

Digestive Physiology and the Role of Microorganisms¹

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Primary Audience: Researchers, Nutritionists, Feed Manufacturers, Producers

SUMMARY

The gastrointestinal tract contains within it a microenvironment of bacteria that influences the host animal in many ways. The microflora can metabolize several nutrients that the host cannot digest and converts these to end products (such as short-chain fatty acids), a process that has a direct impact on digestive physiology. The microbiota directs the assembly of the gut-associated lymphoid tissue, helps educate the immune system, affects the integrity of the intestinal mucosal barrier, modulates proliferation and differentiation of its epithelial lineages, regulates angiogenesis, modifies the activity of the enteric nervous system, and plays a key role in extracting and processing nutrients consumed in the diet. Despite these important effects, the mechanisms by which the gut microbial community influences host biology remain almost entirely unknown. Recent molecular-based investigations have confirmed the species diversity and metabolic complexity of gut microflora, although there is much work to be done to understand how they relate to each other as well as the host animal. It is almost a century ago that Eli Metchnikoff proposed the revolutionary idea to consume viable bacteria to promote health. Since that time, the area known as probiotics has made dramatic progress, particularly during the past 2 decades. The last 20 yr have also seen the emergence of a new, related area of study—prebiotics. Use of these 2 ideas—providing live nonpathogenic bacteria as well as substrates for their growth—have potential to help optimize the health of animals by manipulating the gastrointestinal tract in positive ways.

Key words: digestive physiology, microorganism, probiotic, prebiotic, production

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DESCRIPTION OF PROBLEM

There is a tendency to regard all microorganisms as harmful, to equate bacteria with germs. Nothing could be further from the truth. The number of nonpathogenic species far exceeds the number of pathogenic species, and many of the known bacteria are in fact useful, even essential for the continued existence of life on earth. One example of a beneficial group of mi-

croorganisms are those that inhabit the gastrointestinal tract (**GIT**) of animals. The GIT harbors an incredibly complex and abundant ensemble of microbes [1, 2]. The intestine is in contact with components of this microflora from birth, yet little is known about their influence on normal development and physiology. The GIT is more densely populated with microorganisms than any other organ and is an interface where the microflora may have a pronounced impact

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on animal and human biology [2]. The bacterial population of the human cecum and colon is numerically large with at least 10^{13} cfu/g [3]. Similar values have been reported for other omnivores such as pigs [4]. Bacteria compose about 40 to 55% of solid stool matter [5]. Throughout millions of years of evolution, animals have developed the means for supporting complex and dynamic consortia of microorganisms during their life cycle. A transcendent view of vertebrate biology, therefore, requires an understanding of the contributions of these indigenous microbial communities to host development and adult physiology. The fragile composition of the gut microflora can be affected by various factors such as age, diet, environment, stress, and medication [6]. As with most complex ecosystems, it appears that most species cannot be cultured when removed from their niches [6, 7]. Although a full definition of biodiversity awaits systematic application of molecular enumeration techniques, such as genotyping DNA encoding 16S rRNA [rDNA] genes [8, 9, 10]. More than 50 genera and at least 500 to 1,000 different species are distributed along the length of the GIT [11]. The dominant organisms in terms of numbers are anaerobes including bacteroides, bifidobacteria, eubacteria, streptococci, and lactobacilli, and others, such as enterobacteria, also may be found, usually in fewer numbers [11, 12]. Generally, bacteroides (including those that can utilize a wide range of polysaccharides) are most numerous and can compose more than 30% of the total. Recent evaluation of the microflora ecology of the chicken intestine using 16s rDNA determined that *Lactobacilli* is the predominate organism in young birds, and the population of *Bifidobacterium* dominates in older birds [13].

Colonization begins at birth and is followed by progressive assembly of a complex and dynamic microbial society [14]. Assembly is presumably regulated by elaborate and combinatorial microbial-microbial and host-microbial interactions predicated on principles refined over the course of animal evolution. Comparisons of rodents raised without exposure to any microorganisms to animals that have assembled a microbiota since birth, or those that have been colonized with components of the microbiota during or after completion of postnatal development, have revealed a range of host functions affected

by indigenous microbial communities. For example, the microbiota directs the assembly of the gut-associated lymphoid tissue [15], helps educate the immune system [16, 17], affects the integrity of the intestinal mucosal barrier [18, 19, 20], modulates proliferation and differentiation of its epithelial lineages [21, 22], regulates angiogenesis [23], modifies the activity of the enteric nervous system [24], and plays a key role in extracting and processing nutrients consumed in the diet [25]. The microflora can metabolize proteins and protein degradation products, sulfur-containing compounds, and endogenous and exogenous glycoproteins [12]. Some organisms grow on intermediate products of fermentation such as H₂, lactate, succinate, formate, and ethanol and convert these to end products including short-chain fatty acids (SCFA), a process that has a direct impact on digestive physiology [26]. Although the mechanisms by which bacteria assert these effects on the GIT remain essentially unknown, research in this area is focusing on elucidating these mechanisms as well as manipulating the bacteria and the gastrointestinal environment toward achieving optimal health through probiotics and prebiotics.

THE ROLE OF MICROORGANISMS ON DIGESTIVE PHYSIOLOGY

SCFA Production

The SCFA increase from undetectable levels in the ceca of day-of-hatch chicks to the highest concentration at d 15 of age as the enteric microflora becomes established [27]. The basic fermentative reaction in the human colon or chicken cecum is similar to that in obligate herbivores: hydrolysis of polysaccharides, oligosaccharides, and disaccharides to their constituent sugars, which are then fermented, resulting in an increased biomass [28]. Carbohydrate hydrolysis is affected by a number of bacterial cell-associated and secreted hydrolases that can digest a range of carbohydrates, which monogastric animals cannot. Fermentation yields metabolizable energy for microbial growth and maintenance and also metabolic end products. Nitrogen for protein synthesis can come from urea (via the urease reaction), undigested dietary protein, or endogenous secretions. The principal prod-

ucts are SCFA together with gases [CO_2 , CH_4 , and H_2] and some heat [29]. Carbohydrates entering the large intestine can alter gut physiology in 2 ways: physical presence and fermentation. Effects of SCFA can be divided into those occurring in the lumen and those arising from their uptake and metabolism by the cells of the large bowel wall. The SCFA are the principal luminal anions. They are relatively weak acids with pKa values of 4.8, and increasing their concentrations through fermentation lowers digesta pH [29]. The SCFA also serve as an important source of energy for the gut wall, providing up to 50% of the daily energy requirements of colonocytes [28, 30]. Fermentable carbohydrates can alter the microbial ecology greatly by acting as substrates or supplying SCFA. Much attention has been directed toward the study of specific beneficial lactic acid bacteria, rather than the flora as a whole [30], however, the SCFA have diverse functions with regard to host and microbial physiology.

Blood Flow and Muscular Activity

Studies in vitro have shown that incubation with SCFA (as the sodium salts) at concentrations as low as 3 mM dilate precontracted colonic resistance arterioles in isolated human colonic segments [31]. Greater colonic blood flow has been observed with infusion of acetate, propionate, or butyrate (separately or as a mixture) into the denervated canine large bowel [32]. The mechanism of action of SCFA on blood flow does not involve prostaglandins or α - or β -adrenoreceptor linked pathways [31]. The mechanisms of action may involve local neural networks as well as chemoreceptors together with direct effects on smooth muscle cells [33]. The SCFA produced in the colon and entering the portal circulation seem to influence the upper gut musculature. These actions are important for the maintenance of the function of the whole gastrointestinal system, not just the colon. It is expected that greater blood flow enhances tissue oxygenation and transport of absorbed nutrients.

Enterocyte Proliferation

In rats, SCFA stimulate the growth of colorectal and ileal mucosal cells when they are delivered colorectally or intraperitoneally [34, 35].

In addition to promoting growth, the major SCFA (especially butyrate) appear to lower the risk of malignant transformation in the colon [36]. Secondary bile acids are cytotoxic, and in rats fed deoxycholate plus cholesterol, cell proliferation as measured by incorporation of [^3H]thymidine was increased [37]. Some of the effects of SCFA may be due to low intracolorectal pH rather than any specific SCFA. At a pH of 6, bile acids are largely protonated and insoluble and so would not be taken up by colonocytes [38]. Additionally, lower pH inhibits the bacterial conversion of primary to secondary bile acids [39, 40] and, therefore, lowers their carcinogenic potential.

Mucin Production

Evidence has been presented that mucus production and release is stimulated locally by endogenous production of SCFA by gut microflora [41, 42, 43]. Additionally, some studies have been completed evaluating the influence of specific beneficial or probiotic organisms on mucin production. In vitro studies with *Lactobacillus plantarum* 299v suggest that the ability of organisms to inhibit adherence of attaching and effacing organisms to intestinal epithelial cells is mediated through their ability to increase expression of MUC2 and MUC3 intestinal mucins [44, 45]. The benefits of probiotics mediated through intestinal mucin upregulation may have broader applicability than enteropathogen intervention in poultry. Several investigators have shown that the increase in mucin production following probiotic administration inhibited replication, disease symptoms and shedding of rotavirus in humans [46, 47, 48, 49]. In the proximal colon, an increase in the butyrate concentration altered crypt depth and the number of mucus-containing cells; the increase in butyrate was highly correlated with the number of neutral-mucin-containing cells [50, 51].

Probiotic, Prebiotic, and Synbiotic

The use of lactic acid bacteria as feed supplements goes back to pre-Christian times when fermented milks were consumed by humans. It was not until last century that Eli Metchnikoff, working at the Pasteur Institute in Paris, evaluated the subject from a scientific basis. Metchni-

koff, documented a direct link between human longevity and the necessity of maintaining a healthy balance of the beneficial and pathological microorganisms residing in the human gut. Metchnikoff's 1908 Nobel Prize in physiology was awarded for his discovery of phagocytes and other immune system components, but his accurate description of vital elements in the body's intestinal flora is equally notable. He developed and prescribed to his patients bacteriotherapy (i.e., the use of lactic acid bacteria in dietary regimens) [52]. In support of this he cited the observation that Bulgarian peasants consumed large quantities of soured milk and also lived long lives. He had no doubt about the causal relationship, and subsequent events have, in part, confirmed his thesis. He isolated what he called the Bulgarian bacillus from soured milk and used this in subsequent trials. This organism was probably what became known as *Lactobacillus bulgaricus* and is now called *Lactobacillus delbrueckii* subsp. *bulgaricus* which is one of the organisms used to ferment milk and produce yogurt [52]. After Metchnikoff's death in 1916 the center of activity moved to the United States. Knowledge available at that time suggested the use of *Lactobacillus acidophilus* and many trials were carried out using this organism. Encouraging results were obtained, especially in the relief of chronic constipation [53]. In the late 1940s interest in the gut microflora was stimulated by 2 research developments. The first was the finding that antibiotics included in the feed of farm animals promoted their growth. A desire to discover the mechanism of this effect led to increased study of the composition of the gut microflora and the way in which it might be affecting the host animal. Second, the more readily availability of germ-free animals provided a technique for testing the effect that the newly discovered intestinal inhabitants were having on the host. This increased knowledge also showed that *L. acidophilus* was not the only *Lactobacillus* in the intestine, and a wide range of different organisms came to be studied and later used in probiotic preparations [54].

A probiotic is defined as a live microbial food supplement that benefits the host by improving its intestinal microbial balance [55]. The presence of normal gut microflora may improve the metabolism of the host bird in various ways,

including absorptive capacity [56], protein metabolism [57], energy metabolism and fiber digestion [58], energy conversion [59], and gut maturation [60]. Balanced colonic microflora and immunostimulation are major functional effects attributed to the consumption of probiotics [55]. Many probiotic effects are mediated through immune regulation, particularly through balance control of proinflammatory and anti-inflammatory cytokines [61, 62]. However, probiotics can only be effective if the requirements for their growth are present in the GIT. The concept of prebiotics is relatively new; it was developed in response to the notion that nondigestible food ingredients (e.g., nondigestible oligosaccharides) are selectively fermented by one or more bacteria known to have positive effects on gut physiology. Bacteria fed by a preferential food substrate have a proliferative advantage over other bacteria [63]. Some prebiotics have shown to selectively stimulate the growth of endogenous lactic acid bacteria and *Bifidobacteria* in the gut to improve the health of the host [63]. Probiotic numbers have been enhanced by prebiotics that selectively stimulate the growth and activity of one or a limited number of bacterial species already resident in the large intestine, and, thus, improve host health [12]. In this way, prebiotics selectively modify the colonic microflora and can potentially influence gut metabolism [63]. However, the bacterial nutrient package will not be advantageous without the presence of the targeted, beneficial bacteria, and likewise the live microbial product will not succeed if the environment into which it is introduced is unfavorable [64]. The concept of synbiotic has been proposed recently to characterize foods with both prebiotic and probiotic properties as health-enhancing functional foods [42].

Role of Microorganisms in Poultry Production

The GIT serves as the interface between diet and the metabolic events that sustain life. In poultry, intestinal villi, which play a crucial role in digestion and absorption of nutrients, are underdeveloped at hatch [65], and maximum absorption capacity is attained by 10 d of age [66]. Understanding and optimizing the maturation and development of the intestine in poultry will improve feed efficiency, growth, and overall

health of the bird. In the immediate posthatch period birds must undergo the transition from energy supplied by the endogenous nutrients of the yolk to exogenous carbohydrate-rich feed. During that critical time dramatic changes occur in the intestinal size and morphology [65]. Maturation changes also affect the epithelial cell membranes, a major mechanical interface between the internal environment of the host and the luminal contents [67]. Studies on nutrition and metabolism during the early phase of growth in chicks may help in optimizing nutritional management for maximum growth [68]. By dietary means it is possible to affect the development of the gut and the competitiveness of beneficial and harmful bacteria, which can alter not only gut dynamics but also many physiologic processes due to the end products metabolized by symbiotic gut microflora. Additives such as enzymes, probiotics, and prebiotics are now extensively used throughout the world. The chemical nature of these additives are well understood, but the manner by which they benefit the animal is not [69].

Probiotics as an Alternative to Antibiotics for Control of Bacterial Pathogens in Poultry

Bacterial antimicrobial resistance in the medical and agricultural fields has become a serious problem worldwide. Antibiotic-resistant strains of bacteria are an increasing threat to animal and human health, with resistance mechanisms having been identified and described for all known antimicrobials currently available for clinical use. There is currently increased public and scientific interest regarding the administration of therapeutic and subtherapeutic antimicrobials to animals, due primarily to the emergence and dissemination of multiple antibiotic-resistant zoonotic bacterial pathogens [70]. Social pressures have led to creation of regulations to restrict antibiotic use in poultry and livestock production. There is a need to evaluate potential antibiotic alternatives to improve disease resistance in high intensity food animal production. Nutritional approaches to counteract the debilitating effects of stress and infection may provide producers with useful alternatives to antibiotics. Improvement of disease resistance of animals grown without antibiotics will not only benefit

the animals' health, welfare, and production efficiency but is also a key strategy in the effort to improve the microbiological safety of poultry products [70].

During the last 4 yr, our laboratory has worked toward the identification of probiotic candidates for poultry that can actually displace *Salmonella* and other enteric pathogens that have colonized the gastrointestinal tract of chicks and poults. Published studies [71] have indicated that after more than 8 million enteric organisms were screened for competition in vitro, 36 organisms were identified that had the ability to exclude *Salmonella* in neonatal poultry. Additional *Lactobacillus*-related isolates [72] were eventually found that were even more efficacious in the treatment of *Salmonella*-infected chicks and poults. In laboratory challenge studies, 80 to 90% reductions in *Salmonella* recovery rates from challenged chicks treated with Floramax probiotic culture were typical. By selecting flocks infected with *Salmonella* preslaughter, we have demonstrated that treating such flocks, approximately 2 wk prior to slaughter, with Floramax can markedly reduce environmental *Salmonella* recovery from commercial turkeys and broilers [73]. Treatment of idiopathic enteritis in commercial poults with Floramax also compares favorably to selected antibiotic therapy in recent studies [74]. Large-scale commercial trials have indicated that appropriate administration of this probiotic mixture to turkeys increased body weight gain at processing by approximately 230 g with over 120 flocks evaluated [75], with similar performance gains observed in more limited commercial trials with broilers. Administration of dietary lactose at a very low concentration (0.1%) greatly enhanced the growth rates of probiotic turkeys under commercial conditions and furthered reduced total production costs [75]. These data indicate that selection of therapeutically efficacious probiotic cultures with marked performance benefits in poultry is possible and that defined cultures can sometimes provide an attractive alternative to conventional antimicrobial therapy.

Comparisons Among Genotypic 16S rRNA, MIDI, and Biolog Identifications of FM-B11 Lactic Acid Bacteria

As a blend of facultative and obligate bacteria, the composition of lactic acid bacteria poses

Table 1. Comparisons between MicroSeq, MIDI, and Biolog identifications of FM-B11 (Floramax) lactic acid bacteria (LAB)

LAB ID	16S rRNA sequencing (first 500 bp)	MIDI system ID Micro Test Lab Inc.	MIDI system ID Microbial ID Inc.	Biolog ID
	Microbial ID Inc.			Department of Poultry Science, University of Arkansas
18	<i>Pediococcus parvulus</i>	<i>Enterococcus cecorum</i>	<i>Lactobacillus gasseri</i>	Unable to identify
24	<i>Weissella confusa</i>	<i>Lactobacillus casei</i>	<i>Lactobacillus casei</i>	<i>Clostridium clostridioforme</i>
27	<i>Weissella confusa</i>	<i>Lactobacillus casei</i>	<i>Lactobacillus casei</i>	<i>Weissella confusa</i>
29	<i>Pediococcus parvulus</i>	<i>Lactobacillus delbrueckii-bulgarius</i>	<i>Lactobacillus delbrueckii-bulgarius</i>	<i>Lactobacillus hamsteri</i>
36	<i>Lactobacillus salivarius</i>	<i>Lactobacillus cellobiosus</i>	<i>Lactobacillus casei</i>	<i>Weissella confusa</i>
37B	<i>Weissella confusa</i>	<i>Pediococcus acidilactici</i>	<i>Pediococcus ruminis</i>	Unable to identify
40	<i>Weissella confusa</i>	<i>Lactobacillus casei</i>	<i>Lactobacillus cellobiosus</i>	<i>Weissella paramesenteroides</i>
44	<i>Weissella paramesenteroides</i>	<i>Lactobacillus fermentum</i>	<i>Lactobacillus fermentum</i>	Unable to identify
46	<i>Lactobacillus salivarius</i>	<i>Lactobacillus helveticus</i>	<i>Lactobacillus sanfranciscensis</i>	<i>Lactobacillus salivarius</i>
48	<i>Lactobacillus salivarius</i>	<i>Lactobacillus helveticus</i>	<i>Lactobacillus gasseri</i>	<i>Lactobacillus salivarius</i>
52	<i>Pediococcus parvulus</i>	Unable to identify	<i>Lactobacillus cellobiosus</i>	Unable to identify

a unique problem for microbial identification. The identification techniques of choice for facultative anaerobes are biochemical analyses, but the standard identification system for lactic acid bacteria is cellular fatty acid profiling. However, these phenotypic methods can yield variable results. Genotypic methods that rely on comparisons of 16S rRNA sequences from unknown bacteria are proving to be valuable for use in a wide range of genera and are not sensitive to variable culture conditions. Genotypic 16S rRNA identification of organisms from probiotic cultures may be more reliable than the current standard microbial techniques applied separately to different microbial groups. Although there are many new experimental molecular identification techniques, such as microarray hybridization and matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry, sequence analysis of 16S rRNA is the predominant molecular technology currently available for microbial identification [76]. The 16S rRNA sequences of many bacteria species in a population may be analyzed simultaneously with denaturing gradient gel electrophoresis or random fragment length polymorphism analyses. The detailed information needed to identify each species represented in the complex microbial population of a probiotic product can only be fully obtained from the 16S rRNA at the level of the nucleotide sequence. As an example, we have devised an identification scheme using the MIDI System ID from 2 different private laboratories [77, 78] and the Biolog ID System [79] and the 16S rRNA Sequence Analyses [80] for identification of the individual component bacteria present in the commercial probiotic Floramax (Table 1). The results of that study show that the complex populations of bacteria present in Floramax are not easy to accurately identify, especially with phenotypic techniques. Genotypic identification by 16S rRNA gene sequence analysis has potential to improve the accuracy of bacterial identification, especially as the contents of sequence databases become more comprehensive. Conventional technologies can detect human pathogens, because they are well-established in comparative databases, but emerging and opportunistic pathogens are not. These results support a suggestion by the MIDI company [81] to use 16S rRNA sequence analysis to identify obligate and

facultative anaerobes, such as those in FM-B11 (Floramax). Although ambiguity exists between different methods of identification of nonpathogenic probiotic bacteria, identification of known pathogens is much more consistent. Therefore, the use of fully defined cultures for competitive exclusion or probiotic use are still inherently safer than undefined cultures or those where organisms are identified after the culture has been produced.

CONCLUSIONS

The interest in digestive physiology and the role of microorganisms has generated data whereby human and animal well being can be enhanced and the risk of disease reduced. New molecular techniques allow accurate assessment of the flora composition, resulting in improved strategies for elucidating mechanisms. Given the recent international legislation and domestic consumer pressures to withdraw growth-promoting antibiotics and limit antibiotics available for treatment of bacterial infections, probiotics and prebiotics can offer alternative options. New advances in the application of synbiotics (compatible probiotics and prebiotics) are directed

toward producing significant changes in gut physiology and provide even higher levels of health as well as increasing performance parameters.

Metchnikoff founded the research field of probiotics, which is aimed at modulating the intestinal microflora. However, other parts of the body containing endogenous microflora or problems relating to the immune system may also be candidates for probiotic therapy. Research has shown that probiotics have potential for addressing human health issues such as vaginal candidiosis [82], dental caries [83, 84], allergies [85], autoimmune diseases [86], urogenital infections [87], atopic diseases [88], rheumatoid arthritis [89], and respiratory infections [90]. Current research is still heavily biased toward gastrointestinal applications for probiotics, such as chronic constipation [53], chronic diarrhea [91], inflammatory bowel disease [92], irritable bowel syndrome [93], and food allergy [94]; but the possibilities for affecting many areas of health are numerous. Much research has been completed in efforts to understand and apply the natural benefits of nonpathogenic bacteria, but there is much still to do.

REFERENCES AND NOTES

1. Savage, D. C. 1977. Microbial ecology of the gastrointestinal tract. *Annu. Rev. Microbiol.* 31:107–133.
2. Eckburg, P. B., P. W. Lepp, and D. A. Relman. 2003. Archaea and their potential role in human disease. *Infect. Immun.* 71:591–596.
3. Hill, M. J. 1995. Bacterial fermentation of complex carbohydrates in the human colon. *Eur. J. Cancer Prev.* 4:353–358.
4. Butine, T. J., and J. A. Leedle. 1989. Enumeration of selected anaerobic bacterial groups in cecal and colonic contents of growing-finishing pigs. *Appl. Environ. Microbiol.* 55:1112–1116.
5. Cummings, J., and G. T. Macfarlane. 1991. The control and consequences of bacterial fermentation in the human colon. *J. Appl. Bacteriol.* 70:443–459.
6. Suau, A., R. Bonnet, M. Sutren, J. J. Godon, G. R. Gibson, M. D. Collins, and J. Dore. 1999. Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. *Appl. Environ. Microbiol.* 65:4799–4807.
7. Salzman, N. H., H. de Jong, Y. Paterson, H. J. Harmsen, G. W. Welling, and N. A. Bos. 2002. Analysis of 16S libraries of mouse gastrointestinal microflora reveals a large new group of mouse intestinal bacteria. *Microbiology* 148:3651–3660.
8. Harmsen, H. J., G. C. Raangs, T. He, J. E. Degener, and G. W. Welling. 2002. Extensive set of 16S rRNA-based probes for detection of bacteria in human feces. *Appl. Environ. Microbiol.* 68:2982–2990.
9. Hayashi, H., M. Sakamoto, and Y. Benno. 2002. Phylogenetic analysis of the human gut microbiota using 16S rDNA clone libraries and strictly anaerobic culture-based methods. *Microbiol. Immunol.* 46:535–548.
10. Blaut, M., M. D. Collins, G. W. Welling, J. Dore, J. Van Loo, and W. de Vos. 2002. Molecular biological methods for studying the gut microbiota: The EU human gut flora project. *Br. J. Nutr.* 87(Suppl. 2):S203–S211.
11. Xu, J., and J. I. Gordon. 2003. Inaugural Article: Honor thy symbionts. *Proc. Natl. Acad. Sci. USA* 100:10452–10459.
12. Gibson, G. R., and M. B. Roberfroid. 1995. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *J. Nutr.* 125:1401–1412.
13. Amit-Romach, E., D. Sklan, and Z. Uni. 2004. Microflora ecology of the chicken intestine using 16S ribosomal DNA primers. *Poult. Sci.* 83:1093–1098.
14. Favier, C. F., E. E. Vaughan, W. M. De Vos, and A. D. Akkermans. 2002. Molecular monitoring of succession of bacterial communities in human neonates. *Appl. Environ. Microbiol.* 68:219–226.
15. Cebra, J. J. 1999. Influences of microbiota on intestinal immune system development. *Am. J. Clin. Nutr.* 69:1046S–1051S.
16. Braun-Fahrlander, C., J. Riedler, U. Herz, W. Eder, M. Waser, L. Grize, S. Maisch, D. Carr, F. Gerlach, A. Bufe, R. P. Lauener, R. Schierl, H. Renz, D. Nowak, and E. von Mutius. 2002. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N. Engl. J. Med.* 347:869–877.
17. Kelly, D., J. I. Campbell, T. P. King, G. Grant, E. A. Jansson, A. G. Coutts, S. Pettersson, and S. Conway. 2004. Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. *Nat. Immunol.* 5:104–112.
18. MacPherson, A. J., D. Gatto, E. Sainsbury, G. Harriman, H. Hengartner, and R. M. Zinkernagel. 2000. A primitive T cell

independent mechanism of intestinal mucosal IgA responses to commensal intestinal bacteria. *Science* 288:2222–2226.

19. Hooper, L. V., M. H. Wong, A. Thelin, L. Hansson, P. G. Falk, and J. I. Gordon. 2001. Molecular analysis of commensal host-microbial relationships of the intestine. *Science* 291:881–884.

20. Hooper, L. V., T. S. Stappenbeck, C. V. Hong, and J. I. Gordon. 2003. Angiogenins: A new class of microbicidal proteins involved in innate immunity. *Nat. Immunol.* 4:269–273.

21. Uribe, A., M. Alam, T. Midtvedt, B. Smedfors, and E. Theodorsson. 1997. Endogenous prostaglandins and microflora modulate DNA synthesis and neuroendocrine peptides in the rat gastrointestinal tract. *Scand. J. Gastroenterol.* 32:691–699.

22. Bry, L., P. G. Falk, T. Midtvedt, and J. I. Gordon. 1996. A model of host-microbial interactions in an open mammalian ecosystem. *Science* 273:1380–1383.

23. Stappenbeck, T. S., L. V. Hooper, and J. I. Gordon. 2002. Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. *Proc. Natl. Acad. Sci. USA* 99:15451–15455.

24. Husebye, E., P. M. Hellstro, and T. Midtvedt. 1994. Intestinal microflora stimulates myoelectric activity of rat small intestine by promoting cyclic initiation and aboral propagation of migrating myoelectric complex. *Dig. Dis. Sci.* 39:946–956.

25. Hooper, L. V., T. Midtvedt, and J. I. Gordon. 2002. How host-microbial interactions shape the nutrient environment of the mammalian intestine. *Annu. Rev. Nutr.* 22:283–307.

26. Macfarlane, G. T., and G. R. Gibson. 1995. Microbiological aspects of the production of short-chain fatty acids in the large bowel. Page 87 In *Physiological and Clinical Aspects of Short-Chain Fatty Acids*. J. H. Cummings, J. L. Rombeau, and S. Sakata, ed. Cambridge Univ. Press, Cambridge, UK.

27. van der Wielen, P. W., S. Biesterveld, S. Notermans, H. Hofstra, B. A. P. Urlings, and F. van Knapen. 2000. Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. *Appl. Environ. Microbiol.* 66:2536–2540.

28. Savage, D. C. 1986. Gastrointestinal microflora in mammalian nutrition. *Annu. Rev. Nutr.* 6:155–178.

29. Topping, D. L., and P. M. Clifton. 2001. Short-chain fatty acids and human colonic function: Roles of resistant starch and nonstarch polysaccharides. *Physiol. Rev.* 81:1031–1064.

30. Bird, A. R., I. L. Brown, and D. L. Topping. 2000. Starches, resistant starches, the gut microflora and human health. *Curr. Issues Intest. Microbiol.* 1:25–37.

31. Mortensen, F. V., and H. Nielsen. 1995. In vivo and in vitro effects of shortchain fatty acids on intestinal blood circulation. Page 391 In *Physiological and Clinical Aspects of Short-Chain Fatty Acids*. J. H. Cummings, J. L. Rombeau, and T. Sakata, ed. Cambridge Univ. Press, Cambridge, UK.

32. Kvietyspr, A., and D. N. Granger. 1981. Effect of volatile fatty acids on blood flow and oxygen uptake by the dog colon. *Gastroenterology* 80:962–969.

33. Cherbut, C. 1995. Effects of short-chain fatty acids on gastrointestinal motility. Page 191 In *Physiological and Clinical Aspects of Short-Chain Fatty Acids*. J. H. Cummings, J. L. Rombeau, and T. Sakata, ed. Cambridge Univ. Press, Cambridge, UK.

34. Kripke, S. A., A. D. Fox, J. M. Berman, R. G. Settle, and J. L. Rombeau. 1989. Stimulation of intestinal mucosal growth with intracolonic infusion of short chain fatty acids. *J. Parententeral Nutr.* 13:109–116.

35. Sakat, T., and T. Yajima. 1984. Influence of short chain fatty acids on the epithelial cell division of digestive tract. *Q. J. Exp. Physiol.* 69:639–648.

36. Velazquez, C., R. W. Seto, A. M. Bain, J. Fisher, and J. L. Rombeau. 1997. Deoxycholate inhibits in vivo butyrate-mediated BrDU labeling of the colonic crypt. *J. Surg. Res.* 69:344–348.

37. Lapre, J. A., and L. Van Der Meer. 1992. Diet-induced increase in colonic bile acids stimulates lytic activity of fecal water and proliferation of colonic cells. *Carcinogenesis* 13:41–44.

38. Rafter, J. J., V. W. Eng, R. Furrer, A. Medline, and W. R. Bruce. 1986. Effects of calcium and pH on the mucosal damage produced by deoxycholic acid in the rat colon. *Gut* 27:1320–1329.

39. Macdonald, I. A., G. Singh, D. E. Mahony, and C. E. Meier. 1978. Effect of pH on bile salt degradation by mixed fecal cultures. *Steroids* 32:245–256.

40. Nagengast, F. M., M. P. Hectors, W. A. Buys, and J. H. Van Tongeren. 1988. Inhibition of secondary bile acid formation in the large intestine by lactulose in healthy subjects of two different age groups. *Eur. J. Clin. Invest.* 18:56–61.

41. MacFarlane, G., T. S. Hay, and G. R. Gibson. 1989. Influence of mucin on glycosidase, protease and arylamidase activities of human gut bacteria grown in a 3-stage continuous culture system. *J. Appl. Bacteriol.* 66:407–417.

42. Gibson, G. R., and M. B. Roberfroid. 1995. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *J. Nutr.* 125:1401–1412.

43. Sakata, T., and H. Setoyama. 1995. Local stimulatory effect of short-chain fatty acids on the mucus releases from the hindgut mucosa of rats (*Rattus norvegicus*). *Comp. Biochem. Physiol.* 111A:429–432.

44. Johansson, M. L., G. Molin, B. Jeppsson, S. Nobaek, S. Ahrne, and S. Bengmark. 1993. Administration of different *Lactobacillus* strains in fermented oatmeal soap: In vivo colonization of human intestinal mucosa and effect on the indigenous flora. *Appl. Environ. Microbiol.* 59:15–20.

45. Li, J.-D., W. Feng, M. Gallup, J.-H. Kim, J. Gum, Y. Kim, and C. Basbaum. 1998. Activation of NF- κ B via a Src-dependent Ras-MAPK-pp90rk pathway is required for *Pseudomonas aeruginosa* induced mucin overproduction in epithelial cells. *Proc. Natl. Acad. Sci. USA* 95:5718–5723.

46. Saaverda, J. M., N. A. Bauman, I. Oung, J. A. Perman, and R. H. Yolken. 1994. Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhea and shedding of rotavirus. *Lancet* 344:1046–1049.

47. Yolken, R. H., C. Ojeh, I. A. Khatri, U. Sajjan, and J. F. Forstner. 1994. Intestinal mucins inhibit rotavirus replication in an oligosaccharide-dependent manner. *J. Infect. Dis.* 169:1002–1006.

48. Guarino, A., R. B. Canani, M. I. Spagnuolo, F. Albano, and L. Di Benedetto. 1997. Oral bacterial therapy reduces the duration of symptoms and of viral excretion in children with mild diarrhea. *J. Pediatr. Gastroenterol. Nutr.* 25:516–519.

49. Shornikova, A. V., I. A. Casas, E. Isolauri, H. Mykkanen, and T. Vesikari. 1997. *Lactobacillus reuteri* as a therapeutic agent in acute diarrhea in young children. *J. Pediatr. Gastroenterol. Nutr.* 24:399–404.

50. Sakata, T., and V. Engelhardt. 1981. Influence of short-chain fatty acids and osmolality on mucin release in the rat colon. *Cell Tissue Res.* 219:371–377.

51. Jean-Claude, M., B. Martine, P. Françoise, and A. Claude. 2001. Comparative differential influence of butyrate concentration on proximal and distal colonic mucosa in rats born germ-free and associated with a strain of *Clostridium*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 128:379–384.

52. Metchnikoff Ilya, I. 1908. *Prolongation of Life: Optimistic Studies*. Putnam & Sons, New York, NY.

53. Koebnick, C., I. Wagner, P. Leitzmann, U. Stern, and H. J. Zunft. 2003. Probiotic beverage containing *Lactobacillus casei* Shirata improves gastrointestinal symptoms in patients with chronic constipation. *Can. J. Gastroenterol.* 7:655–659.

54. Hughes, D. B., and D. G. Hoover. 1991. *Bifidobacteria*: Their potential for use in American dairy products. *Food Technol.* 45:74–83.

55. Isolauri, E., Y. Suias, P. Kankaanpaa, and S. Salmiinen. 2001. Probiotics: Effects on immunity. *Am. J. Clin. Nutr.* 73:444S–450S.

56. Yokota, H., and M. E. Coates. 1982. The uptake of nutrients from the small intestine of gnotobiotic and conventional chicks. *Br. J. Nutr.* 47:349–356.

57. Salter, D. N., M. E. Coates, and D. Hewitt. 1974. The utilization of protein and excretion of acid uric in germ free and conventional chicks. *Br. J. Nutr.* 31:307–318.
58. Muramatsu, T., S. Nakajima, and J. Okumura. 1994. Modification of energy metabolism by the presence of the gut microflora in the chicken. *Br. J. Nutr.* 71:709–717.
59. Furuse, M., and H. Yokota. 1984. Protein and energy utilization in germ free and conventional chicks given diets containing different levels of dietary protein. *Br. J. Nutr.* 51:255–264.
60. Furuse, M., S. I. Yang, N. Niwa, and J. Okumura. 1991. Effect of short chain fatty acids on the performance and the intestinal weight in germ free and conventional chicks. *Br. Poult. Sci.* 32:159–165.
61. Ghosh, S., M. J. May, and E. B. Kopp. 1998. NF-kappa B and Rel proteins: Evolutionarily conserved mediators of immune responses. *Annu. Rev. Immunol.* 16:225–260.
62. Neish, A., T. Gewirtz, H. Zeng, and A. N. Young. 2000. Prokaryotic regulation of epithelial responses by inhibition of I β B-ubiquitination. *Science* 289:1560–1563.
63. Gibson, G. R., and X. Wang. 1994. Regulatory effects of Bifidobacteria on the growth of other colonic bacteria. *J. Appl. Bacteriol.* 77:412–420.
64. Apajalahti, J., and M. R. Bedford. 1999. Improve bird performance by feeding its microflora. *World's Poult. Sci. J.* 55:20–23.
65. Uni, Z., Y. Noy, and D. Sklan. 1995. Development of the small intestine in heavy and light strain chicks before and after hatching. *Br. Poult. Sci.* 36:63–71.
66. Noy, Y., and D. Sklan. 1997. Post hatch development in poultry. *J. Appl. Poult. Res.* 6:344–354.
67. Rozee, K. R., D. Cooper, K. Lam, and J. W. Costerton. 1982. Microbial flora on the mouse ileum mucous layer and epithelial surface. *Appl. Environ. Microbiol.* 43:1451–1463.
68. Nir, I. 1995. The uncertainties of the young broiler growth. *Page 19-28 In Proc. 10th Eur. Symp. Poult. Nutr. Eur. Fed. WPSA Branches, Antalya, Turkey.*
69. Bedford, M. R. 2000. Exogenous enzymes in monogastric nutrition—Their current value and future benefits. *Anim. Feed Sci. Technol.* 86:1–13.
70. McDermott, P. F., J. W. Zhao, X. Wagner, D. D. Simjee, R. D. Walker, and D. G. White. 2002. The food safety perspective of antibiotic resistance. *Anim. Biotechnol.* 13:71–84.
71. Bielke, L. R., A. L. Elwood, D. J. Donoghue, A. M. Donoghue, L. A. Newberry, N. K. Neighbor, and B. M. Hargis. 2003. Approach for selection of individual enteric bacteria for competitive exclusion in turkey poults. *Poult. Sci.* 82:1378–1382.
72. Wynco- IVS, Rogers, AR.
73. Vicente, J. L., A. Torres-Rodríguez, S. E. Higgins, C. Pixley, G. Tellez, and B. M. Hargis. 2005. Effect of a probiotic culture on horizontal transmission of *Salmonella enteritidis* in turkey poults. *Poult. Sci.* 84(Suppl. 1):101. (Abstr.)
74. Higgins, S. E., A. Torres-Rodríguez, J. L. Vicente, C. D. Sartor, C. M. Pixley, G. M. Nava, G. Tellez, J. T. Barton, and B. M. Hargis. 2005. Evaluation of intervention strategies for idiopathic diarrhea in commercial turkey brooding houses. *J. Appl. Poult. Res.* 14:345–348.
75. Torres-Rodríguez, A., S. Higgins, L. Salvador, A. Wolfenden, L. Bielke, C. Pixley, N. Neighbor, G. Gaona, X. Hernández, G. Tellez, and B. Hargis. 2005. Evaluation of a *Lactobacillus*-based probiotic on turkey performance under field conditions. *Poult. Sci.* 84(Suppl. 1):100. (Abstr.)
76. Wagner, R. D., D. D. Paine, and C. E. Cerniglia. 2003. Phenotypic and genotypic characterization of competitive exclusion products for use in poultry. *J. Appl. Microbiol.* 94:1098–1107.
77. Micro Test Lab Inc., Agawam, MA.
78. Microbial ID Inc., Newark, DE.
79. Biolog, Inc., Hayward, CA.
80. Microbial ID Inc., Newark, DE.
81. Sasser, M. 1990. Identification of bacteria by gas chromatography of cellular fatty acids. Pages 165–171 in *MIDI technical note 101*. MIDI, Newark, DE.
82. Concetta, B., M. Rinaldo, M. Boccanera, M. R. Spinosa, T. Maggi, S. Conti, W. Magliani, F. De Bernardis, G. Teti, A. Cassone, G. Pozzi, and L. Polonelli. 2000. Therapy of mucosal candidiasis by expression of an anti-idiotype in human commensal bacteria. *Nat. Biotechnol.* 18:1060–1064.
83. Krüger, C. Y., and Q. P. Hu. 2002. In situ delivery of passive immunity by lactobacilli producing single-chain antibodies. *Nat. Biotechnol.* 20:702–706.
84. Nase, L., K. Ataca, and E. Savilahti. 2001. Effect of long-term consumption of a probiotic bacterium, *Lactobacillus rhamnosus* GG, in milk on dental caries and caries risk in children. *Caries Res.* 35:412–420.
85. Pochard, P., H. Hammad, C. Ratajczak, A. S. Charbonnier-Hatzfeld, N. Just, A. B. Tonnel, and J. Pestel. 2005. Direct regulatory immune activity of lactic acid bacteria on Der p 1-pulsed dendritic cells from allergic patients. *J. Allergy Clin. Immunol.* 116:198–204.
86. Martínez, B., J. Sllanpää, E. Smit, T. K. Korhonen, and P. H. Pouwels. 2000. Expression of cbsA Encoding the Collagen-Binding S-Protein of *Lactobacillus crispatus* JCM5810 in *Lactobacillus casei* ATCC 393T. *J. Bacteriol.* 182:6857–6861.
87. Cadieux, P., J. Burton, and G. Gardiner. 2002. *Lactobacillus* strains and vaginal ecology. *JAMA* 287:1940–194.
88. Kalliomaki, M., S. Salminen, H. Arvilommi, P. Kero, P. Koskinen, and E. Isolauri. 2001. Probiotics in primary prevention of atopic disease: A randomised placebo-controlled trial. *Lancet* 357:1076–1079.
89. Vanderhoof, J. A. 2001. Probiotics: Future directions. *Am. J. Clin. Nutr.* 73:1152S–1155S.
90. Villena, J., S. Racedo, G. Agüero, E. Bru, M. Medina, and S. Alvarez. 2005. *Lactobacillus casei* improves resistance to pneumococcal respiratory infection in malnourished mice. *J. Nutr.* 135:1462–1469.
91. Xiao, S. D., Z. Zhang, and H. Lu. 2003. Multicenter, randomized, controlled trial of heat-killed *Lactobacillus acidophilus* LB in patients with chronic diarrhea. *Adv. Ther.* 20:253–260.
92. Schultz, M., A. Timmer, H. H. Herfarth, R. B. Sartor, J. A. Vanderhoof, and H. C. Rath. 2004. *Lactobacillus* GG in inducing and maintaining remission of Crohn's disease. *BMC Gastroenterol.* 4:5.
93. Saggiaro, A. 2004. Probiotics in the treatment of irritable bowel syndrome. *J. Clin. Gastroenterol.* 38(Suppl. 6):S104–S106.
94. Majamaa, H., and E. Isolauri. 1997. Probiotics: a novel approach in the management of food allergy. *J. Allergy Clin. Immunol.* 99:179–185.