Microflora of the digestive tract : critical factors and consequences for poultry

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Summary

The microflora of the digestive tract of poultry is still incompletely known. Microbial populations of varying size and complexity occur throughout the digestive tract and the highest and most complex floras are found in the crop and the caeca. The upper part of the digestive tract is predominantly settled by facultative anaerobes, whereas the caeca are mainly the site of obligate anaerobes. The types, numbers and metabolic activities of the organisms are affected by numerous factors such as individual, animal age, environment, and diet. Bacteria produce various metabolites that can be useful or detrimental to the host. Interactions between bacteria and the gastrointestinal epithelium lead to various structural and functional modifications of the digestive tract. Bacteria can impair lipid digestion and may modify carbohydrate and protein digestion. They cause an increase in energy and amino acid requirements. They have a negative effect on vitamin nutrition. Beneficial bacteria can protect birds against pathogens through a competitive exclusion process. Moreover, the flora is involved in the development of the intestinal immune system. Overall, bacteria have a negative effect on bird growth. They may also have an effect on meat and egg quality. Improved knowledge of the microflora of the digestive tract and its consequences may contribute to its control and beneficial use for birds as well as breeders, consumers and the environment.

Keywords : poultry, microflora, modification of bacterial community; digestive tract, digestion, metabolism, health, intestinal immune system, growth, product quality

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Introduction

To date, the flora of the digestive tract of birds has been considered as playing a minor role compared to the flora of the colon of mammals. Moreover, it has been subjected to the influence of antibiotic growth promotants (AGPs). Subsequently to the development of bacterial resistances to antibiotics, particularly in human medicine, the dietary supplementation with AGPs has been called into question by legislation and consumers. Their withdrawal has resulted in an increased interest in the role of the microflora of the digestive tract. Therefore, it is necessary to gain a better knowledge of the microflora of the digestive tract and its effects to be able to propose effective AGP alternatives.

The following text gives an account of the current state of knowledge of the microflora of the digestive tract of poultry. First, we present the type of flora typically found in the digestive tract of these animals and the various factors influencing its profile. Secondly, we address the effects of the flora on digestive physiology, nutritive value of feed, animals' health, and consequences of the presence of or changes in the microflora on the performance and quality of animal products.

Characterization of the digestive flora of chickens

DESCRIPTION AND LOCATION IN THE DIGESTIVE TRACT

Generally speaking, the digestive flora includes the unicellular microorganisms housed in the digestive tract, i. e. bacteria, fungi and protozoans. Bacterial populations, which are the predominant microorganisms, represent a wide range of interacting metabolic and morphologic types.

The digestive flora of birds has been largely studied and has proved to be different from that of monogastric mammals (Smith, 1965, Perez de Rozas et *al.,* 2004), which is probably due to anatomical and physiological differences. In particular, the colon is much more developed in monogastric mammals than in birds. The majority of the studies led on the digestive flora of birds involved cultures on a variety of selective and non-selective media; however, a great part of bacteria (up to 90% according to some estimations) are unable to grow under these conditions (Lan *et al.,* 2002). Therefore, standard microbiological methods only very partially reflect the digestive ecosystem. In order to solve this problem, molecular techniques have been developed. They enable microorganisms to be revealed using their 16 S ribosomal DNA, whatever their viability conditions. These techniques have technical limitations such as DNA isolation, biased DNA amplification or cloning. They give however a more precise and complete image of the microbial diversity than cultures. Nevertheless, they may also lead to underestimation or overestimation of certain species. As theses techniques are only in their initial development in the case of birds, the available information is still very incomplete. Thus, the digestive flora of birds and its variations are still poorly known, and hence remain to be investigated.

The current knowledge on the digestive flora of birds is the following. The total number of bacteria in the digestive tract is higher than the number of eukaryotic cells constituting the body of the host. Three types of bacteria are distinguished: dominant bacteria ($>10^6$ colony-forming units (CFU)/g content), subdominant bacteria (10^6 to 10^3 CFU/g content) and residual bacteria ($<10^3$ CFU/g content). In chickens, the main sites of bacterial activity are the crop and the caeca and, to a lesser extent, the small intestine. A large proportion of these bacteria are Gram positive and mainly include facultative anaerobes from the crop to the terminal ileum, while caeca additionally contain strict anaerobes which are dominant (Fuller, 1984, Gong *et al.,* 2002, Lu *et al.,* 2003, *Tables 1 et 2*). The crop flora is mainly composed of

lactobacilli attached to the epithelium and forming an almost continuous layer, and enterococci, coliforms and yeasts. In the gizzard and proventriculus, the low pH is responsible for the reduction in the bacterial population (*Figure 1*). In the duodenum, conditions are not favourable to flora development because of the presence of numerous enzymes, high oxygen pressure, presence of high concentrations of antimicrobial compounds such as bile salts, and reflux movements from the jejunum to the gizzard which result in a rapid change in environmental conditions. Further in the small intestine, the environment becomes more favourable to bacterial growth because of the lower oxygen pressure and lower enzyme and bile salt concentration (reabsorbed by the host and partially broken down by the microflora). Thus, the ileum contains 10^9 bacteria per g of contents (Apajalahti *et al.,* 2004). These are mainly facultative anaerobes, such as lactobacilli which make up the majority, enterococci and coliforms. In the caeca, the slow turnover of contents (1 to 2 times/day) facilitates bacterial development and results in an increase in their number (10^{11} bacteria per g of contents, Apajalahti *et al.,* 2004) and diversity. Strict anaerobes are in majority, but facultative anaerobes are also present. Thus, *Clostridiaceae* are a particularly large population (Lu *et al.,* 2003).

The digestive flora is composed of a broad diversity of bacteria. In the caeca, Zhu *et al.* (2002) identified 243 different sequences representing 50 phylogenetic groups or subgroups of bacteria, with 89% of the sequences belonging to 4 phylogenetic groups. For their part, Apajalahti *et al.* (2004) found 640 different species and 140 different bacterial genera in the gastrointestinal tract. Very large proportions, up to 90% of species according to some authors (Gong *et al.,* 2002, Lan *et al.,* 2002, Zhu *et al.,* 2002, Apajalahti *et al.,* 2004, Bjerrum *et al.,* 2004), have never been described, yet.

The microorganisms of the digestive flora may be located in the gut lumen, buried in the mucus layer or adhering to the digestive mucosa where they can form very important cell

layers (Fuller, 1984). The luminal flora is a function of available nutrients, transit rate and the presence or absence of antimicrobial substances. The mucosal flora depends on whether specific adherence sites are expressed on enterocyte membranes by the host, on mucus production rate, secretory antibody (Ig) production, and cellular material extrusion from the membrane. These mucosal bacteria are in close contact with the host and probably play a role of high importance. Yet, this flora has been little studied although it is actually different from the luminal flora (Gong *et al.*, 2002, Zhu *et al.*, 2002).

Although the greatest part of the work performed on the digestive flora of birds is based on chickens, a small part has addressed other avian species of economic interest (Smith, 1965, Barnes, 1979, Mead, 1989). Thus, compared to chickens, the flora of ducks that is housed from their dilated oesophagus up to the small intestine comprises few lactobacilli and numerous coliforms, clostridia and enterococci in the small intestine, and is more abundant in the caeca. In turkeys, the caecal flora bears some similarities with that of chickens.

CRITICAL FACTORS

The digestive flora is influenced by animal strain and sex. Moreover, each individual houses a digestive bacterial community that is its own (Zhu *et al.*, 2002).

The digestive flora evolves with age. At hatching, the digestive tract is a sterile environment where the flora grows rapidly after hatching. Settlement of the microflora depends on the egg's microbial environment at hatching, which determines the order in which animals are exposed to microorganisms, their ability to colonize the intestine and their interactions. Thus, from day old, the ileum and the caeca house 10^8 and 10^{10} bacteria per g of digestive contents, respectively (Apajalahti *et al.*, 2004). Their number reaches 10^9 and 10^{11} bacteria per g at 3 days of age and remains relatively steady until 30 days of age. The caecal

as well as intestinal flora undergoes changes and diversifies with age (Knarreborg *et al.*, 2002; Lu *et al.*, 2003).

The microflora is influenced by the rearing environment. Overall, increased breeding density and thermal stress seem to increase adverse bacteria to the detriment of beneficial bacteria (Suzuki *et al.*, 1989). The presence of intestinal parasites, such as coccidia, leads to damage of the intestinal mucosa and thereby produce new substrates for the microflora leading to its modification (Kimura *et al.*, 1976). However, the flora might be only slightly modified in animals from similarly managed farms.

Aside from the modulating effect of feed AGPs (Knarreborg *et al.*, 2002), the digestive flora is a function of the diet itself as dietary ingredients are potential substrates for bacterial growth. The digestive flora can be modified by the presentation as well as type of cereals, particularly by the presence of water-soluble non-starch polysaccharides (WS-NSP). Thus, Mathlouti *et al.* (2002) found an increase in facultative anaerobic bacterial populations, including lactobacilli and coliforms, in birds fed a wheat and barley-based diet instead of a maize-based diet. Consumption of a whole wheat-based diet compared with a ground wheat-based diet induces a change in the flora (Gabriel *et al.*, 2003, Engberg *et al.*, 2004). According to Engberg *et al*. (2002), pelleting of feed contributes to an increase in coliforms and enterococci in the ileum, and a reduction of *Clostridium perfringens* and lactobacilli in the distal parts of the digestive tract. Similarly, the origin of fats, starch or proteins can modify the flora. Minerals and vitamins may also have an effect. Thus, Orban *et al.* (1997) found an increased bifidobacterial number with a twofold vitamin-mineral premix supplementation level (1% instead of 0.5%). Similarly, Xia *et al.* (2004) showed a decrease in the total viable counts of *Escherichia coli* and *Clostridium* in the intestine and caeca of chickens fed supplementation with copper-bearing montmorillonite, the supplementation with copper alone having no effect.

These various factors account for flora differences found between flocks reared under different conditions. Thus, the digestive flora differs between fast-growing animals reared according to standard management practices for broilers and animals reared in more extensive conditions, i. e., slow-growing strains, feed without antibiotics, lower rearing density and with access to outdoor areas (Bjerrum *et al.*, 2004). Animals from the same hatchery and fed the same feed may also show flora differences due to flock management differences.

Impact on digestive physiology

The interaction of bacteria with the intestinal mucosa and the production of various metabolites such as short-chain fatty-acids (SCFAs) and polyamines result in anatomical and physiological changes in the digestive tract (Coates, 1980, Furuse and Okumura, 1994). Thus, the relative weight of the small intestine is higher in conventional animals compared with germ-free animals. This is due to the increased relative length of the intestine and the thickening of the wall, mainly associated to connective tissues, particularly the *lamina propria*, but also to the lymphoid tissue. In conventional birds, intestinal villi are higher in the jejunum and ileum compared to germ-free birds, but the surface area developed by microvilli per surface unit is smaller. Besides, intestinal villi have a less regular form. Crypts are also deeper all along the small intestine and the number of dividing cells is higher, thereby leading to an increased cell turnover from the distal duodenum to the ileum. Enterocytes reach the tops of the villi more rapidly and are less mature. Consequently, the total activity (per g of tissue) of intestinal digestive enzymes, such as maltase and saccharase, eventually decreases. However, these disaccharidases show similar activities when expressed per animal weight. The presence of a flora does not induce changes in other enzymatic activities involved in digestion, such as amylase, lipase or pancreatic trypsin found in small intestine contents

(Lepkowsky *et al.*, 1964, Philips and Fuller, 1983). Similarly, *in vivo* absorption of nutrients, such as methionine and glucose, is not modified (Yokota and Coates, 1982).

In the caeca, the presence of microorganisms induces a higher relative weight and a thicker wall (Furuse and Yokota, 1984). Although the caeca are the main site housing the digestive flora, very few studies on flora-related mucosal changes have been published. Increasing the flora by introducing lactose in the diet decreases the *lamina propria* thickness and increases cell proliferation (Tellez *et al.*, 1993). Cell turnover time is shorter in the distal part of the caeca versus the proximal part, probably because of the flora largely present in this portion (Takeuchi *et al.*, 1998).

In the presence of a flora, digestive contents are generally more acid and the redox potential lower than in germ-free animals. The microflora induces an increase in the production of mucins (Sakata and Setoyam, 1995), and a change in the proportions of the various types of glycoproteins that compose them. In germ-free birds, there is no change in intestinal transit compared to conventional animals, unlike in laboratory mammals which show an enlarged caecum resulting in a slower transit (Coates, 1980). However, the effect of the flora on transit might be a function of the type of diet as this has been observed in the case of diets containing WS-NSP-rich raw materials, which increase digestive content viscosity (Nahashon *et al.*, 1994b). Flora-produced SCFAs enhance ileal motility (Cherbut, 2003).

Consequences on nutritive value of feed

FEEDSTUFF DIGESTION

Microorganisms are in competition with the host for the use of dietary ingredients in the digestive tract. The feedstuffs mostly concerned are those that are poorly digestible by the host. Besides, in the case of WS-NSP-rich diets, the flora is believed to play a role in the

negative effect observed on feedstuff digestion, although this role is controverted (Maisonnier *et al.*, 2003). Conversely, the microorganisms of the digestive tract might have a positive effect by releasing nutrients that the host can absorb in the intestine and the caeca, the latter also being able to transport carbohydrates and amino acids (Moreto and Planas, 1989).

Among the digestible carbohydrates, maize starch does not show any difference in digestibility in the presence of the microflora (Kussaibati *et al.*, 1982a), although some microorganisms are able to hydrolyze it, particularly in the crop. Most non-digestible carbohydrates are fermented by the microflora in the crop, but mainly in the caeca (Mead, 1989). As for cellulose however, the microflora of chickens does not seem to produce enzymes capable of hydrolyzing it, contrary to turkey.

In chickens under three weeks of age, the flora reduces the faecal apparent digestibility of vegetable fats by 2 points and that of animal fats by 10 points (Boyd and Edwards, 1967, Kussaibati *et al.*, 1982a). This is mostly due to deconjugation of bile salts by bacteria, and also in part to the endogenous excretion of cellular lipids (enterocyte desquamation, bacterial biomass). As conjugated bile salts are used for micelle formation, their low concentration reduces lipid solubilization and hence lipid absorption, particularly those containing long-chain saturated fatty acids. As a consequence, digestibility of saturated fatty acids, such as palmitic and stearic acids, is highly reduced, while that of unsaturated fatty acids, such as oleic and linoleic acids, is not modified by the presence of the microflora (Boyd and Edwards, 1967).

The effect of the microflora on protein digestibility depends on diet compositions. With a casein, gelatin and egg white-based diet, Salter and Fulford (1974) found no difference in apparent faecal digestibility between germ-free and conventional animals, whereas with a maize and soybean-based diet, Kussaibati *et al.* (1982a) found a reduced digestibility in conventional animals. This drop in digestibility may be due to the increased endogenous

protein production from mucus, cellular debris and bacterial biomass. However, the microflora reduces the amount of proteins in digesta because it uses these endogenous proteins, as well as the food proteins not hydrolyzed by the host.

NITROGEN AND ENERGY METABOLISM

The flora can have a beneficial effect on nitrogenous metabolism. Indeed, dietary and urinary (e. g. uric acid) nitrogenous compounds which persist in the caeca are broken down by bacteria into SCFAs and ammonia, which are then absorbed (Braun and Campbell, 1989, Braun, 2003). Ammonia is partly incorporated into the glutamate that is used for protein or glucose synthesis. Conversely, protein needs are higher for conventional chickens than germ-free chickens. In the presence of a microflora in the digestive tract, protein synthesis increases by 25% in the liver (metabolism and detoxification of bacterial products) and 45% in the gut, i. e., a 6 to 8% increase in total protein syntheses (Muramatsu *et al.*, 1987). Besides, with a diet poor in metabolisable energy (2,800 kcal/kg), the presence of a microflora induces a reduction in protein utilisation (Furuse and Okumura, 1994).

The microflora also negatively (Kussaibati *et al.*, 1982b) or positively (Furuse and Okumura, 1994) affects the metabolisable energy content of the diet. As the type of diet influences the flora, this could account for these differences. The negative effect of the flora can be explained by the reduced nutrient digestibility, particularly that of lipids, fermentation-related losses of the carbohydrates available for the animal and increased endogenous losses. On the other hand, the flora has a beneficial effect associated to the fermentation of carbohydrates not used by the host. It therefore produces SCFAs that can be an energy source for enterocytes and the animal after being absorbed in the caeca in particular. However, estimates of energy benefits related to SCFAs vary greatly depending on authors (Jozefiak *et al.*, 2004). Besides, the flora increases the energy requirement for the maintenance (Furuse

and Okumura, 1994) as a consequence of the increased intestinal protein synthesis, but mostly because of dietary energy being taken over by bacteria, and consumption of the energy required to detoxify the numerous substances produced by the microflora.

MINERALS AND VITAMINS

The microflora has a negative effect on the absorption or transport of calcium absorbed by intestinal tissues (Smith and Soares, 1984). It induces an increase in magnesium and phosphorus needs. It reduces manganese absorption, but has no effect on other trace minerals such as copper, zinc and iron (Henry *et al.*, 1987). On the other hand, the flora, because of its production of SCFAs, contributes to the absorption of minerals, like sodium, in the caeca and colon (Braun, 2003).

Intestinal bacteria synthesize vitamins B, K and E, but it is thought that only folic acid (vitamin B9) is available for the animal (Coates, 1980). Besides, in the presence of an intestinal flora, the needs for some vitamins, like pantothenic acid (vitamin B5), are increased for bacterial product detoxification. In addition, vitamins B are more poorly absorbed *in vitro* in the gut of conventional chickens than in the gut of germ-free chickens. However, these results have not been confirmed *in vivo*. The flora might also have a negative effect on the absorption of fat-soluble vitamins which require bile salts.

Consequence on the animal's health

ADVERSE EFFECT OF SOME BACTERIA

The digestive tract of birds can house pathogenic bacteria, such as *Salmonella*, some *Escherichia coli*, *Clostridium perfringens*, etc. Gram-negative bacteria produce endotoxins that are released during the lysis of the lipopolysaccharides that are part of their cell walls.

These endotoxins cause fever and the release of endogenous pyrogenes, which act on thermoregulation centres in the hypothalamus. Other toxins may affect intestinal motility, thereby causing diarrhoeas.

Fermentations, particularly of the amino acids founds in litters, by the digestive flora lead to the production of irritating components, like ammonia which causes conjunctivitis and results in respiratory problems in animals (Thomke and Elwinger, 1998). Besides, the use of AGPs has often been reported to reduce humidity in excreta, with beneficial consequences on the health of poultry: decrease in leg problems and restriction of pathogen development in litters.

PROTECTION AGAINST ADVERSE MICROORGANISMS

The first flora that settles hampers other microorganisms from settling. This phenomenon is called “competitive exclusion” (Ducluzeau and Raibaud, 1979). Accordingly, a beneficial flora can prevent pathogenic bacteria from settling. Investigations have mainly concerned *Salmonella*, but also *Campylobacter spp*., *Yersinia, E. coli*, *Clostridium perfringens*, *Listeria*, etc. It has been demonstrated that *Salmonella* colonisation of the caeca is limited by the treatment of chicks just after hatching with a caecal flora from healthy adult chickens. The use of numerous bacterial species is more effective than mixtures containing few species (Stavric and D’aoust, 1993). Similarly, lactobacilli impair coliform growth (Fuller, 1984).

There is a variety of mechanisms at the origin of competitive exclusion. Certain beneficial bacteria create a microenvironment hostile to other bacterial species by producing antimicrobial metabolites. Indeed, in the crop, lactobacilli produce a large amount of lactic acid beneficial to them, but deleterious to coliforms and most other bacteria (Fuller, 1984). Bacteria produce SCFAs which also have a bacteriostatic, and even bactericidal, effect variable according to the types of acids and bacteria (Wielen *et al.*, 2000). Besides, certain

bacteria, such as lactobacilli, produce bacteriocins which have a wide spectrum of activity. Thus, reuterin, secreted by *L. reuteri*, is effective against salmonellae, coliforms and campylobacters (Mulder *et al.,* 1997). Metabolites of oxygen (hydrogen peroxide, free radicals) are also produced (Gilliland and Speck, 1977, Piard and Desmazeaud, 1991). They can exhibit bacteriostatic or bactericidal activity against lactic or non-lactic acid bacteria. Hydrogen peroxide can also lead to the formation of inhibitory compounds, which are bacteriostatic for lactic acid bacteria and bactericidal for Gram-negative bacteria. Beneficial bacteria also have an effect by modifying the receptors used by adverse bacteria or their toxins, thereby hampering their development in the digestive tract (Rolfe, 1991). In addition, the beneficial flora intervenes through the competitive use of essential nutrients (Rolfe, 1991) and plays a role in the modulation of the immune system.

REGULATION OF THE IMMUNE SYSTEM

The intestinal flora participates in the development and maintenance of an effective intestinal immune system (Salminen *et al.,* 1998). It is involved in the development and regulation of the immune response by influencing the number, distribution and degree of activation of cell populations of the intestinal immune system.

Bacteria stimulate innate immunity by activating phagocytosis and cytokine synthesis by macrophages. However, these regulate the inflammatory response, which must be functional without being excessive. Continuous activation of the immune system by the digestive flora results in decreased zootechnical performances (Klasing *et al.,* 1991). However, bacteria can also attenuate the inflammatory response (Neish *et al.,* 2000).

The digestive flora also modulates specific immunity at a local and systemic level. In particular, oral tolerance to dietary and bacterial antigens may be profoundly modified by the

commensal flora. The digestive flora is also involved in the modulation of the immune response against pathogens.

Intestinal bacteria have different immunomodulating properties according to species (Maassen *et al.*, 1998), probably associated to the composition of their cell wall (Herich and Levkut, 2002). Therefore, the consequences on the animal's immune response depend on the composition of the flora.

Consequences for animal productions

GROWTH

Conventional animals usually show a reduced growth compared to germ-free animals (Kussaibati *et al.*, 1982a, Furuse and Okumura, 1994) because of the various negative effects of the flora mentioned above (decreased digestibility, nutrient takeover, increased gut development and immune system stimulation).

This negative effect on growth is related to the presence of certain microorganisms. Thus, two bacterial types belonging to the common caecal flora have proved responsible: *Enterococcus faecium*, now known to be *Enterococcus hirae* (Fuller, 1984), and *Clostridium perfringens*; but other bacteria to be identified might be involved.

QUALITY OF PRODUCTS

The intestinal microflora has effects on the bacteriological quality of products and on their composition and organoleptic qualities.

Carcass contamination at slaughter by pathogenic bacteria from the digestive tract affects the hygienic quality of poultry products. Thus, *Salmonella*, *Campylobacter spp*., *Helicobacter*

pullorum, *Listeria spp*., *Clostridium perfringens*, *Yersinia* and *Hafnia* can be found. These bacteria represent a hazard as well for animals as for humans in the case of *Salmonella* for example, or only for humans, e. g. with *Campylobacter jejuni*.

The composition and organoleptic quality of meat and eggs are altered by the digestive flora. Certain probiotics increase meat protein content and reduce its fat content, including cholesterol (Wambeke and Peeters, 1995, Haddadin *et al.,* 1996). The intestinal flora modifies meat flavour. Thus, meat maturation by hanging an uneviscerated bird leads to the development of gamey flavours which might be in part a result of the microflora of the digestive tract (Barnes, 1979).

Regarding the egg, its surface as well as contents are modified by changes in the intestinal microflora. Some authors have observed that probiotics increased eggshell thickness (with identical egg weights) and breaking strength (Mohan *et al.,* 1995, Tortuero and Fernandez, 1995, Panda *et al.,* 2000). Egg composition, appearance and taste may be altered. Thus, egg white quality (albumen height) is improved by adding on certain probiotics (Nahashon *et al.,* 1994a). The presence of a flora brings about a change in the fatty acid composition of the egg yolk (Furuse and Okumura, 1994). Its cholesterol content can be reduced by the use of certain probiotics (Mohan *et al.,* 1995). The fishy or undesired taste of eggs from brown layers found in the presence of critical feed compounds, such as rapeseed or fish meal, is due to Gram-positive bacteria from the intestinal flora that convert choline into trimethylamine which builds up in the egg yolk.

Conclusions

The intestinal flora affects the animal at numerous levels. The current objective is to be able to take advantage of its positive effects (e. g., competitive exclusion, development and modulation of the immune system), while minimizing its negative effects (e. g., metabolic cost induced by the increased gut development and continuously activated immune system). These negative or positive effects vary according to the flora composition, which itself varies according to numerous parameters. This is a complex balance that needs to be further investigated. There is still relatively little information on the identity and in vivo activity of those organisms that appear to influence host nutrition and growth performance. Such information is likely to be gained in the future from nucleic acid analyses currently being developed. These new approaches might allow in the future to be able to direct the flora toward a beneficial purpose for the animal, the production system and the consumer's health.

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