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Science and Technology
Meri-Rastilantie 3 B, FI-00980
Helsinki, Finland
e-mail: info@world-food.net

Journal of Food, Agriculture & Environment Vol.8 (2): 695-702. 2010

www.world-food.net

Functions of microbiota in monogastric gastrointestinal tract and new practices in animal production

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Received 23 November 2009, accepted 27 March 2010.

Abstract

The mammalian gastrointestinal (GI) tract harbors a complex micro-ecosystem consisting of countless bacteria. As an essential 'organ', the gut microbiota plays an important role in host nutrition and health by promoting supply, digestion and absorption of nutrient, preventing pathogen's colonization, and shaping and maintaining normal mucosal immunity. Antibiotics used as dietary additive not only interrupt the commensal microbiota in animal GI tract, but also lead to microbial resistance and antibiotics residues in animal products, which has become a worldwide problem to the health of human being and animals. So antibiotics have been banned as feed additives in many countries. Therefore, animal nutritionists and feed manufacturers are becoming increasingly interested in the topic. This paper mainly focuses on the development and important functions of microbes in monogastric GI tract as well as the factors affecting them. Applications of some novel alternatives in practice to keep GI tract ecosystem healthy, such as probiotics, prebiotics, Chinese herbal additives and exogenous enzymes, and their mechanisms were also introduced. The new alternatives to keep health of GI tract have great ecological, environmental issues and economic social effects. It may lead to the rational design of supplementation for animal production to study the functions of the microbiota, which has profound implications to support continuous development in livestock production in the future.

Key words: Gastrointestinal tract, microbiota, animal production, microbial ecological agents, Chinese herbal medicine.

Introduction

Science understandings of mammalian gut microbial ecology was limited and based primarily on microbial culture studies that were biased towards organisms that could survive outside the gut. Nevertheless, almost all of the analyses to date indicate that the animal stomach and digestive tracts, as the biggest immune tissue, harbor a complex ecosystem consisting of countless bacteria [1-4]. With the development of biotechnology, the complex interactions between microbiota in the GI tract and host's health have been recognized recently [5-9].

There has been a close relationship between microbes residing in the gastrointestinal (GI) tract and the mammalian host during the long course of evolution [1-3]. The mammalian GI tract is inhabited by more than 400 species of bacteria, and the total number of microbes is approximately 10^{14} which is about 10 times more than their tissue cells [2, 4-6]. The commensal microflora in the GI tract plays an essential role in influencing the nutrition and health of the host [5], by promoting supply, digestion and absorption of nutrients, and improving growth performance, preventing pathogen colonization, and shaping and maintaining normal mucosal immunity [7]. To some degree, gut microbiota could now be considered an essential 'organ' placed within a host organ [8-12].

This paper reviews mainly on the development and important functions of microbes in GI tract of livestock especially pig, their working mechanisms, and application of some novel alternatives to keep animal healthy in practice. It also aims to illustrate some examples of innovative trends in animal feeding, along with the discussion on the analytical challenges for reliable assessment of the risks/benefits ratios along the food chain.

Development of the Microbes in the GI Tract

Microbial colonization in early life contributes to GI tract development, which may affect the individual's health throughout life [1, 13]. Animal's GI tract is non-microbial before its birth. Immediately after a sterile birth, bacteria originating from the mother, environment or diet will colonize in it [14]. The piglets' GI tract microflora develops rapidly and characteristic microbial populations including *E. coli*, *Lactobacillus*, *Streptococcus*, *Clostridium* and *Bifidobacterium* (Fig. 1). Then profound changes occur in the intestinal ecosystem when the young mammals are weaned from their mother's milk onto solid food [15]. Once solid food is digested, obligate anaerobes increase in number and diversity, especially in the hindgut, and the composition of the microbial varies in different parts of the GI tract [16]. The mammals are initiated into an organized and lifelong process of colonization by foreign organisms. A substantial microbial fermentation takes place in the stomach of swine, and in the lower intestinal part, chyme retention time is increased; so more time is available for bacteria to multiply, resulting in extensive and diverse anaerobic microbial populations [15].

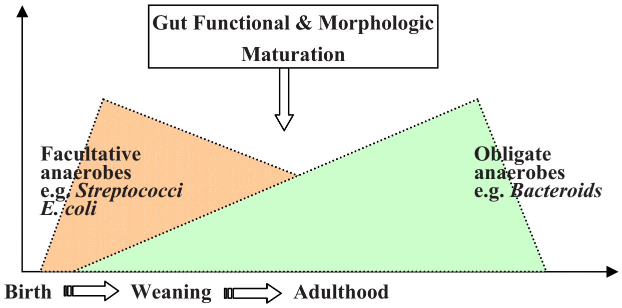

Figure 1. Development of the microbes in GI tract.

It is non-microbial before animals are born, and immediately after a sterile birth, bacteria originating from the mother, the environment or the diet will colonize in the GI tract. Then profound changes occur in the intestinal ecosystem when young mammals are weaned from their mother's milk onto solid food. Once solid food is digested, obligate anaerobes increase in number and diversity, and mammals are initiated into an organized and lifelong process of colonization by foreign organisms [4, 14].

The intestinal maturation occurs during suckling to weaning phase transition. The mammalian intestinal epithelium undergoes marked structural and functional changes during postnatal development such as increase in mucus production, immunological adaptation to new microbes and nutritional antigens, and digestive adaptation to new nutriments, resulting in a functionally mature intestine containing the digestive enzymes necessary to cope with the diet of the adult [17]. The effects appear greatest in young fast growing animals during specific periods when microbial community is subject to large change, such as weaning, and diminish as aging. The age effect is consistent with the capacity of the normal gut microbiota to resist the change as the animal grows. At the meantime, the numbers of all kinds of microbes in GI tract change quickly until it becomes relatively sustainable.

Normal microflora mainly comprises bacteria, and also includes viruses, fungi and protozoa. These microbes can be divided into two parts upon the time they live in the GI tract, autochthonous (or indigenous) and allochthonous (or transient) [18]. The former are those that can establish a stable population consisting predominantly of obligate anaerobes, including *Bacteroides* and *Clostridium* species, and some of them have long been appreciated for potential benefits to the host: provision of essential nutrients, metabolism of indigestible compounds, defense against colonization by opportunistic pathogens, and contributions profoundly to the development of the intestinal architecture as well as providing nutrients to maintain nutritional status and homeostasis of the whole organism [19]. Anaerobic bacteria make up most of the microbes in intestine. More than 90% of the bacterial population is obligate anaerobes, predominant species including *Bacteroides, Eubacterium, Bifidobacterium, Fusobacterium, Peptostreptococcus*, etc.

Important Positive Effects of Microbes to Host

As early as 1885, Louis Pasteur firstly proposed that microorganisms might influence the human immune system. Studies showed that bacteria could play a crucial inductive role in the normal development of animal organs by affecting fundamental developmental processes, such as cell death and differentiation. Using germ-free animals, the impact of the microbiota on mammalian gut development has been studied [20-22]. For decades, science has been able to demonstrate the importance of the microbiota for the nutrition, development and maintenance of the immune system and other positive effects to their hosts, which are increasingly evident [1, 13, 23].

Microbes and gut barrier development: The indigenous bacteria play a crucial inductive role in gut development during early postnatal life and greatly affect the structure and function of the intestinal mucosa [4, 21, 24]. It is showed that the presence of limited kinds of intestinal bacteria is responsible for the development of the gut immune system, such as secretory IgA, major histocompatibility complex molecules and intraepithelial lymphocytes [25].

Several key similarities are exhibited by the developmental transitions that are represented by fucosylated glycan synthesis, expression of the microbicidal protein Ang4, and intestinal angiogenesis[26]. First, all are normally induced during weaning in conventional mice but are arrested in germ-free mice. Second, completion of all three developmental programs can be reinitiated in adult germ-free mice upon colonization with either a complex gut microflora or with a single member of this community. The intestine may be genetically pre-programmed to receive bacterial signals during weaning, and when these signals are lacking gut development is arrested, and resident bacteria are both necessary and sufficient to reverse this arrest in adulthood. Thus, the postnatal mammalian intestine appears to be poised for interaction with its microbial partners, which are essential for its normal development [27].

Microbes and maintenance of immune tolerance: Microflora plays an important role in the development and expansion of lymphoid tissues and in the maintenance and regulation of gut immunity [28]. Many people concluded that the gut microorganism bacterial polysaccharide (PSA) directs the cellular and physical maturation of the developing immune system [5-6, 26, 29].

The intestine is the primary immune organ of the body represented by the gut-associated lymphoid tissue (GALT) through innate and acquired immunity [30, 31]. GALT, being the predominant source of sensitized T and B cells distributed throughout the body, provides the host with protective mechanisms against invasion by potential pathogens across the mucosal surface, and on the other hand, it plays a role in the development of induced tolerance against harmless products of digestion and the normal intestinal microbiota, all of which are potentially immunogenic [32,33]. Another critical feature of the mucosal immune system is the ability to discriminate between harmful pathogens and the harmless members of the commensal microbiota. This is achieved in part, by an evolutionary-conserved family of cell surface and cytosolic receptors, referred to as Toll-like receptors (TLRs), which function in microbial recognition. TLRs play a crucial role in host defense against microbial infection. Activation of TLRs by commensal microflora is critical for the protection against gut injury and associated mortality [28, 34].

In fact, some bacteria that are commonly found residing as part of the microflora are actually opportunistic pathogens that can cause disease when growth or containment within their ecological niche is uncontrolled. Perhaps the most important effect is their ability to thwart the colonization and overgrowth of potential pathogens. This is most evident on mucosal surfaces where species such as lactobacilli and bifidobacteria have been shown to inhibit the colonization of pathogenic bacteria such as *Escherichia coli* or *Shigella* spp. [5, 35-37].

Microbes and nutrient availability and digestibility: The microflora in GI tract has important functions for nutrients and their digestion and absorption [38]. It is suggested that the modulation of gut microbiota affects host metabolism and has an impact on energy harvest from the diet and energy storage [8, 38]. Conventionalization of adult germ-free C57BL/6 mice with a normal microbiota harvested from the distal intestine (cecum) of conventionally raised animals produces a 60% increase in body fat content and insulin resistance within 14 days despite reduced food intake [39]. Gut microbiota may be an important environmental factor in affecting nutrition storage.

The microbes can produce for hosts' nutrition proteins, AA, enzymes, VFA, VB_2, VB_6, VB_{12} and so on [18, 40-42]. Intestinal bacteria themselves are rich sources of protein (as much as 60-65%), which can be also used by hosts.[7] In pigs, up to 30% of energy for maintenance could be retained due to microbial biodegradation, particularly in the large intestine [43]. In all, bacterial and hosts have complex cell communication system. The mucosal surface of the GI tract represents a major entry point and ecological niche for many microbes, and the normal microbes form an important immune barrier, absorbing nutrients, whilst preventing invasion by organisms.

Negative Effects of Microbes to Host and Problems of Antibiotics

Normal microbes have many positive effects above-mentioned to their hosts, but the microflora is not always beneficial to the host's health [44]. When the balance is interrupt, it may lead to many diseases. Weaned piglets often suffer from post-weaning diarrhea followed by impaired growth performance and high mortality. In order for the intestine to function optimally, the 'balance' of the bacteria must be maintained, and this appears to be increasingly difficult as lifestyles change. For a century, science has known that not only does the colonic microflora play an essential role in digestion and provide substrates for metabolism in animal tissues, but also diet can dramatically alter the microbial composition of the microbiota and influences bacterial metabolism, and specific items of the diet may have selective effects on the microbiota[45]. Also many other factors, such as the antibiotics, weaning, pH of the intestinal content and environment are responsible for the types of bacteria, population and metabolic activity in the GI tract (Table 1) [46].

Some antibiotics have been used as growth promoting in feed for over five decades, and they are still effective. However, over prescription and misuse of traditional antibiotics as dietary additive not only interrupted the commensal microflora in animal GI tract but also continuously led to microbial resistance and antibiotics residues in animal products, which has become a worldwide problem to the health of human being and animals [47, 48]. So a restricted use of antibiotics and even a total ban of using antibiotics as growth promoters is needed. A better understanding of bacterial pathogenesis has grown increasingly important because of the emergence of new pathogens and the growing problems of resistance among enteric pathogens and other enteric bacteria [49]. Outbreak of diseases caused by pathogens or enteric floras with resistance were more and more. A recent prominent example was the outbreak in the summer of 2005 in Ziyang County, Sichuan Province, China, where severe invasive *suis serotype* 2 infection occurred in humans and pigs, and killed 38 persons in 204 infected persons [50]. An earlier, smaller outbreak in Jiangsu Province in 1998 killed 14 of 25 reported human patients [51].

Thus, antibiotics have been banned as feed additives in many countries in EU and Swiss because of their toxicological and resistance risk, and this is a trend of modern living, which prompts the market to propose some other alternatives to farmers, such as the administration of immuno-stimulants (i.e. colostrums) and/or natural substances having a direct or indirect anti-bacterial effect. The EU had imposed a total ban on the use of the 4 remaining feed antibiotic growth promoters by January 2006. Therefore, animal nutritionists and feed manufacturers are becoming increasingly interested in finding novel materials to substitute antibiotics to keep animal GI tract healthy and increase animal production, which will contribute to the sustainable development in livestock production [52].

This is particularly relevant, as microorganisms have developed resistance to antibiotics, thus prompting the need for alternative treatments for GI diseases [53, 54]. Non-antimicrobial approaches to therapy have become increasingly important with the emergence of serious antimicrobial resistance. Recently, attention has turned to indigenous non-pathogenic microorganisms and the ways in which they benefit the host.

New Practices to Keep Health of the GI Tract

The term of growth promotion applies to the increase in performance or productivity achieved in food producing animals following the addition to their diets of feed antibiotics or growth promoters. The mechanisms by which growth promotion is produced are only poorly understood. It is now generally accepted that any improvement in performance is directly related to a variety of direct effects on the gut microbial flora and associated indirect effects on intestinal tissues [55]. The direct effects include a reduction in the thickness of the intestinal mucosal layer, inhibition of bacterial growth, interference with bacterial cell wall development, induction of filament formation and interference with the

Table 1. Examples of factors that may affect the composition of the gut microflora in animal.

Environmental factors	Factors of animals themselves
Type of feeding	Weaning
Redox potential	Peristalsis
Amount, chemical composition and availability of growth substrates	Individual fermentation strategies by the bacteria
Immunological interactions	Production of bacterial metabolites
Presence of antimicrobial compounds	Availability of inorganic electron acceptors
Intestinal host-related	Gut pH
Xenobiotic compounds	Availability of colonisation sites
Others	Age of the host

metabolism of intestinal bacteria [56]. It is clear from available data that conventional animals are depressed in performance and this depression can be alleviated by the administration of growth promoting agents in the diet of these animals. The lifting of this depression results in an improvement in performance, especially daily live weight gain and feed conversion efficiency. The GI tract microflora impacts growth efficiency and disease susceptibility through some mechanisms. So according to the factors affected the normal microbes as discussed above, new practical methods available for the different animal species have been found to keep the GI health [52, 57]. There has been a wide range of substances (i.e. microbial ecological agents, Chinese medicine herbals) used in human being, swine, poultry and other animals to influence the GI microflora.

Microbial ecological agents: Microbial ecological agents are the successful attempts to manipulate of the composition of the gut microbes towards a potentially more remedial community, which may be increase of *Lactobacillus* and *Bifidobacterium*, and decrease of *E. coli* in the intestine or apparent selection for *Lactobacillus*, commensals known to competitively exclude potentially pathogenic species from colonizing the intestine [58-60]. Microbial ecological agents include at least three, which are probiotics, prebiotics and synbiotics [61].

Probiotics: Probiotics, which according to the old definition are viable micro-organisms (bacteria or yeast) beneficial to the host[40], containing living beneficial bacteria are used as the new animal feed additives that have been used to change the composition of colonic microbiota, and beneficially affect the host by improving its intestinal microbial balance. They can adhere to the intestinal epithelial cells, stimulate gut mucosal immune tissue and induce cellular or humoral immune response [60, 62].

Probiotics, an emerging alternative therapy, are intestinal bacteria that promote health by stimulating optimal mucosal immune responses and can promote animal growth and development, enhance immune function and improve animal microbial flora [40]. The mechanisms by which probiotics prevent or ameliorate diarrhea are through stimulation of the immune system, competition for binding sites on intestinal epithelial cells with pathogens and secrete natural antibiotic substances [59]. These effects are more evident when treatment with probiotics, such as *Bifidobacterium* and *Lactobacillus* that are perceived as exerting health-promoting properties, is initiated early in the course of disease (Fig. 2)[23]. We

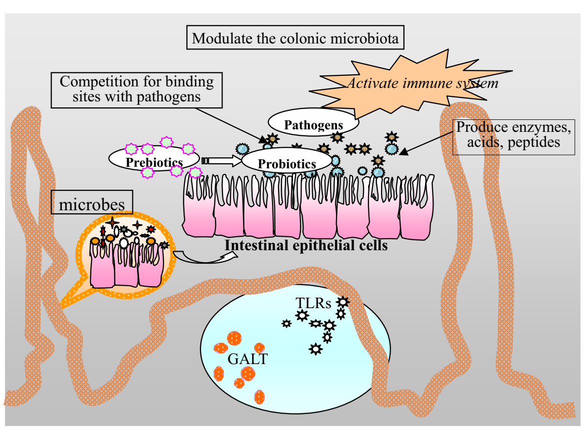

Figure 2. Functions of probiotics and prebiotics.

can use genetic engineering and molecular and genomics-based knowledge to modify probiotics that have special effect to host in feed and (or) food industry.

Current observations suggest that normal flora (or probiotic 'enhancement' of normal flora) may contribute to the resistance to frank pathogens, though in certain host contexts, the flora may be pathogenic/proinflammatory in and of itself. Probiotics can stimulate optimal mucosal immune responses, promote animal growth and development, enhance immune function and improve animal microbial flora [40,63]. The mechanisms by which probiotics prevent or ameliorate diarrhea are through stimulation of the immune system, competition for binding sites on intestinal epithelial cells with pathogens and secrete natural antibiotic substances. Prebiotics can significantly modulate the colonic microbiota by increasing the number of specific bacteria and thus changing the composition of the microbiota [61]. Gut-associated lymphoid tissue (GALT) provides the host with protective mechanisms against invasion by potential pathogens across the mucosal surface, and plays a role in the development of induced tolerance against harmless products of digestion and the normal intestinal flora. Toll-like receptors (TLRs), which function in microbial recognition, plays a crucial role in host defense against microbial infection.

Prebiotics: Prebiotics is a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of a limited number of bacteria species already resident in the colon [61]. Gibson [61] concluded that intake of prebiotics can significantly modulate the colonic microbiota by increasing the number of specific bacteria and thus changing the composition of the microbiota, and thus attempt to improve host health. Nondigestible oligosaccharides in general and fructooligosaccharides in particular, are prebiotics. There are many prebiotic oligosaccharides known which can stimulate the growth and colonisation of probiotic bacteria nowadays, e.g. mannanoligosaccharides (MOS), xylooligosaccharides (XOS), fructooligosaccharides (FOS) and galactooligosaccharides (GAS) [64-67].

Prebiotics are not only the most promising but also best investigated substances with respect to a bone-health-promoting potential, compared with probiotics and synbiotics [68]. More recently, as the viability of live bacteria in food products and during transit through the GI tract may be variable, the prebiotic concept has been developed.[60] The oligosaccharide mixture strongly inhibited the attachment of enterohepatic *Escherichia coli* ($P < 0.01$) and *Salmonella enterica* serotype Typhimurium ($P < 0.01$) to HT29 cells. Addition of the novel mixture at 4% to a commercial diet increased the density of bifidobacteria ($P < 0.001$), and decreased the pH ($P < 0.001$) compared with the control diet and the control diet supplemented with inulin.

The concept of synbiotics is proposed to characterize some colonic foods with interesting nutritional properties that make these compounds candidates for classification as health-enhancing functional food ingredients [61]. Synbiotics take advantage of both the addition of beneficial bacteria and the encouragement of the growth of resident beneficial bacteria.

Generally, pre- and probiotic products have provided inconsistent results, and research to better define optimal feed processing and application in animals is ongoing. As the microbiota management tools, probiotics, prebiotics and synbiotics have been developed and, indeed, commercialized over the past few decades with the expressed purpose of increasing numbers of bifidobacteria and/or lactobacilli within the gastrointestinal tract.These components can have effects not only locally but also systemically beyond the gut, and their effects are multiple but not classically nutritional.

Chinese herbal medicine (CHM): In Chinese culture, Chinese herbal medicine (CHM) is often used to maintain good health rather than to cure illness once it has developed, much in the same way that vitamin or mineral supplements or herbal preparations are used in Western countries. CHM have been used for centuries and their potential benefits identified by empirical usage. CHM are natural, safe, cheap and highly effective. So it would be expected as a dietary additive as a substitute of antibiotics.

CHM offers a very complex combination of bioactive compounds in the midst of proteins and sugars. Polysaccharides from various CHM have been shown to be immuno-potentialing both *in vivo* as well as *in vitro*. Polysaccharides from natural sources are a class of macromolecules that can profoundly affect the immune system and therefore have the potential as immuno-modulators with wide clinical applications. In our experiment, CHM ultra-fine powder could enhance digestion and absorption of protein or amino acids in early-weaned piglets [69, 70]. As potential antimicrobial alternatives, CHM may suppress the growth of bacteria in early-weaned piglets [69, 71]. We found that the CHM ultra-fine powder could effectively promote a healthy intestinal environment after weaning to improve the growth performance and reduce the incidence of diarrhea in the weaned piglets [69]. Further, one of our studies investigated the mechanisms of dietary supplementation with *Acanthopanax senticosus* (AS) extract in inhibiting weanling stress and promoting growth performance in weaned piglets. By metabolic activity of lactobacilli (detection of 16s rRNA by hybridization) in the small intestine of piglets, we demonstrated changes in composition and subsequent activity of several lactobacilli species and supplemental AS and antibiotics could improve disorder of microbial flora, while AS had a better effective tendency than antibiotics [72-79].

What is worth mentioning is that the main effective constituents of some CHM are prebiotics, e.g. inulin, so that sometimes CHM is used as or added into microbial ecological agents. The consensus is, however, the exact chemical composition of many plant extracts has not yet been established and the quality of raw herbs is frequently different in different cultivation areas and times of harvest. This may result in changes in the quantity of the bioactive components present in the raw material. Furthermore, in many cases, the bioactive components and their mechanisms of actions are unknown. To ensure quality, safety and efficacy of CHM, modern technology for analysis, research and production is necessary [72]. Understanding the action mechanism further can provide insights into how CHM provides beneficial and protective effects to the human and animal body [80]. The essence of traditional Chinese medicine is ecological medicine, so that the research of gastrointestinal flora microecology should be combined with the basic theory of traditional Chinese medicine [81].

Antibodies or peptide: Antibodies directed against key pathogens can be combined with probiotic strains and additional nutrients to foster their intestinal proliferation. In addition to the observed improved feed intake and growth in young animals, these products may reduce the dependence on antibiotics for disease control. Antibody products, similar to pre- and probiotics, would be expected to alter the gut microbial flora and prevent adhesion of pathogenic organisms to the gut mucosa. Several different approaches in providing animals with protective antibodies through the diet are being studied. The egg rich in disease-specific antibodies is spray-dried and fed to weaning pigs with the added advantage that a highly nutritious product is provided, which can be vaccinated against specific viral and bacterial pathogens commonly responsible for high morbidity and mortality in weaning pigs [81, 82].

Anti-microbial peptide can exert a host-protective effect such as antibacterial, antifungal, antiviral, anti-tumor, anti-inflammatory, and immuno-regulatory properties in various animals and humans[83, 84]. We have produced a new-type antimicrobial peptide bovine lactoferricin by gene engineering, which has a similar function as natural bovine lactoferricin. The peptide could decrease the concentration of *E. coli* in the ileum, caecum and colon ($P<0.01$) and increased the concentration of lactobacilli and bifidobacteria in the ileum, caecum and colon ($P<0.01$) [85].

Others: Feed enzyme supplementation also has good potential for broader application. Supplementation of endogenous enzymes can improve feed quality, increase digestibility of existing nutrients and reduce nutrient content in waste. Enzyme supplementation influenced the distribution of organic acids in the ileum, indicating a shift in dominating bacteria, could be effective means for promoting gut health in piglets. Our former experiments showed that xylanase or xylanase and protease supplementation to the corn/wheat bran diet can improve ADG and feed conversion rate and obviously facilitate digestive rate of crude protein, crude fat and dry matter ($P < 0.05$) and can improve GI tract [86-94].

Other products under development include dietary immuno-stimulants that enhance mucosal immunity in the gut, hormone-modulating antibodies and hormones.

Conclusions

This review has discussed the major problems that face scientists trying to improve health of GI tract covered the various attempts and strategies that have been employed to improve microbes in the GI tract. The new alternatives to keep health of GI tract have great ecological, environmental issues and economic social effects. It seems clear from this review that several major challenges are presented and improvement of approach is possible providing that it is based on a rational scientific basis, but which until now has not been obvious due to our lack of understanding of the very complex system. Future development and routine implementation of them will pave the way for a new era in the field of feed additives to keep health of GI tract in animal production.

In retrospect, interaction and co-effects between those products should be studied further. The effective products should have a significant and sustainable beneficial impact on animal production and health, be proven safe for both the animal and human population, be easy to apply and store and provide a substantial return on investment. Renewed interest in microbial physiology and in the isolation of 699 ‘unculturable’ or ‘not-yet-cultured’ microbes is also necessary. New technologies such as terminal restriction fragment length polymorphism (T-RLFP), (Gen)omics including metagenomes/cryosequencing, now provide microbiologists with their best opportunity to see the iceberg in its entirety, rather than just its tip, in both a comparative and functional system [63, 83]. The composition and functions of the microbiota, as well as deviations from the balanced microbiota, will advance the selection of new and specific probiotics. This is both an exciting and daunting prospect. Clearly, new technologies will provide a massive increase in the rate of information acquisition for the potential to be fully realized. One expectation is that such technologies will eventually lead to the identification of novel, natural chemical entities that microbes have evolved to manipulate host biology in ways that enforce health and prevent various diseases from arising within or outside of the GI tract.

Acknowledgements

This research was jointly supported in part by grants from the Chinese Academy of Science Knowledge Innovation Project (#KSCX2-YW-N-051), National 863 project (2008AA10Z316), NSFC (#30901040, #30901041, #30928018, #30828025), the National Basic Research Program of China (#2008AA10Z316) and the CAS/SAFEA International Partnership Program for Creative Research Teams.

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