic livestock, improved productive performance should also result. There is more quantitative evidence in the case of the latter due to the nature of the experimentation which is conducted. However, the use of fermented milk products by man is an age-old
custom. Figure 9.14 summarises the main effects of non-pathogenic bacteria with the gut of farm animals.

![Diagram]

**Figure 9.14** The effects of non-pathogenic bacteria in the gastrointestinal tract of farm animals.

**Animal responses to probiotics supplementation**

In recent years, interest has grown in the feeding of micro-organisms, particularly *Lactobacilli*, as an alternative to the use of antibiotics and also following antibiotic therapy. Antibiotic therapy often lowers the *Lactobacilli* population in the intestinal tract and it is claimed that replenishing the intestinal tract with, for example, *L. acidophilus* results in accelerated return to a beneficial intestinal population. Modern day farming often stops the young animal from obtaining its normal bacterial loading. It has been suggested that the most satisfactory method to overcome this is to feed bacteria. As stated before, the best results are claimed to be obtained from the ingestion of $1 \times 10^8$ to $1 \times 10^9$ viable *L. acidophilus* daily, but ingestion of excessive numbers may induce diarrhoea. Relative to humans, there is considerable information on farm animals, with further results appearing constantly. Improved gut health will result in more efficient digestibility (Table 9.6).
Table 9.6 The influence of the inclusion in the diet of a mixture of *Lactobacillus acidophilus*, *Streptococcus faecium* and *Saccharomyces cerevisae* in pig diets on ileal digestibility (%).

<table>
<thead>
<tr>
<th>Feed ingredient</th>
<th>Digestibility (%)</th>
<th>Control</th>
<th>Treated</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>Protein</td>
<td>78.9</td>
<td>80.6</td>
<td>2.1</td>
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<tr>
<td></td>
<td>Lysine</td>
<td>84.2</td>
<td>89.9</td>
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<td></td>
<td>Methionine</td>
<td>93.9</td>
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<td></td>
<td>Threonine</td>
<td>77.5</td>
<td>75.3</td>
<td>10.1</td>
</tr>
<tr>
<td>Wheat</td>
<td>Protein</td>
<td>80.7</td>
<td>86.2</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>Lysine</td>
<td>63.5</td>
<td>82.1</td>
<td>29.3</td>
</tr>
<tr>
<td></td>
<td>Methionine</td>
<td>72.7</td>
<td>83.1</td>
<td>14.3</td>
</tr>
<tr>
<td></td>
<td>Threonine</td>
<td>74.5</td>
<td>80.2</td>
<td>7.6</td>
</tr>
<tr>
<td>Soyabean meal (48% crude protein)</td>
<td>Protein</td>
<td>77.2</td>
<td>85.0</td>
<td>10.1</td>
</tr>
<tr>
<td></td>
<td>Lysine</td>
<td>84.2</td>
<td>88.3</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>Methionine</td>
<td>75.1</td>
<td>88.4</td>
<td>17.7</td>
</tr>
<tr>
<td></td>
<td>Threonine</td>
<td>79.36</td>
<td>85.3</td>
<td>7.6</td>
</tr>
</tbody>
</table>

*(Tossenberger, quoted by Gombos, 1991)*

When considering the various classes of farm livestock, it is possible to highlight particular occasions when probiotics are most likely to have a positive effect (Ewing and Haresign, 1989) (Table 9.7).

**Challenge**

It has been suggested that, in the case of bacterial feed additives and probiotic-like materials (and other additives generally), “response is dependent on challenge” (Cole, 1991). In other words, the greater the problem, the greater the response. For example, in a herd where weaner pigs had shown responses to organic acids in the drinking water, inconclusive responses were obtained when haemolytic *E. coli* populations had been reduced and diarrhoea problems diminished (Cole *et al.*, 1970).

In an attempt to rationalise the differences in response relating to the extent of the problem, data from a number of trials have been collected (Figure 9.15). Suckling piglets of sows fed probiotics based on lactic acid bacterial in the diet had lower mortality than those which did not.

The degree of response to a probiotic is markedly influenced by the level of performance already existing in the herd. It is proposed that little or no response can be expected when pig mortality is below about 10% (Cole, 1991). Presumably below 10% piglet mortality problems were not entirely due to enteric factors. It is worth noting that the trials analysed all used *Lactobacillus* spp. Each point represents an experiment. The slope of response above 10% mortality: \( Y = 0.553 \times X - 4.63 \).
Table 9.7 Times at which bacterial additives may be most effective

<table>
<thead>
<tr>
<th>Animals</th>
<th>Times and Reasons</th>
</tr>
</thead>
</table>
| Calves | - After birth – to encourage the early establishment of beneficial micro-flora.  
          - Change to bucket feeding  
          - Before and after transportation  
          - At weaning  
          - Following over eating or antibiotic administration |
| Adult Cattle; to restore the desirable microbial balance and appetite following: | - Ketosis  
          - Antibiotic treatment for therapeutic reasons  
          - Bloat  
          - Difficult calvings |
| Lambs | - Weak or poorly mothered lambs should be treated as soon as possible after birth to encourage the early establishment of beneficial micro-flora  
          - During changeover to milk  
          - If digestive upsets occur  
          - Tailing and castration  
          - Weaning onto concentrate feeds at young ages |
| Adult sheep: to restore the microbial balance and appetite following: | - Twin lamb disease  
          - Difficult lambings  
          - Antibiotic treatment for therapeutic reasons |
| Piglets | - To prevent scouring in young piglets often associated with such stress as teeth clipping/iron injection, castration, weaning and transportation  
          - Following antibiotic treatment for therapeutic reasons |
| Older pigs: to restore the gut microbial balance and appetite following: | - Antibiotic treatment for therapeutic reasons  
          - Farrowing, particularly after a difficult farrowing  
          - Transportation/mixing |

(Ewing and Haresign, 1989)

In this case, only the sow’s food contained probiotic as the piglets were not offered supplementary or creep feed for their nutrients. The object was to make the sow’s faeces less harmful by reducing the populations of harmful *E. coli* etc. as suckling pigs are known to eat considerable quantities of faeces and bedding. Thus, it is postulated that the probiotics were having a beneficial effect on the micro-flora of the sow’s digestive tract and consequently the faeces, which would cause less enteric problems to the piglets.
Probiotics have been particularly effective in young animals, for example rabbits (Hollister et al., 1991), chickens (Wiseman, 1990) calves (Rosell, 1987) and pigs (Cole, 1991). Presumably the developing digestive tract is vulnerable to microbial stimulation and any move to improved gut health is reflected in better general health, together with enhanced animal performance, with particularly beneficial responses in terms of reduced mortality.

As far as the pig is concerned, perhaps one of the most essential times to maintain gut health is in the period immediately after weaning. This is well known as a period in which diarrhoea, growth checks and mortality can be a problem. Certainly one of the predisposing factors is the change from milk to a solid diet and its associated changes in the digestive system. At the same time, of course, there are dramatic changes in the enzyme system. In creating weaner diets, nutritionists generally aim to include some dairy product e.g. dried whey. Such materials contain lactose and there is evidence that the combination of a probiotic based on lactic acid bacteria, together with a lactose source, presents a more powerful way of controlling haemolytic *E. coli* than either given alone. Furthermore, a combination of lactic acid bacteria, organic acids and a source of lactose has been shown to be particularly effective in the control of haemolytic *E. coli*.

In broiler chickens research into the usefulness of commercial probiotics has been conducted since the 1980’s, however they have so far not attained the
same degree of commercial success compared to other nutricines for this species such as enzymes and oligosaccharides. This may be due to the high levels of processing temperatures associated with feed production, especially where salmonella requires controlling. Trials with growing broilers have shown performance benefits (Table 9.8) (Priyankarage et al., 2003) in comparison with antibiotic growth promoters, however this was only significant for broilers up to 21 days old.

**Table 9.8 Comparison of probiotics for young broiler chickens (Priyankarage et al., 2003)**

<table>
<thead>
<tr>
<th>Parameter (21 days)</th>
<th>Control</th>
<th>Antibiotic (zinc bacitracin)</th>
<th>Probiotic&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Probiotic&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Probiotic&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain (g)</td>
<td>524a</td>
<td>561b</td>
<td>548ab</td>
<td>565b</td>
<td>552b</td>
</tr>
<tr>
<td>FCR</td>
<td>1.736ab</td>
<td>1.695a</td>
<td>1.744b</td>
<td>1.710ab</td>
<td>1.70ab</td>
</tr>
</tbody>
</table>

<sup>1</sup> *Lactobacillus, Streptococcus and Bifidobacterium* spp.

<sup>2</sup> *Lactobacillus acidophilus, Saccharomyces cerevisiae* and *Streptococcus faecium*

<sup>3</sup> *Saccharomyces cerevisiae*

In this trial, although there were benefits in growth performance in the younger birds, the results at 42 days showed no statistical differences. There were no significant differences observed between *Lactobacilli* or *Streptococci* populations colonising the gut wall or within the digesta from samples taken at 42 d, although samples from the 21 d old animals may have revealed a different result.

**Adult animals**

Adult animals which have a well balanced and stable gastro-intestinal micro-flora are less likely to be colonised by additional micro-organisms which enter the tract. (Savage, 1979), as the adult animal’s existing micro-flora is more likely to exclude the additional bacteria than *vice versa*. Consequently, there is less effect of probiotics in older animals than in young animals.

Laboratory inoculation of chicks has been shown to differ from commercial situations (Weinack et al., 1981). The native gut micro-flora is more effective in influencing the demonstrable level of *E. coli* following experimental inoculation than in altering the levels of those *E. coli* naturally existing in the chick. Thus, ‘normal’ *E. coli* may be part of the natural microbial flora necessary for exclusion of other foreign bacteria. Probiosis within the gut will naturally try to avoid the colonisation of any foreign organisms, whether pathogenic or not. In the literature there have also been studies showing no correlation between gastro-intestinal
and faecal bacterial populations (Muralidhara et al., 1977; Paul and Hoskins, 1972; Pollman et al., 1980a) which would question the use of faecal analysis as a measure of gastrointestinal bacterial change. Also, much of the in vivo research work has been carried out on germ-free animals. However, as an animal’s typical environment is far from germ-free this is not necessarily a relevant comparison.

Just as all antibiotics do not have the same mode of action it is likely that all probiotics do not either. Therefore, stating that probiotic results are variable may not reflect fairly the achievable response to all of the products, and this has already been discussed in relation to the suckling piglet.

Further reasons given to explain the variability in response to direct-fed “live” microbials are:

- Failure of the bacteria to survive storage, processing and gastric acids.
- Failure of the bacteria to implant in the gut
- Destruction of the bacteria by antibiotics.
- Lack of stress in the animals and a Lactobillus population in the gut which was not deficient, with the consequence that performance was already at a maximum.

Bacterial supplements are not only sold for inclusion in livestock feed but are also sold on the human health market. A comprehensive review of the health benefits from such supplements has been made by Sandine et al., 1972 and include the following:

- Reduction in candida infections.
- Reduction in constipation (Alm, 1983; Graf, 1983).
- Reduced lactose intolerance (Goodenough and Kleyn, 1976; Savaiano et al., 1984)
- Stimulation of immunity (Perdigon et al., 1986a; Perdigon et al., 1986b).
- Reduction of serum cholesterol levels and consequently cardiovascular disease (Mann, 1977; Gilliland et al., 1985; Nahaisi, 1986; Danielson et al., 1989).
- Prevention of carcinogen production, such as nitrosamines (Nahaisi, 1986; Rowland and Grass, 1975)
- Protection against bowel cancer (Goldin and Gorbach, 1984).
- Reduction in gastro-intestinal pathogens (Gandhi and Nambrudripad, 1978).

130  The Living Gut
Special attention is given to the lactic acid bacteria because of their importance in the nutrition of animals and man. In nature, their occurrence is related to their high demands for nutrients and to the fact that their energy generation is purely by fermentation. They are rarely found in soil or water. Examples of their occurrence are:

- In milk, its products and the places where they are produced e.g. *Lactobacillus lactis*, *L. bulgaricus*, *L. helveticas*, *L. casei*, *L. fermentum*, *L. brevis*, *Streptococcus lactis*
- In the mucous membranes and the gastrointestinal tract of animals, e.g. *L. acidophilus*, *Bifidobacterium*, *S. faecalis*, *S. salivarius*, *S. bovis*, *S. pyogenes*, *S. pneumoniae*.
- In both intact and fermented plants such as silage. They may be naturally present or be introduced to suppress the growth of spoilage bacteria e.g. *Lactobacillus plantarum*, *L. delbruckii*, *L. fermentum*, *L. brevis*, *S. lactis*, *Leuconostoc mesenteroides*.

*Streptococcus* (also known as *Enterococcus*) *faecalis* is a normal inhabitant of human intestines with many *Streptococci* found in the mucous membranes of the oral cavity, and the respiratory, urinary and genital organs, while some are blood parasites and very virulent pathogens.

Lactic acid is the main acid in sour milk. It was first identified as a fermentation product in 1847 by Blondneau, and in 1877, a pure culture of lactic acid producing bacteria, *S. lactis*, was isolated.

Feeding lactic acid was shown to lead to improved growth in weaned pigs and a shift in the bacterial population against *E. coli* (Cole, Beal and Luscombe, 1968), and other work supports this phenomenon (Herrick, 1972; Pollman *et al*., 1980b; Wu, 1987). Lactic acid has also been found to be inhibitory towards *S. typhimurium*, *in vitro*, and this is important in the control of *Salmonella* on a routine basis.

Probiotics are growing in popularity in the human food, animal and companion animal markets. As combinations of bacteria become optimised for use in feeds to promote health and growth, more will be taken up commercially. There will always be challenges regarding heat and processing resistance, however these may be overcome by encapsulation or similar technologies.
The Living Gut
Carbohydrates are becoming increasingly recognised as important contributors in promoting and maintaining the living gut environment. They can be components of feed raw materials, such as soyabean meal. They occur in milk sugars, or they may be specifically derived from sources such as yeast. Many of them, such as lectins, have the ability to bind to the gut wall, where they may stimulate immune responses or cause irritation. Bacterial attachment to the enterocytes on the gut wall can be restricted by the addition of isolated fimbriae/lectins and their analogues (Pusztai et al., 1990) (Figure 10.1)

Lectins are a class of proteins that combines with sugars rapidly, selectively and reversibly. Like oligosaccharides, they are ubiquitous in nature and are found in plants.
Isolated fimbriae or plant lectins which have been present in the diet can occupy the binding site and prevent attachment of the bacteria. Dietary lectins may cause a change in the carbohydrate side chains of surface receptors of the small intestine brush border epithelium and, by creating favourable conditions for the attachment of selected bacterial species, lead to selective overgrowth. These principles show the flexibility open to nutritionists in beneficially controlling the micro-flora of the digestive tract.

Oligosaccharides of most interest to nutrition and health research in recent years have been in the form of the oligosaccharides (mainly mannan- and fructo-) oligosaccharides, which are associated with maintaining the microflora.

It is interesting that different bacteria attach preferentially in different organs. For example, while some settle in the digestive tract others may prefer the urinary tract or other organs. Consequently, cell recognition is important to bacteria. It is suggested that carbohydrates (sugars) are the primary markers for cell recognition with all cells carrying a sugar coat. The micro-organism has glycoproteins (lectins/fimbriae) on the surface of cells, which can recognise and combine rapidly/selectively and reversibly with the sugar (oligosaccharide) of the gut wall (Figure 10.2).

![Figure 10.2 Mode of attachment of bacteria to the gut wall](image-url)
The lectin/carbohydrate combination is specific to a particular organism. However, if the same carbohydrate (e.g., an oligosaccharide) is provided in the diet, harmful bacteria can be encouraged to attach to these instead, with potential beneficial consequences.

The microbial community in most animals requires a certain level of fermentable fibre as a substrate. Carbohydrates such as fructo-oligosaccharides (FOS) fulfil this function by acting as a substrate for certain non-pathogenic bacteria resident in the caeca or colon. Trials have shown that supplementation with FOS can aid the colonisation and efficacy of probiotics addition to the diet, as it helps maintain the introduced populations at viable levels within the host animal. Such combinations have proved effective in the establishment of an intestinal ecosystem with a high ‘barrier effect’ against pathogens. Bailey, Blankenship and Cox (1991) reported that the addition of 0.75% FOS to a probiotic-supplemented diets fed to broilers challenged with *Salmonella typhimurium* had much lower *Salmonella* colonisation in the gastrointestinal tract (19% versus 61% fed the probiotics alone).

Chambers, Spencer and Modler (1997) supplemented broiler diets with FOS and lactose derivates and their data showed that feeding either of these oligosaccharides resulted in a decline in *Salmonella* infection in broilers that had been challenged with this pathogen at 5 days of age. Caecal digesta pH was also reduced, indicating a higher proportion of acid-producing bacteria in this gastric region. These benefits disappeared within one week following the withdrawal of the carbohydrate treatments when the birds reached five weeks of age.

More unusual oligosaccharides include isomalto-oligosaccharides (IMOS), isolated from leucanostoc fermentation. This is selectively fermented by *Bifidobacteria* and *Lactobacilli*, and not used by undesirable salmonella or *E. coli*. When fed to growing chickens, IMOS promoted the growth of isolated bacteria from chicken caeca and *Bifidobacteria*, whilst reducing the *Salmonella* populations (Chung and Day, 2004).

Research in pigs has seen similar results. Trials comparing FOS and trans-galacto-oligosaccharides (TGOS) showed a trend for lower faecal dry matter compared to an unsupplemented control diet when fed to 9 week old piglets for 35 days (Houdijk et al., 1998). Trials conducted in China reported significant improvements in FCR (37%) and a 71% reduction in the occurrence of diarrhoea in weaned piglets (Xudong et al., 2005). Examination of the VFA content within the gut from animals in the same experiment showed significant increases in total VFA, propionic and butyric acid, acetic acid, isobutyric acid, isovaleric acid and total VFAs in faeces. The increase in levels of certain VFAs, such as butyrate,
is known to be important in the maintenance of gut absorptive structures, such as villi. Other studies have reported improvements in ion transport across the gut wall and gut enzyme activity (Correa-Matos et al., 2003), promoting the availability of nutrients to the host animal.

Inulin is another carbohydrate-based compound, composed of fructose which has been tested in agricultural and companion animal species with some success. A comprehensive review of the usefulness of inulin has been published by Verdonk et al., (2005). As well as performance benefits in pigs and poultry, calves show improved daily gain, changes in bacterial population (significant increases in Bifidobacter) and improved faecal scores. In dogs, some of the benefits reported included the numbers of anaerobic species of bacteria in the gut declining with inulin supplementation, higher digestibility of protein, organic matter and improved VFA profiles. In cats, data has shown an improvement in the numbers of beneficial bacteria and a decrease in potential pathogens and higher short-chain fatty acid (SCFA) production, alongside increased propionate levels.

Other types of carbohydrates have a different mode of action. Mannan-oligosaccharides (MOS) have been shown to prevent gastric disorders by binding pathogenic bacteria (Spring et al., 2000), and promote improved immunity through interaction with the gut associated lymphatic tissue (Kelly, 2004). Many research trials have shown the benefits of MOS in binding pathogens such as E. coli and Salmonella spp. that rely on mannose-sensitive fimbrae for gut wall attachment during the establishment of colonies. They have common fimbrae (type 1) that recognise and bind to mannose receptors on enterocytes (Sharon, 1987). This adhesion can be reversed by the presence of D-mannose or D-mannopyranoside (α-D-methyl-pyranoside) (Ofek et al., 1977). This has practical implications, for the addition of mannose (2.5%) to the drinking water of broilers has been shown to significantly reduce the level of colonisation by Salmonella typhimurium and total bacterial levels in the bird’s caecum (Oyofo et al., 1989). D-mannopyranose is found naturally in yeasts (Saccharomyces cerevisiae) as well as other mannans. It has been reported (Miles, 1993) that mannose can interfere with the attachment of Salmonella, E. coli and Vibrio cholera which have a mannose specific substance on the surface. The fimbrae are equally attracted to mannoligosaccharides, which prevents them from adhering to the gut lining, reducing colonisation opportunities and facilitating excretion and reducing caecal population density.

It has also been pointed out that carbohydrate directed interactions between cells are not restricted to pathological phenomena but are also crucially important in the healthy operation of the immune system (Sharon and Lis, 1993). These interactions have a role in directing leucocytes to specific parts of the body.
Studies have been conducted to compare the ability of different mannose-type sugars to block bacterial attachment by pathogenic bacteria expressing type 1 fimbriae to D-mannose. D-mannose-6-phosphate or β-bonded 1,4 mannan do not bind *E. coli* to any appreciable degree, but those bound in α-1,3 linkage demonstrate higher binding capacity than pure D-mannose (Spring, 2000). Cabib *et al.* (1982) have illustrated the importance of the chemical structure of MOS in its efficacy in binding fimbriated bacteria. MOS is also known to be resistant to digestion and fermentation (Spring, 2000).

In early *in vivo* studies MOS was shown to reduce levels of *Salmonella* as well as *E. coli* in the caecum of challenged broiler chicks. (Figure 10.3), (Spring *et al*., 2000).

![Figure 10.3](image)

**Figure 10.3** Effect of dietary mannoligosaccharide MOS on caecal *E. Coli* concentrations of chicks maintained in microbial isolators (p<0.05) (Spring *et al*., 2000)

Improving bacterial population profiles in the gut can influence characteristics of the gut wall lining. Trials in grower-finisher pigs (fed mannoligosaccharide from 21-100 kg bodyweight) have shown that the number or replicating cells in the crypts of villi lining the gut wall increased in animals receiving the oligosaccharide (Rekiel *et al*., 2005), (Figure 10.4).
In the same trial the number of different bacterial types present in the gut was monitored. Results showed that pigs fed the oligosaccharide had much lower numbers of yeasts and *Proteus vulgaris* bacteria and ten times fewer *Enterobacteriaceae* spp. (Table 10.1).

**Table 10.1 Bacterial numbers in the gut of pigs fed either MOS or flavomycin (Rekiel et al., 2005)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Gut section</th>
<th><em>Enterobacteriaceae</em></th>
<th>Yeasts &amp; fungi</th>
<th><em>Proteus vulgaris</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavomycin</td>
<td>Duodenum</td>
<td>$1.15 \times 10^8$</td>
<td>$3.40 \times 10^7$</td>
<td>$1.46 \times 10^8$</td>
</tr>
<tr>
<td></td>
<td>Jejenum</td>
<td>$1.14 \times 10^8$</td>
<td>$2.30 \times 10^6$</td>
<td>$1.46 \times 10^8$</td>
</tr>
<tr>
<td></td>
<td>Ileum</td>
<td>$8.70 \times 10^7$</td>
<td>$4.33 \times 10^7$</td>
<td>$7.10 \times 10^7$</td>
</tr>
<tr>
<td></td>
<td>Colon</td>
<td>$1.63 \times 10^6$</td>
<td>$1.15 \times 10^7$</td>
<td>$1.19 \times 10^7$</td>
</tr>
<tr>
<td>MOS</td>
<td>Duodenum</td>
<td>$5.65 \times 10^7$</td>
<td>$2.58 \times 10^6$</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Jejenum</td>
<td>$3.23 \times 10^7$</td>
<td>$3.56 \times 10^6$</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Ileum</td>
<td>$1.58 \times 10^6$</td>
<td>$8.01 \times 10^4$</td>
<td>$8.10 \times 10^2$</td>
</tr>
<tr>
<td></td>
<td>Colon</td>
<td>$2.86 \times 10^6$</td>
<td>$8.60 \times 10^4$</td>
<td>-</td>
</tr>
</tbody>
</table>

Improvements in immune response stimulation by MOS promotes a general improvement in immune response, evidenced in pig trials where improved control to non-fimbriated bacteria such as *Clostridia perfringens* has been demonstrated (Geliot, pers. comm., Figure 10.5).
For the pig farmer, the main observation in pigs receiving such benefits from technical feed ingredients is in the form of improved herd health and growth. Trials run in Nanjing Agricultural University, China, showed how the incidence of diarrhoea was reduced and performance improved in weaned piglets fed mannoligosaccharide for 21 days compared to a zinc bacitracin control diet (Huang et al., pers. comm.), (Table 10.2).

**Table 10.2 Effect of mannoligosaccharide on performance and incidence of diarrhoea in weaned piglets (Huang et al., pers. comm.)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>MOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numbers of pigs</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>Average daily feed intake (g)</td>
<td>407</td>
<td>407</td>
</tr>
<tr>
<td>ADG (g)</td>
<td>258&lt;sub&gt;b&lt;/sub&gt;</td>
<td>274&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>FCR (g)</td>
<td>1.58</td>
<td>1.48</td>
</tr>
<tr>
<td>Day-heads of diarrhoea</td>
<td>22</td>
<td>13</td>
</tr>
<tr>
<td>Incidence of diarrhoea, %</td>
<td>2.9</td>
<td>1.7</td>
</tr>
</tbody>
</table>

<sup>as</sup>means with different superscripts are significantly different (P<0.05);

Several trials have been conducted to examine the impact of MOS on dog digestion and immune parameters. These trials have chiefly concerned the examination of
a commercial preparation of MOS available for use in pet foods, supplements and treats.

Kappel (1998) tested the effect of MOS on fibre digestibility in 40 beagles. Diets were supplemented with extra fibre (beet pulp, soyabean hulls or cellulose) in order to create treatments with different levels of insoluble and soluble fibre (Table 10.3), which the dogs’ own endogenous enzyme secretions are not able to break down. Diets containing MOS showed greater soluble fibre digestibility, probably as a result of changes in fermentation profile in the gut bacterial populations. Reductions in faecal ammonia concentrations (Marquart, 1999) have also been reported under similar trial conditions.

### Table 10.3 Effect of MOS on fibre digestibility in dogs (Kappel, 1998)

<table>
<thead>
<tr>
<th>Digestibility (%)</th>
<th>Dry matter</th>
<th>Organic matter</th>
<th>Fibre</th>
<th>Insoluble fibre</th>
<th>Soluble fibre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>82.0</td>
<td>83.6</td>
<td>25.6</td>
<td>15.7</td>
<td>61.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MOS</td>
<td>81.8</td>
<td>83.1</td>
<td>25.7</td>
<td>15.2</td>
<td>72.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> means not sharing a letter differ significantly (P<0.05)

Several other studies have been conducted to investigate the influence of fibre and carbohydrates such as MOS. Strickling <i>et al.</i> (2000) conducted a trial where they compared groups of dogs fed diets with and without 5 kg/t MOS. The researchers observed a strong trend for reduced ileal butyrate (p=0.07) and <i>Clostridia perfringens</i> numbers (p=0.09) in the dogs fed MOS, and ileal propionate was enhanced (p=0.09). MOS can be used to promote a better fermentation profile in the gastric tract of canine species, which in turn suggests an improved bacterial population profile, and less chance of developing digestive disorders.

Researchers at the University of Vienna (Zentek <i>et al.</i>, 2002) have shown that MOS can change fibre digestion as well as levels of ammonia excretion and volatile fatty acid production (Table 10.4).

### Table 10.4 Effect of feeding MOS on volatile fatty acid production, fibre digestion and ammonia excretion in dogs (Zentek <i>et al.</i>, 2002)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>MOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>VFA (µmol/ml)</td>
<td>26.8</td>
<td>41.3</td>
</tr>
<tr>
<td>Ammonia (µmol/ml)</td>
<td>116.0a</td>
<td>78.4b</td>
</tr>
<tr>
<td>Crude fibre digestion (%)</td>
<td>61.8b</td>
<td>69.1a</td>
</tr>
</tbody>
</table>
Reductions in ammonia levels, whilst being useful regarding odour control, may also be related to changes in protein digestion. Improved crude fibre digestion is important in gut health and motility, and increased VFA production is related directly to changes in bacterial fermentation patterns, although the individual fatty acids were not reported in this work.

Different oligosaccharide products may be combined, particularly where their modes of action are known to be different. Swanson et al. (2002) examined the effect of MOS and FOS in combination, fed at 0.5% of the diet on total anaerobe, total aerobe, *E. coli*, *Clostridium perfringens*, *Bifidobacteria* spp. and *Lactobacilli* spp. populations. Dogs fed MOS showed decreased (p≤0.05) total aerobes, and numeric increases (p=0.13) for *Lactobacilli* spp. populations in faeces; trends that are used as positive indicators of colonic health. Dogs fed FOS and MOS in combination showed a trend for decreased (p<0.10) total anaerobe populations, whereas *Bifidobacterium*, *E. coli*, and *C. perfringens* populations were maintained across treatment groups.

Other feeding experiments with dogs (Table 10.5) have shown trends for reductions in faecal clostridial concentrations when receiving food supplemented with MOS (Finucane et al., 1999; Strickling, 1999). These findings were of particular interest, as *Clostridia perfringens* do not possess mannose-specific, type 1 fimbriae, so the effect would be mediated by immune responses or other factors, as discussed above.

**Table 10.5 Effect of manmoligosaccharide (MOS) and fructoligosaccharide (FOS) addition to dog diets on faecal bacteria (log CFU/g) (Strickling, 1999)**

<table>
<thead>
<tr>
<th>Faecal bacteria</th>
<th>Control</th>
<th>MOS</th>
<th>FOS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. perfringens</em></td>
<td>3.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Bifidobacteria</em></td>
<td>9.36</td>
<td>9.27</td>
<td>9.54</td>
</tr>
<tr>
<td><em>Lactobacilli</em></td>
<td>7.81</td>
<td>8.82</td>
<td>8.30</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Means not sharing a superscript tend to differ (P=0.09).

Holo-analysis of all published trial data (up to 2005) have been carried out (Rosen, 2006a,b; 2007 a,b) for turkeys, broilers and pigs for mannoligosaccharides. In pigs and broiler chickens positive responses occur in around 70% of reported cases for performance parameters, whereas in turkeys, it is rather lower at 52%. This work illustrates that carbohydrates are as effective in animal feed as more established and mainstream feed ingredient products, such as antibiotics and enzymes.
Although less work has been conducted in this area for humans, there remains a high level of interest in the applications of gut-active carbohydrates in functional foods and supplements. There are several papers relating to studies on oligosaccharides such as FOS in human subjects, showing useful improvements. The combination of carbohydrates and probiotics, for example, may prove a useful therapy for patients requiring re-establishment of their microflora following antibiotic therapy (Hidaka et al., 1990; Tuohy et al., 2001).
It is well known that the nature of the diet can influence the processes of digestion and absorption, and that the conditions in the digestive tract and levels of microbes such as *Lactobacilli* change after feeding. Acidifiers seek to exploit this by providing dietary materials that influence the gut environment and hence the micro-organisms that grow there. This has an impact on the absorption of nutrients and can improve productivity.

**Mode of action**

Acids have three main modes of action as feed ingredients:

- As hygiene promoters, where they reduce pH and act as a complexing agent for ions thereby inhibiting microbial growth.
- Reducing pH within the intestinal tract to improve enzyme activity.
- As an energy source in metabolism (Roth and Kirchgessner, 1987).

Acids may be strong or weak, from an inorganic (chemical) or organic (biological) source. Any compound that dissociates into its constituent ions, including acids, is classified as an electrolyte. A strong electrolyte will dissociate completely or nearly completely, but a weak electrolyte will only dissociate to a limited extent (Table 11.1). Consequently, a solution of strong electrolytes will consist mostly of ions, and in a weak solution there will be a large proportion of undissociated particles. The level of dissociated hydrogen ions dictates acidity, and hence a strong electrolyte will exert a larger effect on pH than a weak one.

**Table 11.1 Some common electrolytes and non-electrolytes (Patience, 1989)**

<table>
<thead>
<tr>
<th>Strong electrolytes</th>
<th>Weak electrolytes</th>
<th>Non-electrolytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen chloride</td>
<td>Hydrogen fluoride</td>
<td>Glucose</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>Ammonia</td>
<td>Sucrose</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>Acetic acid</td>
<td>Ethanol</td>
</tr>
<tr>
<td>Potassium fluoride</td>
<td>Mercuric chloride</td>
<td>Oxygen</td>
</tr>
<tr>
<td></td>
<td>Acetone</td>
<td></td>
</tr>
</tbody>
</table>
Individual electrolytes have specific roles. For example, sodium is found extensively throughout the body and has major roles in the maintenance of osmotic pressure and acid-base equilibrium, control of water metabolism and transportation of ions across cell boundaries. In the context of acids as feed ingredients, they are primarily included to control the growth of undesirable microbes and by establishing an optimal pH in the digesta for the catalysis of food substrates, i.e. efficient digestion.

A recent approach has been to consider electrolyte balance, because the balance of dietary cations and anions is closely related to performance in farm animals. There are two major estimates of electrolyte balance (Patience, 1989). These are dietary undetermined anion (dUA) and dietary electrolyte balance (dEB).

\[
\text{dUA} = (\text{Na}^+ + \text{K}^+ + \text{Ca}^{2+} + \text{Mg}^{2+}) - (\text{Cl}^- + \text{H}_2\text{PO}_4^{-})
\]

\[
\text{dEB} = \text{Na}^+ + \text{K}^+ - \text{Cl}^-
\]

Both are expressed in millequivalents (mEq) or milliosmoles (mOsm) as it is the electrical and osmotic properties that are of interest. For example, in a feedstuff dEB is often used for convenience as it involved only three analyses and in many situations is useful. dUA is often more accurate but is laborious.

Improved growth rate has been reported in weaner pigs when dEB was approximately 155mEq/kg (Cole et al., 1992a) (Figure 11.1). A value of 250 mEq/kg has been suggested for 4 week old chicks and there are well known effects of dEB on egg shell quality in laying hens (Mongin, 1981).

![Figure 11.1](image_url)  
**Figure 11.1** Electrolyte balance (dEB) and growth rate in pigs (8-30 kg live weight) (after Cole et al., 1992a)
Organic acids exert their effect directly on microbial cells (Freitag, 2007). Many pathogens are inhibited at pH of less than 5. Small acid molecules, which are lipophilic, can pass across the cell membrane and dissociate within the alkaline conditions of the cytoplasm. This reduces the internal pH of the bacterial cell, preventing enzyme activity, metabolism and thereby prevent the correct functioning of the cell. This has the effect of reducing bacterial populations within the digestive tract for acid sensitive bacteria. Lactobacilli spp. appear to be unaffected (Hellweg et al., 2006), and certain bacteria are sensitive to acids of specific chain lengths, e.g. gram negative bacteria are sensitive to acids comprising less than 8 carbon atoms (Strauss and Hayler, 2001).

The impact on metabolism by acids in the diet is due to the absorption into the gut wall (Freitag, 2007). The enterocytes lining the gut can utilise short chain acids for respiration and energy is produced via ATP generation. Long chain fatty acids and sorbic acid are metabolised via the beta-oxidation pathway. Different types of acids liberate different levels of energy, which can be used in the ration formulation when calculating the amount of energy available from an acidified feed.

**Practical benefits of acidified diets**

Different types of inorganic and organic acids are available commercially. Trials have shown that HCl can significantly increase apparent digestibility of nitrogen, increasing retention in weaned piglets (Mahan et al., 1999). This has a knock-on effect in terms of growth performance. (Table 2).

**Table 11.2 Effects of added HCl on performance and digestibility in weaned piglets (Mahan et al., 1999)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dietary chlorine level (%) from HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.20</td>
</tr>
<tr>
<td>Daily gain (14-21 d post weaning) (g)</td>
<td>372</td>
</tr>
<tr>
<td>N retention (g/d)</td>
<td>6.09</td>
</tr>
<tr>
<td>Apparent digestibility (%)</td>
<td>88.6</td>
</tr>
</tbody>
</table>

Acidification of diets for monogastric animals has increased in use as a hygiene and gut modifying agent in the last 10-15 years, especially for piglets just after weaning. This period of the pig’s life is typically characterised by poor growth, diarrhoea and mortality. The occurrence of such problems is a function of conditions post-weaning rather than actual chronological age or weight of
the piglet, and outbreaks occur about two weeks after weaning. The shedding of haemolytic \textit{E. coli} is implicated in such problems and it has proved difficult to induce these conditions when the piglet is being suckled. A major difference in the suckled pig and the weaned pig is the lower levels of lactic acid in the gut of the latter.

There are now many acidifying products available commercially, and the main market for them is piglets, with some use in poultry. There are published texts now available that are specifically devoted to the topic of acids in animals feed (Lückstäd, 2006). Early attempts at acidification were set against this background of post-weaning conditions. They involved the use of organic acids which were often supplied in the drinking water in the belief that sick pigs would drink during a period of inappetance. Several acids have proven beneficial in controlling haemolytic \textit{E. coli} and improving growth performance, but lactic acid is probably predominant (Table 11.3). However, the effects of these materials were only evident during the period of administration.

\begin{table}[h]
\centering
\begin{tabular}{lllll}
\hline
 & \multicolumn{2}{c}{Pigs killed before treatment applied (i.e. at weaning)} & \multicolumn{2}{c}{Pigs killed after 4 week treatment period} \\
Litter & Duodenum & Jejunum & Control & Lactic acid & Propionic acid \\
\hline
 & & & & \\
Duodenum & 1 & 444.20 (H) & 112 (h) & 0.30 & 1.00 \\
 & 2 & 0.60 (H) & 145 (h) & 0.02 & 0.04 \\
 & 3 & 0.60 (H) & 2.70 (h) & 0 & 2.00 \\
 & 4 & 0.68 (H) & 0.40 (h) & 0 & 0 \\
Jejunum & 1 & 0.30 (H) & 140 (h) & 0.20 & 0.06 \\
 & 2 & 14.50 (H) & 2.30 (h) & 0.04 & 0.10 \\
 & 3 & 5.50 (H) & 0.20 (h) & 0 & 3.40 \\
 & 4 & 6.00 (H) & 1.50 (h) & 0 & 0.40 \\
\hline
\end{tabular}
\caption{Effect of lactic acid and propionic acid (0.8\%) in the drinking water for 4 weeks after weaning on the \textit{E. coli} population of the small intestine (million organisms/ml intestinal contents).}
\end{table}

\textit{H} were haemolytic \textit{E. coli} and \textit{h} is a mixture of haemolytic and non-haemolytic strains of \textit{E. coli}. For other pigs in which \textit{E. coli} were found there were non-haemolytic strains only.

(Cole \textit{et al.}, 1968)
A mature pig is able to adjust stomach pH by secretion of hydrochloric acid from the parietal cells, with highly acidic values being reached (as low as pH 2.0). Young pigs are different, although the newborn pig produces some hydrochloric acid, its secretory capacity is severely limited. As a result, the young pig has a stomach pH of 4-7, which is much higher than the optimum for enzyme activity and the initiation of digestion. The enzyme pepsin has two pH optima, pH 2.0 and pH 3.5, and, at higher levels, protein digestion is reduced. These effects are quite separate from the effects reported by Cole et al. (1968) regarding a large beneficial change in the bacterial flora as a result of the addition of 0.8% lactic acid to the drinking water. A number of organic acids have been used in the diet of weaned pigs, e.g. fumaric acid, citric acid, propionic acid. Generally good responses have been reported in terms of growth performance (Table 11.4).

**Table 11.4** Effects of fumaric acid supplementation on pig performance (Easter, 1988)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fumaric acid level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Diet pH</td>
<td>5.96</td>
</tr>
<tr>
<td>Live weight gain (g/day)</td>
<td>261</td>
</tr>
<tr>
<td>Feed intake (g/day)</td>
<td>501</td>
</tr>
<tr>
<td>FCR</td>
<td>1.92</td>
</tr>
</tbody>
</table>

Clearly the effects of the materials on the pH of the gastro-intestinal tract may have been a factor in the mechanism of response. However, it is interesting to note that in the work of Cole et al. (1968, 1970) lactic acid appears more effective in the improvement of growth performance and it may well have its own specific benefits.

Poultry have also been extensively studied in their response to acidified diets. Trials reported by Desai et al. (2006) have shown improvements when acids have been administered in feed and through drinking water. This may be manifested as improved performance (Table 11.5), reduced wet litter and diarrhoea scores (Table 11.6) or reduced salmonella shedding in faeces (Table 11.7).

The use of other acidic materials, such as medium chain fatty acids, are also known to be able to control and improve the microflora and gut environment (Van Immerseel et al., 2006).
The Living Gut

Table 11.5 Acidified diets reduced diarrhoea scores in poultry fed acid at a rate of 2 kg/t feed (Desai et al., 2006)

<table>
<thead>
<tr>
<th>Performance data</th>
<th>Control</th>
<th>Acidified feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhoea incidence at 6 d (%)</td>
<td>34.0b</td>
<td>25.7a</td>
</tr>
<tr>
<td>Avg scour score at 6 d</td>
<td>0.47b</td>
<td>0.31a</td>
</tr>
<tr>
<td>Mortality (%) at 42 d</td>
<td>3.81</td>
<td>1.43</td>
</tr>
<tr>
<td>FCR at 42 d</td>
<td>1.85</td>
<td>1.84</td>
</tr>
</tbody>
</table>

Means not sharing a letter differ significantly P<0.05

Table 11.6 Acidified drinking water (0.5 l/1000 l) improved performance in poultry kept on deep litter under good management conditions (Desai et al., 2006)

<table>
<thead>
<tr>
<th>Performance data</th>
<th>Control</th>
<th>Acidified feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final weight (kg)</td>
<td>1.347</td>
<td>1.478</td>
</tr>
<tr>
<td>Weight gain (kg)</td>
<td>1.308</td>
<td>1.440</td>
</tr>
<tr>
<td>Feed intake (kg)</td>
<td>2.315</td>
<td>2.389</td>
</tr>
<tr>
<td>FCR</td>
<td>1.77</td>
<td>1.66</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>2.0</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Table 11.7 Impact of feeding different levels of formic and propionic acid blend on chicken faecal salmonella shedding (Hinton and Linton, 1988)

<table>
<thead>
<tr>
<th>Acidifier rate of inclusion in feed (kg/t)</th>
<th>No. positive samples</th>
<th>% positive in flock</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>42</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>55</td>
<td>79</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Acids are also used to improve silage quality for ruminant and semi-ruminant animals, as it controls the development of potentially dangerous clostridial organisms, as well as ensuring a suitable drop in pH to promote the correct fermentation and preservation of the forage to maintain nutritional value.
Many plant-based compounds have been shown to affect gut micro-organisms directly (Murphy Cowan, 1999; Calsamiglia et al., 2007). Phytogenic ingredients may be fed either as complete plants, defined areas of the plant (leaf, root, seed, whole plant) or as extracts, where the plant material undergoes processing to isolate either water, oil or alcohol soluble fractions. The material may be fresh, dried, crushed or in a liquid or powder form.

The very nature of these types of nutricines makes determining a sole active compound difficult, as many appear to act in concert to facilitate the benefits observed in vivo.

Regulations regarding the licensing of botanicals or phytogenics as feed additives are rather interesting, and differ from other feed ingredients. Many are still listed primarily as flavours, although in some countries in Asia, where the use of these types of products is more widely accepted, patents have been issued for certain single or combinations of plant extracts. The generic nature of the source material for phytogenic ingredients has made full zootechnical registration, as for feed enzymes, rather difficult. It is hard to protect the commercial rights of the company that has invested in submitting the required technical data required for making claims. This has left many companies with the dilemma of the high cost of full zootechnical registration against any commercial sales value from the registered product, especially if competitors have free rein to then make the same claims. To rectify this situation, it appears that some rights are being afforded by some authorities to companies who make this investment, thereby protecting their sales and encouraging full registration.

Several commercial products are now available for use in animal feed, milk or water systems or via capsules and tablets. These may contain single or multiple phytogenic sources and active compounds. Certain products have been shown to improve digestion and gut microflora profiles, leading to better nutrient availability for growth and development. Such data is typically taken from monogastric species; calves, pigs and poultry.

Initial screening trials to investigate the beneficial properties of botanical ingredients are available, and commercial products have been developed from
this basis. Botanical ingredients have been shown to facilitate useful changes on the gut environment and bacteria (Rao and Nigam, 1970; Tucker, 2006), however it is important to consider and determine the effective dose needed to bring about the desired benefits. The activity and efficacy on gut microbes can be quite variable, depending on the plant source, extract process and quality consistency. There may also be stability or shelf life issues associated with some compounds and extracts.

Some of the known gut-active phytogenic ingredients are shown in Table 12.1 below.

**Table 12.1 Plant-derived ingredients with known gut activities (Tucker, 2001)**

<table>
<thead>
<tr>
<th>Plant</th>
<th>Active Ingredient</th>
<th>Growth promotion</th>
<th>Digestive function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anise</td>
<td>Anethole</td>
<td></td>
<td>Carminative</td>
</tr>
<tr>
<td>Bloodroot</td>
<td>Sanguinarine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cinnamon</td>
<td>Cinnamaldehyde</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fenugreek</td>
<td>Saponins</td>
<td></td>
<td>Feed intake</td>
</tr>
<tr>
<td>Garlic</td>
<td>Allicin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horseradish</td>
<td>Allyl isothiocyanate</td>
<td></td>
<td>Gastric secretion</td>
</tr>
<tr>
<td>Juniper</td>
<td>Alpha-pinene</td>
<td></td>
<td>Appetite</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Carminative</td>
</tr>
<tr>
<td>Milk Thistle</td>
<td>Silymarin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pine</td>
<td>Bornyl acetate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rosemary</td>
<td>Phenols</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tea tree</td>
<td>Terpinen-4-ol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyme</td>
<td>Thymol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yarrow</td>
<td>Chamazulene</td>
<td></td>
<td>Pancreatic secretions, bloating</td>
</tr>
</tbody>
</table>

It should be remembered that phytogenic or botanical extracts and products may be associated with more than one mode of action, either beneficial or deleterious. For example, many have antioxidant properties, or maybe minor bactericidal effects, even though this is not the main benefit observed in trials.

**Anti-microbial effects**

From the list of phytogenics available, many are reported to have anti-microbial activities in the digestive tract. Laboratory screening of a variety of plant extracts and compounds, has been done against *Campylobacter jejuni* used standard zone of inhibition tests to evaluate antimicrobial activity (Tucker, 2001). The results
below give the distance to which growth was inhibited on the agar plate from the application of the test extracts (Table 12.2). The greater the zone of inhibition, the stronger the antimicrobial effect.

**Table 12.2 Antimicrobial efficacy of phytogenic compounds on plated *Campylobacter jejuni* colonies (Tucker, 2001).**

<table>
<thead>
<tr>
<th>Test compound</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oregano oil</td>
<td>25</td>
</tr>
<tr>
<td>Garlic granules</td>
<td>25</td>
</tr>
<tr>
<td>Rosemary</td>
<td>3</td>
</tr>
<tr>
<td>Grapefruit seed</td>
<td>25</td>
</tr>
<tr>
<td>Millefleurs</td>
<td>25</td>
</tr>
<tr>
<td>Thyme oil</td>
<td>7</td>
</tr>
<tr>
<td>Aniseed</td>
<td>2</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>25</td>
</tr>
</tbody>
</table>

To illustrate this, we can take garlic as an example. Garlic has long been associated with improved gut health, along with other benefits. The key active component, allicin, has been shown to exert an anti-microbial action on gut microflora. Research determining the minimum inhibitory concentration (MIC) required to inhibit growth of various bacteria important in animal production and health, has shown that allicin is more efficacious than other plant extracts with anti-bacterial activities (Figure 12.1). Allicin can be rather unstable in some forms, and needs careful quality control to ensure efficacy is maintained in the final product.

**Figure 12.1** Relative efficacy of allicin, derived from garlic, versus other anti-microbial botanicals against common pathogenic bacteria found in animal production (Tucker, 2001).
Allicin-based products have been used in animal performance studies. In a broiler trial conducted in the UK, the effect of an allicin-based commercial product on *Clostridia* numbers versus an unsupplemented control and a diet containing commercial sub therapeutic levels of avilamycin were compared. Broilers were dissected at the end of the trial and the caeca removed. Anaerobic culture analysis of the caecal contents was performed to quantify clostridial numbers (Figure 12.2).

![Figure 12.2](image)

**Figure 12.2** Broilers fed garlic-based commercial feed ingredients had fewer caecal clostridia colonies than those receiving no supplementation, and similar levels compared to birds receiving avilamycin (Tucker, 2001).

This was attributed to a combined effect of the natural activity against cell enzyme pathways, coupled with improved digestibility in the gut. The mode of action of the garlic-derived component is reported to disrupt key enzyme-mediated reactions within cells. In most cells there exists a repair mechanism that automatically reverses such damage. However in certain pathogenic species, this repair mechanism does not exist, and exposure to the garlic compounds inhibits bacterial protein synthesis and ultimately cell growth and multiplication. This selective effect has been demonstrated in pig trials, where commercial feed was inoculated with known levels of *E.coli* 0157 and 0149 variants and *Lactobacillus plantarum* and *casei*. The feed was monitored for bacterial growth at 0, 7 and 14 days. The results are shown in Figure 12.3 below.
Immediately after inoculation with the bacteria, the antibiotic in the positive control diet reduced the level of *Lactobacilli*, which are important commensal (benign) bacteria that positively contribute to fermentation. They are also an important group for out-competing pathogenic species such as *E. coli*, and establishing stable hind gut function. At this time, there was no change in bacterial loading in the garlic extract diet relative to the negative control.

After one week of storage however, different effects of the antibiotic and the garlic extract diets were observed (Figure 12.4). The antibiotic had an indiscriminate effect on both types of micro-organism in the feed. In contrast the garlic extract only affected the levels of *E. coli*, and this response was sustained to 14 days of storage. The beneficial *Lactobacilli* strains have been allowed to flourish to a similar level as the negative control, and only the *E. coli*, which do not possess the intracellular enzyme repair mechanism, have been reduced in the feed.

Experiments conducted with botanical products and ruminants, such as dairy and veal calves, have reported improvements in performance as well as in scour scores, which are a major problem in young calves. Typical results during the first 6 weeks of life include improved calf weight gain by 6% and compound feed intake by 3% compared to an unsupplemented control. The data from 0 - 3 weeks of age showed more pronounced effects, probably as a result of the higher susceptibility of younger calves to digestive disorders and bacterial challenges (Tucker, 2001). In this younger age group, weight gain was improved by 21%, milk replacer
intake by 3% and weaner feed by 8%. This resulted in improvements in FCR – with a 24% improvement in milk replacer and 17% improvement in weaner diet FCR. Such improvements indicate increased digestive efficiency, linked to several activities of the botanical components, for example increased endogenous enzyme secretion, improved gut environment and microflora balance and increased liver function to better utilize fats and proteins. Scour status results are shown in Figure 12.5 below.

**Figure 12.4** Effect of supplementing commercial pig feed with garlic extract based ingredients on *E. coli* and *Lactobacilli* numbers after 7 days storage at ambient temperature.

**Figure 12.5** Effects of botanical supplementation of milk replacer and weaner diet on calf scour status and body condition score (Tucker, 2001)
Lambs follow a similar feeding regime, and face similar digestive problems to young claves, mostly during the transition period between milk and dry weaner feed. Trials with garlic-base botanical products conducted at a commercial research site in the UK found that supplementation resulted in 35% increased in weight gain \((P<0.001)\), a difference that declined once the lambs began to consume appreciable amounts of dry weaning diet. However feed conversion ratio showed a 2% improvement in overall performance throughout the trial. Uniformity, which is directly related to the production economics of meat animals, was also improved by supplementation.

![Figure 12.6](image_url)  
**Figure 12.6** Effect of botanical supplementation compared to unsupplemented control diet on the performance of growing lambs (Tucker, 2001)

Oregano is another important botanical compound that forms the base of many commercial animal feed products. It is primarily used for its anti-microbial properties, where its active ingredients carvacrol and thymol, both volatile oils, have been shown to inhibit the growth of many bacterial species (Dorman, 2000). It has also been reported to completely arrest the growth of *Aspergillus* spp. fungi (Basilico and Basilico, 1999). The synergism that exists between the two main active components is considered important, and the ration between them may influence the efficacy of oregano *in vivo*. The mode of action of the active volatile oils is via cell membrane disruption, caused by the movement of ions across the cells membrane following the penetration of the active components into the cell. Once within the cell, they change membrane permeability and prevent the synthesis of protein responsible for cellular respiration in the mitochondria. Both these activities are fatal to the microbial cell (Fox, pers comm.).
Performance benefits in animal trials have been shown in commercial studies with several products based on oregano. Feeding experiments with breeding sows have shown that oregano based products significantly improved piglet weight at birth and weaning, along with sow feed intake during lactation and milk yield (Table 12.1).

Table 12.1 Breeding sow and their progeny’s performance can benefit from supplementation with oregano-based botanical supplements (Meriden Animal Health)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Negative control</th>
<th>Oregano-based product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total birth weight per litter (kg)</td>
<td>15.2b</td>
<td>16.3a</td>
</tr>
<tr>
<td>Total weight weaned per litter</td>
<td>64.6b</td>
<td>70.6a</td>
</tr>
<tr>
<td>Piglet feed intake (kg)</td>
<td>0.161b</td>
<td>0.178a</td>
</tr>
<tr>
<td>Daily gain piglets (kg)</td>
<td>0.252b</td>
<td>0.268a</td>
</tr>
<tr>
<td>Feed intake of sow during lactation (kg/d)</td>
<td>5.86b</td>
<td>6.33a</td>
</tr>
<tr>
<td>Milk yield per sow (l/d)</td>
<td>9.53b</td>
<td>10.44a</td>
</tr>
</tbody>
</table>

The results showed similar responses to those previously reported for antibiotic supplementation. Other published trials detail how growing pig herds supplemented with oregano-based products have significantly fewer antibiotic resistant microbes compared to those receiving prophylactic antibiotics (Docic and Bilkei, 2003).

**Digestive secretions**

Some botanical products, such as yarrow and horseradish, stimulate gastric secretion through enzyme production from the pancreas. This is particularly important in young animals, where gut maturation can dictate digestive efficiency. Improved secretion increases digestion of protein and starch in the upper ileum, making more nutrients available for absorption in those areas of high villi population. This reduces the amounts of undigested feed particles or unabsorbed nutrients passing down the gut and being utilized by bacteria within the caeca in birds, or the colon in mammals.

Increasing the availability of nutrients for fermentation by gut microflora is associated with many digestive disorders, due to the uncontrolled proliferation of bacteria. This is especially true for *Clostridia*, which are particularly encouraged by increases in nitrogen availability. *Clostridia perfringens*, the organism associated with necrotic enteritis, requires high levels of nitrogen to multiply. Increasing levels of undigested nitrogen, combined with a coccidiosis challenge, has been
shown to spontaneously increase the incidence of necrotic enteritis without further inoculation with *Clostridia*. Likewise, increased energy sources from undigested starch favours development of other, potentially harmful, microbial species.

Horseradish has certain antimicrobial properties against important food safety bacteria such as *Listeria monocytogenes* and *Salmonella typhimurium* (Delaquis *et al.*, 1995) and can also affect the digestive tract in other ways, as it is associated with increasing gastric secretions. The pungency of allyl isothiocyanate of horseradish which is responsible for providing the hot sensation when eaten, stimulates the appetite and gastric secretion. As with all botanical products, it is important to remember that their components may not always be beneficial. Again, we can use horseradish as an example here – as its high glucosinolate content may cause lesions and production problems in laying hens, akin to those observed for the same compounds derived from oil seed rape. Overdosing with horseradish extracts may be associated with the development of certain gut-associated tumours.

Detailed trials conducted using yarrow extract in broiler chickens has shown that yarrow can improve feed conversion efficiency by up to 8% (Lewis *et al.*, 2003).


Baltimore, USA.


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